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Date: 2005-05

Notes:

- 1) Segmented by Journal which publish articles on Basic Research and Human Disease**
- 2) Journals are listed alphabetically from J to Z**

J Androl (1)

Hayashi, T., A. Yoshinaga, et al. (2004). "Expression of the p63 and Notch Signaling Systems in Rat Testes During Postnatal Development: Comparison With Their Expression Levels in the Epididymis and Vas Deferens." J Androl **25**(5): 692-698.

<http://www.andrologyjournal.org/cgi/content/abstract/25/5/692>

The role of tubular structures that contribute to the passage of spermatozoa is not solely passive; these structures actively contribute to their own functions, although these tubules and ducts are contiguous and collaborate in the development of the male gamete along their lengths. The testis has the specific function to generate spermatozoa and spermatozoa undergo numerous changes as they pass through the epididymis. A member of the p53 family of genes, p63, is highly expressed in the basal layers of epithelial tissues and plays a key role in maintaining their cell populations, whereas Notch 1 and its ligand Jagged 2 have an important role in the differentiation of germ cells and Jagged 2 is up-regulated by TAp63, one of the p63 isoforms, which transactivates p53 target genes and induces apoptosis. Although the presence of p63 in most epithelia is established, the role of p63 and its possible relationship with the Notch system in the seminiferous epithelium have not been examined. Therefore, we investigated the expression of p63, Jagged 2, and Notch 1 in the testis during postnatal development in comparison with their expression levels in the vaso-epididymal epithelium. In the testis, the expression of TAp63 mRNA increased at day 14 after birth and the expressions of Jagged 2 and Notch 1 mRNA increased at day 16 after birth, suggesting that TAp63-mediated Jagged 2 induction activates the Notch signaling system. On the other hand, the strong signal of Δ Np63 mRNA was already recognized in the vas deferens at day 0 after birth and advanced chronologically along the duct to the caput epididymis and p63 protein was expressed in basal cells in their epithelium, whereas the mRNAs of Jagged 2 and Notch 1 were maintained at a low level. Consequently, examination of our data raises the probability that TAp63 has an important role for maintenance of germ cell numbers, triggering or balancing the development, differentiation, and apoptosis of germ cells in the testis, which is completely different from the role of Δ Np63 in other epithelial tissues.

J Biomol Screen (3)

Benjamin, E. R., S. L. Haftl, et al. (2004). "A Miniaturized Column Chromatography Method for Measuring Receptor-Mediated Inositol Phosphate Accumulation." J Biomol Screen 9(4): 343-353.

<http://jbx.sagepub.com/cgi/content/abstract/9/4/343>

Inositol phosphates (IPs), such as 1,4,5-inositol-trisphosphate (IP3), comprise a ubiquitous intracellular signaling cascade initiated in response to G protein-coupled receptor-mediated activation of phospholipase C. Classical methods for measuring intracellular accumulation of these molecules include time-consuming high-performance liquid chromatography (HPLC) separation or large-volume, gravity-fed anion-exchange column chromatography. More recent approaches, such as radio-receptor and AlphaScreen™ assays, offer higher throughput. However, these techniques rely on measurement of IP3 itself, rather than its accumulation with other downstream IPs, and often suffer from poor signal-to-noise ratios due to the transient nature of IP3. The authors have developed a miniaturized, anion-exchange chromatography method for measuring inositol phosphate accumulation in cells that takes advantage of signal amplification achieved through measuring IP3 and downstream IPs. This assay uses centrifugation of 96-well-formatted anion-exchange mini-columns for the isolation of radiolabeled inositol phosphates from cell extracts, followed by low-background dry-scintillation counting. This improved assay method measures receptor-mediated IP accumulation with signal-to-noise and pharmacological values comparable to the classical large-volume, column-based methods. Assay validation data for recombinant muscarinic receptor 1, galanin receptor 2, and rat astrocyte metabotropic glutamate receptor 5 are presented. This miniaturized protocol reduces reagent usage and assay time as compared to large-column methods and is compatible with standard 96-well scintillation counters.

Taylor, M. R. G., M. L. Robinson, et al. (2004). "Analysis of Genetic Variations of Lamin A/C Gene (LMNA) by Denaturing High-Performance Liquid Chromatography." J Biomol Screen 9(7): 625-628.

<http://jbx.sagepub.com/cgi/content/abstract/9/7/625>

The human LMNA gene, when mutated, has been shown to cause at least 7 human diseases: dilated cardiomyopathy, Emery Dreifuss muscular dystrophy, limb girdle muscular dystrophy, familial partial lipodystrophy, Charcot Marie tooth disease type II, mandibuloacral dysplasia, and Hutchinson-Gilford Progeria (OMIM #176670). This article describes a high-throughput method for screening the human lamin A/C (LMNA) gene for genetic mutations and sequence variation using denaturing high-performance liquid chromatography (DHPLC). In the present study, 76 patients with dilated cardiomyopathy were screened for mutations using DHPLC and sequence analysis. Abnormal elution profiles were identified and sequenced on an ABI 377 automatic sequencer. Heterozygous LMNA mutations were detected in 8% of the affected patients. In addition, a number of intronic and exonic single nucleotide polymorphisms were identified. LMNA mutations are clinically relevant in at least 6 human diseases. This study provides a protocol for high-throughput LMNA analysis applicable both in the research and in the clinical diagnostic setting.

Yauch, R. L., E. E. Kadel, III, et al. (2004). "Transcriptional-Based Screens for Pathway-Specific, High-Throughput Target Discovery in Endothelial Cells." J Biomol Screen 9(8): 704-711.

<http://jbx.sagepub.com/cgi/content/abstract/9/8/704>

With the sequence of the human genome at hand, target discovery strategies are needed that can rapidly identify novel gene products involved in human disease pathways. In this article, the authors describe a cell-based, high-throughput assay that can identify gene products capable of modulating the vascular endothelial growth factor (VEGF) and tumor necrosis factor * (TNF α) signaling pathways in human endothelial cells. The assay uses real-time PCR technology to measure downstream reporter mRNA transcripts induced upon cytokine stimulation in a 96-well plate format and has been adapted for use with recombinant adenoviruses. The authors specifically demonstrate modulation of cytokine-driven reporter transcripts using drug inhibitors and through adenoviral-mediated expression of known signaling intermediates of the respective pathways. In addition, they have used an arrayed library of 350 recombinant adenoviruses to screen for novel modulators of the VEGF and TNF* pathways. The high-throughput screening capacity and sensitivity of this system make it a useful tool for new drug target identification.

J Med Virol (1)

Castle, P. E., M. Schiffman, et al. (2002). "Comparisons of HPV DNA detection by MY09/11 PCR methods." J Med Virol **68**(3): 417-23.

Two modifications to the original L1 consensus primer human papillomavirus (HPV) PCR method, MY09-MY011, using AmpliTaq DNA polymerase (MY-Taq), were evaluated for HPV DNA detection on clinical specimens from a cohort study of cervical cancer in Costa Rica. First, HPV DNA testing of 2978 clinical specimens by MY09-MY011 primer set, using AmpliTaq Gold DNA polymerase (MY-Gold) were compared with MY-Taq testing. There was 86.8% total agreement ($\kappa = 0.72$, 95%CI = 0.70-75) and 69.6% agreement among positives between MY-Gold and MY-Taq. MY-Gold detected 38% more HPV infections ($P < 0.0001$) and 45% more cancer-associated (high-risk) HPV types ($P < 0.0001$) than MY-Taq, including 12 of the 13 high-risk HPV types. Analyses of discordant results using cytologic diagnoses and detection of HPV DNA by the Hybrid Capture 2 Test suggested that MY-Gold preferentially detected DNA positive specimens with lower HPV viral loads compared with MY-Taq. In a separate analysis, PGMY09-PGMY11 (PGMY-Gold), a redesigned MY09/11 primer set, was compared with MY-Gold for HPV DNA detection ($n = 439$). There was very good agreement between the two methods ($\kappa = 0.83$; 95%CI = 0.77-0.88) and surprisingly no significant differences in HPV detection ($P = 0.41$). In conclusion, we found MY-Gold to be a more sensitive assay for the detection of HPV DNA than MY-Taq. Our data also suggest that studies reporting HPV DNA detection by PCR need to report the type of polymerase used, as well as other assay specifics, and underscore the need for worldwide standards of testing.

J Natl Cancer Inst (9)

Ahrendt, S. A., Y. Hu, et al. (2003). "p53 Mutations and Survival in Stage I Non-Small-Cell Lung Cancer: Results of a Prospective Study." J Natl Cancer Inst **95**(13): 961-970.

<http://jncicancerspectrum.oupjournals.org/cgi/content/abstract/jnci;95/13/961>

Background: The p53 gene is frequently mutated in non-small-cell lung cancer (NSCLC); however, the effect of p53 gene mutations on patient prognosis remains unclear. Therefore, we initiated a prospective study to determine the association of p53 gene mutations with survival in patients with stage I NSCLC. **Methods:** Tumor samples were collected prospectively from 188 patients with operable NSCLC (stages I, II, and IIIA). p53 mutations were detected by direct dideoxynucleotide sequencing and p53 GeneChip analysis. Association of clinical and pathologic variables (e.g., alcohol consumption, sex, age, pathologic stage) with mutation of the p53 gene was determined by logistic regression. Associations between p53 mutation status, clinical and pathologic variables, and survival were assessed using a Cox proportional hazards regression model. All statistical tests were two-sided. **Results:** p53 mutations were detected in 55% (104/188) of tumors. These mutations were associated with non-bronchoalveolar tumors, a history of alcohol consumption, and younger patient age. The risk of death was statistically significantly higher in patients with p53 mutations in their tumors (hazard ratio [HR] = 1.6, 95% confidence interval [CI] = 1.0 to 2.4; $P = .049$) than in patients with wild-type p53 in their tumors. Tumor stage, the presence of a p53 mutation, and increasing patient age were statistically significant predictors of patient death in the entire patient group; however, the statistically significant prognostic effect of p53 mutation was limited to patients with stage I NSCLC (stage I HR = 2.8, 95% CI = 1.4 to 5.6; stage II HR = 1.8, 95% CI = 0.74 to 4.4; and stage III HR = 0.70, 95% CI = 0.32 to 1.5). Among patients with stage I NSCLC, actuarial 4-year survival was statistically significantly higher in those with wild-type p53 than in those with mutant p53 (78% versus 52%, respectively; difference in 4-year survival = 26%, 95% CI = 6% to 46%; $P = .009$, log-rank test). **Conclusion:** Tumor p53 mutations are a statistically significant predictor of poor outcome in patients with stage I NSCLC.

Burkhardt, C. A., A. J. Cheng, et al. (2003). "Effects of MYCN Antisense Oligonucleotide Administration on Tumorigenesis in a Murine Model of Neuroblastoma." *J Natl Cancer Inst* **95**(18): 1394-1403.

<http://jncicancerspectrum.oupjournals.org/cgi/content/abstract/jnci;95/18/1394>

Background: Human MYCN (hMYCN) oncogene amplification is a powerful predictor of treatment failure in childhood neuroblastoma, and dysregulation of hMYCN protein expression appears to be critically involved in the pathogenesis of this disease. We used hMYCN antisense (AS) oligonucleotides to investigate, both in vitro and in vivo, the therapeutic potential of inhibiting hMYCN expression. **Methods:** We transiently transfected human neuroblastoma IMR-32 cells, which have an amplified hMYCN gene, with fluorescently labeled hMYCN AS or scrambled (SCR) control oligonucleotides and used fluorescence-activated cell sorting to enrich for cell populations containing different levels of the oligonucleotides. We used fluorescence immunocytochemistry or reverse transcription polymerase chain reaction to assay gene expression levels and trypan blue exclusion to assay growth inhibition in the cell populations. We examined the effects of continuous treatment for 6 weeks with AS or SCR oligonucleotides via subcutaneously implanted osmotic pumps on tumor growth in a transgenic mouse model of hMYCN-induced neuroblastoma ($n = 20$ mice per group). All statistical tests were two-sided. **Results:** IMR-32 cells treated with AS oligonucleotides had approximately half as much hMYCN protein and cell proliferation as either SCR oligonucleotide-transfected or mock-transfected controls; the differences were statistically significant. Transgenic mice treated with AS oligonucleotides had lower tumor incidence and statistically significantly lower tumor mass than SCR-treated or untreated control mice. Compared with control treatments, AS oligonucleotide treatment in vitro and in vivo was associated with decreased expression of hMYCN and putative hMYCN target genes but not with that of closely related genes. Several AS oligonucleotide-treated mice developed tumors contralateral to the site of oligonucleotide administration, whereas SCR oligonucleotide-treated or untreated mice displayed bilateral tumor growth. **Conclusions:** Decreased expression of hMYCN protein is achievable with the use of AS oligonucleotide

treatment, even in the presence of hMYCN oncogene amplification. Antisense strategies targeting the hMYCN oncogene in vivo decrease mouse neuroblastoma tumorigenesis. Investigation of their clinical effect in children with neuroblastoma is warranted.

Lango, M. N., K. F. Dyer, et al. (2002). "Gastrin-Releasing Peptide Receptor-Mediated Autocrine Growth in Squamous Cell Carcinoma of the Head and Neck." *J Natl Cancer Inst* **94**(5): 375-383.

<http://jncicancerspectrum.oupjournals.org/cgi/content/abstract/jnci;94/5/375>

Background: Gastrin-releasing peptide receptor (GRPR)-mediated autocrine growth appears to be an early marker of susceptibility to tobacco-related lung cancers. Because expression of GRPR, however, has not been reported in squamous cell carcinoma of the head and neck (SCCHN), we investigated its expression and that of its ligand GRP in normal mucosa and SCCHN tissues and the involvement of these proteins in the proliferation of SCCHN cells. **Methods:** We assessed GRPR messenger RNA (mRNA) expression in specimens from 25 patients with SCCHN, six control noncancer patients, and 14 SCCHN cell lines by use of quantitative reverse transcriptase-polymerase chain reaction. We used neutralizing GRP monoclonal antibody 2A11 to block the GRP-GRPR interaction in SCCHN cell lines and xenografts and assessed the antibody's effect on proliferation by counting cultured cells or measuring xenograft tumor volume in vivo. All statistical tests were two-sided. **Results:** Tumor and mucosa tissues, respectively, from SCCHN patients expressed sixfold and fourfold higher levels of GRPR mRNA than normal mucosa tissue from noncancer patients ($P < .001$). The levels of GRPR expression in the tumor and adjacent normal epithelium of individual patients with SCCHN were correlated ($r = .652$; $P = .001$), suggesting that increased GRPR expression is an early event in SCCHN formation. SCCHN cells expressed fivefold higher levels of GRPR mRNA than did cultured normal mucosal epithelial cells ($P = .005$). GRP stimulated proliferation of SCCHN cells in a dose-dependent fashion ($P = .006$). Neutralizing GRP monoclonal antibody 2A11 inhibited SCCHN cell proliferation in vitro and in vivo. Median survival was 54 months in patients with higher levels of GRPR mRNA and was not reached in those with lower levels. **Conclusions:** GRP and GRPR appear to participate in an autocrine regulatory pathway in SCCHN. Thus, strategies that specifically target GRP and/or GRPR may be effective therapeutic approaches for this disease.

Niell, B. L., G. Rennert, et al. (2004). "BRCA1 and BRCA2 Founder Mutations and the Risk of Colorectal Cancer." *J Natl Cancer Inst* **96**(1): 15-21.

<http://jncicancerspectrum.oupjournals.org/cgi/content/abstract/jnci;96/1/15>

Background: Mutations in BRCA1 and/or BRCA2 (BRCA1/2) profoundly increase the risks of breast and ovarian cancers, but it is unclear whether mutations in these genes increase the risk of colorectal cancer. We investigated BRCA1/2 founder mutations and a family history of breast cancer as potential risk factors for colorectal cancer. **Methods:** In the population-based Molecular Epidemiology of Colorectal Cancer study in northern Israel, 1422 case patients with incident colorectal cancer, diagnosed between March 31, 1998, and December 31, 2002, and 1566 control subjects without colorectal cancer were genotyped for the BRCA1 187delAG, BRCA1 5385insC, and BRCA2 6174delT founder mutations. Genotypes and interview data from all case patients and control subjects and from only those of Ashkenazi Jewish descent (1002 case patients and 1038 control subjects) were used to calculate odds ratios [ORs] from logistic regression. **Results:** Twenty-four (2.4%) case patients and 20 (1.9%) control subjects carried one of the three mutations (OR = 1.24, 95% confidence interval [CI] = 0.68 to 2.26). A family history of breast cancer in a female relative was not associated with an increased risk of colorectal cancer, even after adjustment for the presence of a BRCA founder mutation (OR = 1.03, 95% CI = 0.75 to

1.41). Conclusions: Although weak associations cannot be excluded, Ashkenazi BRCA founder mutations do not confer a strongly elevated risk of colorectal cancer. Similarly, a family history of breast cancer does not appear to be a strong risk factor for colorectal cancer in this population.

Primdahl, H., F. P. Wikman, et al. (2002). "Allelic Imbalances in Human Bladder Cancer: Genome-Wide Detection With High-Density Single-Nucleotide Polymorphism Arrays." J Natl Cancer Inst **94**(3): 216-223.

<http://jncicancerspectrum.oupjournals.org/cgi/content/abstract/jnci;94/3/216>

Background: Bladder cancer is characterized by genomic instability. In this study, we investigated whether genome-wide screening using single-nucleotide polymorphism (SNP) arrays could detect allelic imbalance (loss or gain of at least one allele) in bladder cancers. Methods: For microarray analysis, DNA was isolated from microdissected bladder tumors and leukocytes from 11 patients. The stage T1 tumor (connective tissue invasive) and the subsequent stage T2-4 tumor (muscle invasive) were available from eight of these patients, and only the first muscle-invasive stage T2-4 tumor was available from three of the 11 patients. The microarray contained 1494 biallelic polymorphic sequences. For microsatellite analyses, DNA was isolated from tumors and leukocytes of nine patients with primary T2-4 tumors and 13 patients with Ta (noninvasive) tumors. All statistical tests were two-sided. Results: We assigned a genotype to 1204 loci, 343 of which were heterozygous. Allelic imbalance was detected in known areas of imbalance on chromosomes 6, 8, 9, 11, and 17, and a new area of imbalance was detected on the p arm of chromosome 6. Microsatellite analysis of nine other T2-4 tumors and 13 Ta tumors showed that allelic imbalance was more frequent in T2-4 tumors than in Ta tumors ($P < .001$). We detected 8.5 allelic imbalances (median) in 348 informative loci in T1 tumors and 28 allelic imbalances (median) in 329 informative loci in T2-4 tumors. When pairs of T1 and T2-4 tumors were analyzed from eight patients, 68% of imbalances detected in T1 tumors (146 imbalances) occurred in the subsequent T2-4 tumors (99 imbalances). Homozygous TP53 mutations were more often associated ($P = .005$) with high allelic imbalance than with low allelic imbalance. Conclusion: SNP arrays are feasible for high-throughput, genome-wide scanning for allelic imbalances in bladder cancer.

Steenbergen, R. D. M., D. Kramer, et al. (2004). "TSLC1 Gene Silencing in Cervical Cancer Cell Lines and Cervical Neoplasia." J Natl Cancer Inst **96**(4): 294-305.

<http://jncicancerspectrum.oupjournals.org/cgi/content/abstract/jnci;96/4/294>

Background: Cervical carcinogenesis is initiated by infection with high-risk (i.e., carcinogenic) human papillomavirus (HPV) types. The subsequent progression from premalignant cervical intraepithelial neoplasia (CIN) to invasive cancer is driven by both genetic and epigenetic processes. We assessed the role of the gene encoding the adhesion molecule tumor suppressor in lung cancer 1 (TSLC1) in this progression. Methods: We analyzed TSLC1 gene expression by real-time quantitative reverse transcription-polymerase chain reaction, promoter methylation by sodium bisulfite genomic DNA sequencing, and allelic loss by microsatellite analysis in primary keratinocytes, in four non-tumorigenic HPV-immortalized human keratinocyte cell lines, and in 11 human cervical cancer cell lines that were positive for a high-risk HPV DNA type and in normal cervical epithelial cells. We transfected cervical cancer SiHa cells that did not express TSLC1 mRNA with an expression vector containing the TSLC1 complementary DNA (cDNA) or an empty vector and analyzed transfectants for anchorage-independent growth and tumorigenicity in nude mice. We also examined TSLC1 promoter methylation in premalignant cervical lesions and in cervical carcinomas and smears. All statistical tests were two-sided. Results: TSLC1 mRNA was strongly reduced, relative to levels in primary keratinocytes, or absent in 10 (91%) of 11 cervical

carcinoma cell lines but in none (0%) of the four HPV-immortalized cell lines (difference = 91%, 95% confidence interval [CI] = 74% to 100%; $P = .004$). The TSLC1 promoter was hypermethylated, relative to normal foreskin and cervical epithelial cells, in nine (82%) of the 11 cervical carcinoma cell lines but in none (0%) of the four HPV-immortalized cell lines (difference = 82%, 95 CI = 59% to 100%; $P = .01$). Seven (88%, 95% CI = 47% to 100%) of the eight SiHa/TSLC1 transfectants displayed a marked reduction in anchorage-independent growth (i.e., 0-100 colonies per 5000 cells) compared with none of the four (0%, 95% CI = 0% to 60%) SiHa transfectants bearing the empty vector (i.e., SiHa/hygro transfectants; difference = 88%, 95% CI = 65% to 100%; $P = .01$) or untransfected SiHa cells. All seven mice (100%, 95% CI = 59% to 100%) injected with untransfected SiHa cells or SiHa/hygro transfectants displayed tumors of at least 50 mm³ by 2-6 weeks after injection compared with none of eight mice (0%, 95% CI = 0% to 37%) injected with the SiHa/TSLC1 transfectants (difference = 100%, 95% CI = 68% to 100%; $P < .001$). We detected TSLC1 promoter hypermethylation in seven (35%, 95% CI = 15% to 59%) of 20 high-grade CIN lesions (i.e., CIN II and III) and in 30 (58%, 95% CI = 43% to 71%) of 52 cervical squamous cell carcinomas compared with none (0%, 95% CI = 0% to 34%) of nine normal cervical epithelial biopsy samples and none (0%, 95% CI = 0% to 22%) of 12 CIN I lesions ($P < .001$ for cervical squamous cell cancer versus normal epithelial biopsy samples plus CIN I lesions). Conclusions: TSLC1 gene silencing via promoter hypermethylation is a frequent event in the progression from high-risk HPV-containing, high-grade CIN lesions to invasive cervical cancer.

Strickler, H. D., R. D. Burk, et al. (2005). "Natural History and Possible Reactivation of Human Papillomavirus in Human Immunodeficiency Virus-Positive Women." *J Natl Cancer Inst* **97**(8): 577-586.

<http://jncicancerspectrum.oupjournals.org/cgi/content/abstract/jnci;97/8/577>

Background: Little is known in human immunodeficiency virus (HIV)-positive women about how the combination of plasma HIV RNA level and CD4+ T-cell count is associated with the natural history of human papillomavirus (HPV) infection or about HPV reactivation--whether it occurs and with what frequency in HIV-positive women. Methods: HIV-positive ($n = 1848$) and -negative ($n = 514$) women were assessed at semiannual visits (total person-years = 5661) for cervicovaginal HPV with polymerase chain reaction assays and for squamous intraepithelial lesions (SILs) by Pap smear. We studied the prevalent detection of HPV and SILs with generalized estimating equations and the incident detection and persistence of HPV and SILs with multivariable Cox models. All statistical tests were two-sided. Results: We observed a strong interaction between the associations of CD4+ and plasma HIV RNA strata with both prevalent ($P_{\text{interaction}} = .002$) and incident ($P_{\text{interaction}} = .001$) detection of HPV. Indeed, the hazard ratio for incident HPV detection peaked between 4.0 and 5.0, with either a CD4+ count of less than 200 cells per mm³ or an HIV RNA level of more than 100 000 copies per mL. Although incident HPV detection in all women was associated with the number of recent sex partners ($P_{\text{trend}} < .001$), 22% of sexually inactive HIV-positive women with a CD4+ count of less than 200 cells/mm³ also had at least one incidently detected HPV type. The association between CD4+/HIV RNA strata and HPV persistence was statistically significantly smaller ($P < .001$) than for incident HPV detection. SIL prevalence, incident detection, and persistence had similar associations with CD4+/HIV RNA strata as HPV (above). Conclusion: In HIV-positive women, plasma HIV RNA level and CD4+ count in combination appear to have a strong and statistically interactive association with incident detection of HPV, some of which may reflect HPV reactivation (e.g., in sexually inactive women). The more moderate association between HIV coinfection and HPV persistence could partly explain why cervical cancer rates have not reached more epidemic proportions in HIV-positive women.

Syed, V., G. Ulinski, et al. (2002). "Reproductive Hormone-Induced, STAT3-Mediated Interleukin 6 Action in Normal and Malignant Human Ovarian Surface Epithelial Cells." J Natl Cancer Inst **94**(8): 617-629.

<http://jncicancerspectrum.oupjournals.org/cgi/content/abstract/jnci;94/8/617>

Background: Reproductive hormones are associated with risk for epithelial ovarian cancer. To determine the effect of such hormones on the activation of interleukin 6 (IL-6)/STAT3 (signal transducer and activator of transcription-3) signaling, which may be involved in ovarian cancer, we investigated the status of STAT3, IL-6, and its receptor in immortalized human ovarian surface epithelial (HOSE) and ovarian cancer (OVCA) cell lines. **Methods:** Two immortalized HOSE cell lines and two OVCA cell lines were cultured with gonadotropins, sex steroid hormones, and/or IL-6, alone or with specific inhibitors or IL-6-neutralizing antibodies. Expression of IL-6, the IL-6 receptor {alpha} chain (IL-6R{alpha}), and phosphorylated and unphosphorylated STAT3 messenger RNAs (mRNAs) and proteins in all cells was determined. Cell proliferation and soft-agar colony formation were assessed. STAT3 activity was investigated in OVCA cells transfected with a dominant negative STAT3 (Dn-STAT3), wild-type STAT3, or an empty control vector. All statistical tests were two-sided. **Results:** Levels of IL-6 mRNA and protein increased in all cells treated with follicle-stimulating hormone (FSH), luteinizing hormone (LH), 17{beta}-estradiol, or estrone but increased only in OVCA cells treated with testosterone and 5{alpha}-dihydrotestosterone. For all lines, IL-6 antibodies partially inhibited hormone-stimulated cell proliferation but completely inhibited IL-6-enhanced cell proliferation. IL-6 induced STAT3 phosphorylation and activation in HOSE cells; STAT3 was constitutively activated in OVCA cells. Higher levels of IL-6R{alpha} and STAT3 transcription factors were observed in OVCA cells than in HOSE cells. After transfection, Dn-STAT3 suppressed endogenous STAT3 and inhibited all forms of IL-6-stimulated OVCA cell proliferation (OVCA 429 cells, $P < .001$; OVCA 432 cells, $P < .006$), whereas wild-type STAT3 enhanced HOSE cell proliferation (wild-type STAT3 at 0.5 {micro}g/mL in HOSE 306 cells, $P < .002$; STAT3 at 1.0 {micro}g/mL in HOSE 306 or both concentrations of wild-type STAT3 in HOSE 642 cells, $P < .001$). **Conclusions:** The IL-6/STAT3 signaling pathway may mediate FSH-, LH-, and estrogen-stimulated HOSE cell proliferation. Increased IL-6R{alpha} expression and constitutive STAT3 activation may be associated with ovarian cancer.

Wang, L. L., A. Gannavarapu, et al. (2003). "Association Between Osteosarcoma and Deleterious Mutations in the RECQL4 Gene in Rothmund-Thomson Syndrome." J Natl Cancer Inst **95**(9): 669-674.

<http://jncicancerspectrum.oupjournals.org/cgi/content/abstract/jnci;95/9/669>

Background: Rothmund-Thomson syndrome (RTS) is an autosomal recessive disorder associated with an increased predisposition to osteosarcoma. Children with RTS typically present with a characteristic skin rash (poikiloderma), small stature, and skeletal dysplasias. Mutations in the RECQL4 gene, which encodes a RecQ DNA helicase, have been reported in a few RTS patients. We examined whether a predisposition to developing osteosarcoma among an international cohort of RTS patients was associated with a distinctive pattern of mutations in the RECQL4 gene. **Methods:** We obtained clinical information about and biologic samples from 33 RTS patients (age range = 1-30 years). Eleven patients were diagnosed with osteosarcoma. All 21 exons and 13 short introns of the RECQL4 gene were sequenced from the genomic DNA of all subjects. Kaplan-Meier survival analysis was used to estimate the incidence of osteosarcoma among patients with and without mutations predicted to produce a truncated RECQL4 protein. **Results:** Twenty-three RTS patients, including all 11 osteosarcoma patients, carried at least one of 19 truncating mutations in their RECQL4 genes. The incidence of osteosarcoma was 0.00 per year in truncating mutation-negative patients (100 person-years of observation) and 0.05 per year in truncating mutation-positive patients (230 person-years of observation) ($P = .037$; two-sided

log-rank test). Conclusions: Mutations predicted to result in the loss of RECQL4 protein function occurred in approximately two-thirds of RTS patients and are associated with risk of osteosarcoma. Molecular diagnosis has the potential to identify those children with RTS who are at high risk of this cancer.

J Neurophysiol (2)

Bao, H., W. H. Wong, et al. (2003). "Voltage-Gated Calcium Channel Currents in Type I and Type II Hair Cells Isolated From the Rat Crista." *J Neurophysiol* **90**(1): 155-164.

<http://jn.physiology.org/cgi/content/abstract/90/1/155>

When studied in vitro, type I hair cells in amniote vestibular organs have a large, negatively activating K⁺ conductance. In type II hair cells, as in nonvestibular hair cells, outwardly rectifying K⁺ conductances are smaller and more positively activating. As a result, type I cells have more negative resting potentials and smaller input resistances than do type II cells; large inward currents fail to depolarize type I cells above -60 mV. In nonvestibular hair cells, afferent transmission is mediated by voltage-gated Ca²⁺ channels that activate positive to -60 mV. We investigated whether Ca²⁺ channels in type I cells activate more negatively so that quantal transmission can occur near the reported resting potentials. We used the perforated patch method to record Ca²⁺ channel currents from type I and type II hair cells isolated from the rat anterior crista (postnatal days 4-20). The activation range of the Ca²⁺ currents of type I hair cells differed only slightly from that of type II cells or nonvestibular hair cells. In 5 mM external Ca²⁺, currents in type I and type II cells were half-maximal at -41.1 $\pm$ 0.5 (SE) mV (n = 10) and -37.2 $\pm$ 0.2 mV (n = 10), respectively. In physiological external Ca²⁺ (1.3 mM), currents in type I cells were half-maximal at -46 $\pm$ 1 mV (n = 8) and just 1% of maximal at -72 mV. These results lend credence to suggestions that type I cells have more positive resting potentials in vivo, possibly through K⁺ accumulation in the synaptic cleft or inhibition of the large K⁺ conductance. Ca²⁺ channel kinetics were also unremarkable; in both type I and type II cells, the currents activated and deactivated rapidly and inactivated only slowly and modestly even at large depolarizations. The Ca²⁺ current included an L-type component with relatively low sensitivity to dihydropyridine antagonists, consistent with the α subunit being CaV1.3 (α 1D). Rat vestibular epithelia and ganglia were probed for L-type α -subunit expression with the reverse transcription-polymerase chain reaction. The epithelia expressed CaV1.3 and the ganglia expressed CaV1.2 (α 1C).

Distler, C., P. K. Rathee, et al. (2003). "Fast Ca²⁺-Induced Potentiation of Heat-Activated Ionic Currents Requires cAMP/PKA Signaling and Functional AKAP Anchoring." *J Neurophysiol* **89**(5): 2499-2505.

<http://jn.physiology.org/cgi/content/abstract/89/5/2499>

Distler, C., P. K. Rathee, K. S. Lips, O. Obreja, W. Neuhuber, and M. Kress. Fast Ca²⁺-Induced Potentiation of Heat-Activated Ionic Currents Requires cAMP/PKA Signaling and Functional AKAP Anchoring. *J. Neurophysiol.* 89: 2499-2505, 2003. Calcium influx and the resulting increase in intracellular calcium concentration ([Ca²⁺]_i) can induce enhanced sensitivity to temperature increases in nociceptive neurons. This sensitization accounts for heat hyperalgesia that is

regularly observed following the activation of excitatory inward currents by pain-producing mediators. Here we show that rat sensory neurons express calcium-dependent adenylyl cyclases (AC) using RT-PCR and nonradioactive in situ hybridization. Ionomycin-induced rises in $[Ca^{2+}]_i$ -activated calcium-dependent AC and caused translocation of catalytic protein kinase A subunit. Elevation of $[Ca^{2+}]_i$ finally resulted in a significant potentiation of heat-activated currents and a drop in heat threshold. This was not prevented in the presence of suramin that nonspecifically uncouples G protein-dependent receptors. The sensitization was, however, inhibited when the specific PKA antagonist PKI14-22 was added to the pipette solution or when PKA coupling to A kinase anchoring protein (AKAP) was disrupted with InCELlect StHt-31 uncoupling peptide. The results show that heat sensitization in nociceptive neurons can be induced by increases in $[Ca^{2+}]_i$ and requires PKA that is functionally coupled to the heat transducer, mostly likely vanilloid receptor VR-1. This calcium-dependent pathway can account for the sensitizing properties of many excitatory mediators that activate cationic membrane currents.

J. Am. Soc. Nephrol. (26)

Anders, H.-J., E. Belemezova, et al. (2004). "Late Onset of Treatment with a Chemokine Receptor CCR1 Antagonist Prevents Progression of Lupus Nephritis in MRL-Fas(lpr) Mice." J. Am. Soc. Nephrol. **15**(6): 1504-1513.

<http://www.jasn.org/cgi/content/abstract/15/6/1504>

ABSTRACT. Slowly progressive renal injury is the major cause for ESRD. The model of progressive immune complex glomerulonephritis in autoimmune MRLlpr/lpr mice was used to evaluate whether chemokine receptor CCR1 blockade late in the disease course can affect progression to renal failure. Mice were treated with subcutaneous injections of either vehicle or BX471, a nonpeptide CCR1 antagonist, three times a week from week 20 to 24 of age. BX471 improved blood urea nitrogen levels (BX471, 35.1 $\{+/-\}$ 5.3; vehicle, 73.1 $\{+/-\}$ 39.6 mg/dl; $P < 0.05$) and reduced the amount of ERHR-3 macrophages, CD3 lymphocytes, Ki-67 positive proliferating cells, and ssDNA positive apoptotic cells in the interstitium but not in glomeruli. Cell transfer studies with fluorescence-labeled T cells that were pretreated with either vehicle or BX471 showed that BX471 blocks macrophage and T cell recruitment to the renal interstitium of MRLlpr/lpr mice. This was associated with reduced renal expression of CC chemokines CCL2, CCL3, CCL4, and CCL5 and the chemokine receptors CCR1, CCR2, and CCR5. Furthermore, BX471 reduced the extent of interstitial fibrosis as evaluated by interstitial smooth muscle actin expression and collagen I deposits, as well as mRNA expression for collagen I and TGF- $\{\beta\}$. BX471 did not affect serum DNA autoantibodies, proteinuria, or markers of glomerular injury in MRLlpr/lpr mice. This is the first evidence that, in advanced chronic renal injury, blockade of CCR1 can halt disease progression and improve renal function by selective inhibition of interstitial leukocyte recruitment and fibrosis.

Anglicheau, D., C. Verstuyft, et al. (2003). "Association of the Multidrug Resistance-1 Gene Single-Nucleotide Polymorphisms with the Tacrolimus Dose Requirements in Renal Transplant Recipients." J. Am. Soc. Nephrol. **14**(7): 1889-1896.

<http://www.jasn.org/cgi/content/abstract/14/7/1889>

ABSTRACT. The immunosuppressive drug tacrolimus, whose pharmacokinetic characteristics display large interindividual variations, is a substrate for P-glycoprotein (P-gp), the product of the multidrug resistance-1 (MDR1) gene. Some of the single nucleotide polymorphisms (SNP) of MDR1 reported correlated with the in vivo activity of P-gp. Because P-gp is known to control tacrolimus intestinal absorption, it was postulated that these polymorphisms are associated with tacrolimus pharmacokinetic variations in renal transplant recipients. The objective of this study was to evaluate in a retrospective study of 81 renal transplant recipients the effect on tacrolimus dosages and concentration/dose ratio of four frequent MDR1 SNP possibly associated with P-gp function (T-129C in exon 1b, 1236C>T in exon 12, 2677G>T,A in exon 21, and 3435C>T in exon 26). As in the general population, the SNP in exons 12, 21, and 26 were frequent (16, 17.3, and 22.2% for the variant homozygous genotype, respectively) and exhibited incomplete linkage disequilibrium. One month after tacrolimus introduction, exon 21 SNP correlated significantly with the daily tacrolimus dose ($P \leq 0.05$) and the concentration/dose ratio ($P \leq 0.02$). Tacrolimus dose requirements were 40% higher in homozygous than wild-type patients for this SNP. The concentration/dose ratio was 36% lower in the wild-type patients, suggesting that, for a given dose, their tacrolimus blood concentration is lower. Haplotype analysis substantiated these results and suggested that exons 26 and 21 SNP may be associated with tacrolimus dose requirements. Genotype monitoring of the MDR1 gene reliably predicts the optimal dose of tacrolimus in renal transplant recipients and may predict the initial daily dose needed by individual patients to obtain adequate immunosuppression. E-mail: Dany.Anglicheau@biomedicale.univ-paris5.fr

Benigni, A., D. Corna, et al. (2004). "Targeted Deletion of Angiotensin II Type 1A Receptor Does not Protect Mice from Progressive Nephropathy of Overload Proteinuria." J. Am. Soc. Nephrol. **15**(10): 2666-2674.

<http://www.jasn.org/cgi/content/abstract/15/10/2666>

In experimental and human renal diseases, progression is limited by angiotensin-converting enzyme inhibitors. Whether renoprotection was due to their capacity of reducing proinflammatory and profibrotic effects of angiotensin II (Ang II) or limiting proteinuria and its long term toxicity is debated. For dissecting the relative contribution of Ang II and proteinuria to chronic renal damage, the protein-overload proteinuria model was used in genetically modified mice lacking the major isoform of murine AT1 receptor (AT1A). Uninephrectomized AT1A+/+ and -/- mice received a daily injection of BSA or saline for 4 or 11 wk. AT1A-/-BSA mice acquired a renal phenotype of proteinuria and renal glomerular and tubulointerstitial lesions, albeit attenuated with respect to AT1A+/+BSA. Administration of the calcium channel blocker lacidipine to reduce BP of AT1A+/+BSA mice to levels of AT1A-/-BSA translated into comparable values of protein excretion rate and glomerular and tubulointerstitial injury in both strains. These results confirm that the toxic effect of protein trafficking on renal disease progression is not necessarily dependent on Ang II to the extent that targeted deletion of AT1A does not prevent disease progression. A role of Ang II via AT1B or AT2 receptors is still a possibility that cannot be ruled out by the present experimental approach. These findings provide a clear rationale for specifically targeting proteinuria in pharmacologic interventions of chronic nephropathies.

Blaber, R., E. Stylianou, et al. (2003). "Selective Regulation of ICAM-1 and RANTES Gene Expression after ICAM-1 Ligation on Human Renal Fibroblasts." J. Am. Soc. Nephrol. **14**(1): 116-127.

<http://www.jasn.org/cgi/content/abstract/14/1/116>

ABSTRACT. Leukocyte infiltration of the cortico-interstitium is characteristic of many forms of progressive renal disease. The principal adhesion molecule expressed on resident interstitial cells

and recognized by leukocytes is intercellular adhesion molecule-1 (ICAM-1). ICAM-1 is an inducible transmembrane receptor, which forms the counter-receptor for the leukocyte β_2 integrins. ICAM-1-dependent binding induces the synthesis of the chemokine RANTES and of ICAM-1 itself. This study examines some of the signaling pathways involved in this induction. After ICAM-1 cross-linking on fibroblasts, the mRNA and protein for both RANTES and ICAM-1 were induced. This induction was calcium-dependent and inhibited by BAPTA-AM. The p38, ERK1, and ERK2 MAP kinases were activated in a $[Ca^{2+}]_i$ -dependent manner, with a maximum phosphorylation at approximately 3 min after cross-linking. Through the use of selective inhibitors of p38 MAP kinase (SB203580) or MEKK (PD98059), p38 but not ERK activation was shown to be essential for the induction of ICAM-1. Neither was involved in RANTES activation, however. These mechanisms differed from those initiated by TNF- α , which were not $[Ca^{2+}]_i$ -dependent. Electrophoretic mobility shift analysis demonstrated a time-dependent induction of both AP-1 and NF- κ B binding activity in nuclear extracts, maximal at approximately 15 min after ICAM-1 cross-linking. Only AP-1 activation, however, was calcium-dependent, suggesting the central involvement of this transcription factor in ICAM-1 and RANTES induction after the ligation of ICAM-1. This study suggests an independent mechanism of inflammatory amplification, which may be characteristic of a persistent leukocytic involvement in areas of chronic inflammation rather than in cytokine-induced acute inflammation. E-mail: Steadmanr@cf.ac.uk

Dahan, K., O. Devuyst, et al. (2003). "A Cluster of Mutations in the UMOD Gene Causes Familial Juvenile Hyperuricemic Nephropathy with Abnormal Expression of Uromodulin." J. Am. Soc. Nephrol. **14**(11): 2883-2893.

<http://www.jasn.org/cgi/content/abstract/14/11/2883>

ABSTRACT. Familial juvenile hyperuricemic nephropathy (FJHN [MIM 162000]) is an autosomal-dominant disorder characterized by abnormal tubular handling of urate and late development of chronic interstitial nephritis leading to progressive renal failure. A locus for FJHN was previously identified on chromosome 16p12 close to the MCKD2 locus, which is responsible for a variety of autosomal-dominant medullary cystic kidney disease (MCKD2). UMOD, the gene encoding the Tamm-Horsfall/uromodulin protein, maps within the FJHN/MCKD2 critical region. Mutations in UMOD were recently reported in nine families with FJHN/MCKD2 disease. A mutation in UMOD has been identified in 11 FJHN families (10 missense and one in-frame deletion)--10 of which are novel--clustering in the highly conserved exon 4. The consequences of UMOD mutations on uromodulin expression were investigated in urine samples and renal biopsies from nine patients in four families. There was a markedly increased expression of uromodulin in a cluster of tubule profiles, suggesting an accumulation of the protein in tubular cells. Consistent with this observation, urinary excretion of wild-type uromodulin was significantly decreased. The latter findings were not observed in patients with FJHN without UMOD mutations. In conclusion, this study points to a mutation clustering in exon 4 of UMOD as a major genetic defect in FJHN. Mutations in UMOD may critically affect the function of uromodulin, resulting in abnormal accumulation within tubular cells and reduced urinary excretion. E-mail: Dahan@gmed.ucl.ac.be

Eitner, F., T. Ostendorf, et al. (2003). "PDGF-C Expression in the Developing and Normal Adult Human Kidney and in Glomerular Diseases." J. Am. Soc. Nephrol. **14**(5): 1145-1153.

<http://www.jasn.org/cgi/content/abstract/14/5/1145>

ABSTRACT. PDGF-C is a new member of the PDGF-family and has recently been identified as a rat mesangial cell mitogen. Its expression and function in human kidneys is unknown. Localization of PDGF-C protein was analyzed by immunohistochemistry using a rabbit polyclonal antibody directed against the core-domain of PDGF-C in human fetal kidneys (n = 8), normal

adult human kidneys (n = 9), and in renal biopsies of patients with IgA nephropathy (IgAN, n = 31), membranous nephropathy (MGN, n = 8), minimal change disease (MC, n = 7), and transplant glomerulopathy (TxG, n = 12). Additionally, PDGF-C mRNA was detected in microdissected glomeruli by real-time RT-PCR in cases of normal adult kidneys (n = 7), IgAN (n = 27), MGN (n = 11), and MC (n = 13). In the fetal kidney, PDGF-C localized to the developing mesangium, ureteric bud epithelium, and the undifferentiated mesenchyme. In the adult kidney, PDGF-C was constitutively expressed in parietal epithelial cells of Bowman's capsule, tubular epithelial cells (loops of Henle, distal tubules, collecting ducts), and in arterial endothelial cells. A marked upregulation of glomerular PDGF-C protein was seen in MGN and TxG with a prominent positivity of virtually all podocytes. In MC, PDGF-C localized to podocytes in a more focal distribution. In MGN, increased glomerular PDGF-C protein expression was due to increased mRNA synthesis as a 4.3-fold increase in PDGF-C mRNA was detected in microdissected glomeruli from MGN compared with normal. PDGF-C protein was additionally expressed in individual mesangial cells in TxG. Finally, upregulated PDGF-C protein expression was detected within sclerosing glomerular and fibrosing tubulointerstitial lesions in individual cases from all analyzed groups. We conclude that PDGF-C is constitutively expressed in the human kidney and is upregulated in podocytes and interstitial cells after injury/activation of these cells. E-mail: feitner@ukaachen.de

Esteban, V., O. Lorenzo, et al. (2004). "Angiotensin II, via AT1 and AT2 Receptors and NF- κ B Pathway, Regulates the Inflammatory Response in Unilateral Ureteral Obstruction." J. Am. Soc. Nephrol. **15**(6): 1514-1529.

<http://www.jasn.org/cgi/content/abstract/15/6/1514>

ABSTRACT. Inflammatory cell infiltration plays a key role in the onset and progression of renal injury. The NF- κ B participates in the inflammatory response, regulating many proinflammatory genes. Angiotensin II (Ang II), via AT1 and AT2 receptors, activates NF- κ B. Although the contribution of Ang II to kidney damage progression is already established, the receptor subtype involved in the inflammatory cell recruitment is not clear. For investigating this issue, the unilateral ureteral obstruction (UUO) model was used in mice, blocking Ang II production/receptors and NF- κ B pathway. Two days after UUO, obstructed kidneys of wild-type mice presented a marked interstitial inflammatory cell infiltration and increased NF- κ B activity. Treatment with AT1 or AT2 antagonists partially decreased NF- κ B activation, whereas only the AT2 blockade diminished monocyte infiltration. Obstructed kidneys of AT1-knockout mice showed interstitial monocyte infiltration and NF- κ B activation; both processes were abolished by an AT2 antagonist, suggesting AT2/NF- κ B involvement in monocyte recruitment. In wild-type mice, only angiotensin-converting enzyme inhibition or combined therapy with AT1 plus AT2 antagonists blocked monocyte infiltration, NF- κ B activation, and upregulation of NF- κ B-related proinflammatory genes. Therefore, AT1 and AT2 blockade is necessary to arrest completely the inflammatory process. Treatment with two different NF- κ B inhibitors, pirrolidin-dithiocarbamate and parthenolide, diminished monocyte infiltration and gene overexpression. These data show that Ang II, via AT1 and AT2 receptors and NF- κ B pathway, participates in the regulation of renal monocyte recruitment and may provide a rationale to investigate further the role of AT2 in human kidney diseases.

Furu, L., L. F. Onuchic, et al. (2003). "Milder Presentation of Recessive Polycystic Kidney Disease Requires Presence of Amino Acid Substitution Mutations." J. Am. Soc. Nephrol. **14**(8): 2004-2014.

<http://www.jasn.org/cgi/content/abstract/14/8/2004>

ABSTRACT. Autosomal recessive polycystic kidney disease (ARPKD; MIM 263200) is a hereditary and severe form of polycystic disease affecting the kidneys and biliary tract with an estimated incidence of 1 in 20,000 live births. The clinical spectrum is widely variable: up to 50% of affected neonates die shortly after birth, whereas others survive to adulthood. Mutations at a single locus, polycystic kidney and hepatic disease 1 (PKHD1), are responsible for all typical forms of ARPKD. Mutation detection was performed in PKHD1 by DHPLC in 85 affected, unrelated individuals. Seventy-four amplicons were amplified and analyzed from the PKHD1 genomic locus. Sequence variants were considered pathogenic when they were not observed in 160 control individuals (320 chromosomes). For purposes of genotype-phenotype comparisons, families were stratified by clinical presentation into two groups: the severe perinatal group, in which at least one affected child presented with perinatal disease and neonatal demise, and the less severe, nonperinatal group, in which none of the affected children died in the neonatal period. Forty-one mutations were found in 55 affected disease chromosomes; 32 of these mutations have not been reported previously. Mutations were distributed throughout the portions of gene encoding the predicted extracellular portion of the protein product. The most commonly encountered mutation, T36M, was found in 8 of 55 disease chromosomes. Amino acid substitutions were found to be more commonly associated with a nonlethal presentation, whereas chain terminating mutations were more commonly associated with neonatal demise ($\chi^2 = 11.54$, $P = 0.003$). All patients who survive the neonatal period have at least one amino acid substitution mutation, suggesting that such substitutions produce milder disease through production of partially functional protein products. The nature of the germline mutations in ARPKD plays a significant role in determining clinical outcome. E-mail: lgw@uab.edu

Gogusev, J., I. Murakami, et al. (2003). "Molecular Cytogenetic Aberrations in Autosomal Dominant Polycystic Kidney Disease Tissue." J. Am. Soc. Nephrol. **14**(2): 359-366.

<http://www.jasn.org/cgi/content/abstract/14/2/359>

ABSTRACT. Autosomal dominant polycystic kidney disease (ADPKD) is a genetically heterogeneous disorder characterized by focal cyst formation from any part of the nephron. The molecular bases include germinal mutation of either PKD1 or PKD2 genes, enhanced expression of several protooncogenes, alteration of the TGF- α /EGF/EGF receptor (EGFR) axis, and disturbed regulation of proliferative/apoptosis pathways. To identify new locations of ADPKD related oncogenes and/or tumor suppressor genes (TSG), comparative genomic hybridization (CGH) and loss of heterozygosity (LOH) analyses were performed for a series of individual cysts ($n = 24$) from eight polycystic kidneys. By CGH, imbalances were detected predominantly on chromosomes 1p, 9q, 16p, 19, and 22q in all tissues. DNA copy number gain was seen on chromosomes 3q and 4q in five samples. The CGH data were supplemented by LOH analysis using 83 polymorphic microsatellite markers distributed along chromosomes 1, 9, 16, 19, and 22. The highest frequency of LOH was found on the 1p35-36 and 16p13.3 segments in cysts from seven samples. Allelic losses on 9q were detected in six, whereas deletions at 19p13 and 22q11 bands were observed in three polycystic kidneys. These results indicate that the deleted chromosomal regions may contain genes important in ADPKD initiation and progression. E-mail: gogusev@necker.fr

Grandaliano, G., P. Pontrelli, et al. (2003). "Protease-Activated Receptor-2 Expression in IgA Nephropathy: A Potential Role in the Pathogenesis of Interstitial Fibrosis." J. Am. Soc. Nephrol. **14**(8): 2072-2083.

<http://www.jasn.org/cgi/content/abstract/14/8/2072>

ABSTRACT. An increasing body of evidence suggests that proteases may play a key role in the

pathogenesis of tissue fibrosis. Protease-activated receptor-2 (PAR-2) is cleaved and activated by trypsin-like proteolytic enzymes, including tryptase and activated coagulation factor X (FXa). Both these soluble mediators have been demonstrated, directly or indirectly, at the interstitial level in progressive renal diseases, including IgA nephropathy (IgAN). PAR-2 mRNA and protein levels were investigated by RT-PCR and immunohistochemistry, respectively, in 17 biopsies from IgAN patients and 10 normal kidneys. PAR-2 expression was also evaluated, by RT-PCR and western blotting, in cultured human mesangial and proximal tubular cells. Finally, gene expression of plasminogen activator inhibitor-1 (PAI-1) and TGF- β , two powerful fibrogenic factors, was evaluated in FXa-, trypsin-, and PAR-2 activating peptide-stimulated human proximal tubular cells by Northern blot. In normal kidneys, PAR-2 gene expression was barely detectable, whereas in IgAN biopsies the mRNA levels for this protease receptor were strikingly increased and directly correlated with the extent of interstitial fibrosis. Immunohistochemical staining demonstrated that PAR-2 protein expression in IgAN biopsies was mainly localized in the proximal tubuli and within the interstitial infiltrate. Proximal tubular cells in culture expressed PAR-2. Activation of this receptor by FXa in tubular cells induced a striking increase in intracellular calcium concentration. In addition, incubation of both cell lines with trypsin, FXa, or PAR-2 activating peptide caused a marked upregulation of PAI-1 gene expression that was not counterbalanced by an increased expression of plasminogen activators. Finally, PAR-2 activation induced a significant upregulation of TGF- β gene and protein expression in both mesangial and tubular cells. On the basis of our data, we can suggest that PAR-2 expressed by renal resident cells and activated by either mast cell tryptase or FXa may induce extracellular matrix deposition modifying the PAI-1/TGF- β balance and inducing TGF- β expression. These molecular mechanisms may underlie interstitial fibrosis in IgAN. E-mail: g.grandaliano@nephro.uniba.it

Grone, H.-J., C. D. Cohen, et al. (2002). "Spatial and Temporally Restricted Expression of Chemokines and Chemokine Receptors in the Developing Human Kidney." *J. Am. Soc. Nephrol.* **13**(4): 957-967.

<http://www.jasn.org/cgi/content/abstract/13/4/957>

ABSTRACT. The directed migration of cells, cell-cell adhesion, and the control of proliferation are key events during metanephric development. The chemokines are a family of proteins that selectively control aspects of cell migration, activation, proliferation, and adhesion. The expression of a series of chemokines and chemokine receptors during human renal development was investigated by using immunohistochemical analyses and real-time reverse transcription-PCR assays of defined laser-microdissected metanephric structures. The results demonstrate that mononuclear cell-like cells within the nephrogenic blastema focally express interferon-inducible protein-10/CXCL10, a ligand for CXCR3. Mononuclear-like cells dispersed through the developing organ express CX3CR1. Expression of CXCR4, the receptor for stromal cell-derived factor-1/CXCL12, is also limited to stromal CD34-positive cells. In contrast, the expression of stromal cell-derived factor-1/CXCL12, fractalkine, and CXCR3 is first observed in the comma- or S-shaped body stage. The intensity of this expression becomes stronger in the capillary loop stage, and expression is mainly observed in the mesangial stalk and endothelial cells of the glomeruli. These proteins may play modulatory roles in kidney development. Because genes that are expressed during ontogeny often play a role in tissue regeneration, these embryonal chemokine/chemokine receptor patterns may be important in renal injury and repair.

Guery, B., G. Choukroun, et al. (2003). "The Spectrum of Systemic Involvement in Adults Presenting with Renal Lesion and Mitochondrial tRNA(Leu) Gene Mutation." *J. Am. Soc. Nephrol.* **14**(8): 2099-2108.

<http://www.jasn.org/cgi/content/abstract/14/8/2099>

ABSTRACT. The A3243G mutation of the mitochondrial tRNA(Leu) gene has been recently reported in rare patients with focal and segmental glomerulosclerosis (FSGS). However, the full spectrum of systemic and kidney manifestations in adults presenting with this mutation remains poorly defined. Assessment of renal and nonrenal manifestations was performed in nine patients with A3243G mutation and prominent kidney disease diagnosed in adulthood. At first renal evaluation, median age was 35 years. Renal lesions consisted of FSGS (n = 2), tubulointerstitial nephropathy (n = 3), or bilateral enlarged cystic kidneys (n = 1). All but one patient exhibited extrarenal manifestations: deafness (8 of 9) requiring hearing aid in half the cases, diabetes mellitus (3 of 9), neuromuscular involvement (2 of 9), hypertrophic cardiomyopathy (1 of 9), and macular dystrophy (1 of 9). After a median follow-up of 5 yr, five patients progressed to end-stage renal disease between the ages of 15 and 51 years, four being successfully transplanted. Similarly, extrarenal manifestations progressed since all patients had deafness and diabetes (including three posttransplants), while half had neuromuscular, cardiac, or retinal involvement. In the adult patients with A3243G mutation and renal involvement, preexisting deafness is almost consistently found. While FSGS remains the most typical lesion, tubulointerstitial nephropathy or bilateral, enlarged cystic kidneys may also be encountered. In most cases, diabetes mellitus, macular dystrophy, hypertrophic cardiomyopathy, or neuromuscular features occur later in the course of the disease. The severity of the clinical course is heterogeneous, with end-stage renal failure being reached between the second and sixth decades. Renal transplantation may be offered to these patients, despite a high incidence of steroid-induced diabetes mellitus. E-mail: dominique.chauveau@nck.ap-hop-paris.fr

Hueso, M., P. Alia, et al. (2004). "Angiotensin Converting Enzyme Genotype and Chronic Allograft Nephropathy in Protocol Biopsies." J. Am. Soc. Nephrol. **15**(8): 2229-2236.

<http://www.jasn.org/cgi/content/abstract/15/8/2229>

ABSTRACT. Genotype DD of the angiotensin-converting enzyme (ACE) is not associated with an increased incidence of native renal diseases, although it could modulate progression to renal failure in patients who already display chronic lesions. Because its role in renal allograft degeneration is not well characterized, whether ACE genotype was associated with the prevalence of chronic allograft nephropathy (CAN) was studied, in a group of protocol biopsies from 180 patients, or with the incidence of CAN in 152 patients with at least two sequential biopsies. As a control group, ACE genotype was also studied in 41 donors and 72 healthy subjects. For analyzing the influence of ACE genotype in graft survival, patients were grouped into six categories (II-normal biopsy, ID-normal, DD-normal, II-CAN, ID-CAN and DD-CAN). Finally, relative renal ACE mRNA levels were measured in 67 cases by real-time PCR using the delta threshold cycle method. ACE-DD genotype was more frequent in patients who received a transplant than in control subjects (43.3% versus 30.1%, $P = 0.026$), but prevalence (DD = 42.7% versus non-DD = 42.2%) or incidence (DD = 24.6% versus non-DD = 29.9%) of CAN was not different regarding recipient ACE genotype. Furthermore, patients with the ACE-DD genotype and CAN had the poorest graft survival (II-normal = 100%, ID-normal = 91%, DD-normal = 84%, II-CAN = 100%, ID-CAN = 66%, and DD-CAN = 36%; $P = 0.034$) and higher ACE mRNA levels than non-DD and CAN (DD = -3.36 ± 2.35 versus non-DD = -5.65 ± 1.72 -fold in ACE copies; $P = 0.012$). It is concluded that ACE-DD genotype is not associated with an increased prevalence or incidence of CAN but is actually associated with higher ACE mRNA levels and poorer graft survival in patients who already display CAN.

Imanishi, Y., H. Tahara, et al. (2002). "Clonal Chromosomal Defects in the Molecular Pathogenesis of Refractory Hyperparathyroidism of Uremia." J. Am. Soc. Nephrol. **13**(6): 1490-1498.

<http://www.jasn.org/cgi/content/abstract/13/6/1490>

ABSTRACT. Indirect X chromosome-inactivation analyses have demonstrated that most parathyroid glands from patients with uremic refractory secondary/tertiary hyperparathyroidism are monoclonal neoplasms. However, little is known regarding the specific acquired genetic abnormalities that must underlie such clonal expansion or the molecular pathogenetic features of this disorder, compared with primary parathyroid adenomas. To address these issues in a uniquely powerful manner, both comparative genomic hybridization (CGH) and genome-wide molecular allelotyping were performed with a large group of uremia-associated parathyroid tumors. As indicated by CGH, one or more chromosomal changes were present in 24% of the tumors, which is markedly different from the value for common sporadic adenomas (72%). Two recurrent abnormalities that had not been previously described for sporadic parathyroid adenomas were noted with CGH, i.e., gains on chromosomes 7 (9%) and 12 (11%). Losses on chromosome 11 occurred in only one of the 46 uremia-associated tumors (2%); the tumor also contained a somatic mutation of the remaining MEN1 allele (221del18). A total of 13% of tumors demonstrated recurrent allelic loss on 18q, with 18q21.1-q21.2 being defined as the putative tumor suppressor-containing region. In conclusion, the powerful combination of genome-wide molecular allelotyping and CGH has identified recurrent clonal DNA abnormalities that suggest the existence and locations of genes important in uremic hyperparathyroidism. In addition, genome-wide patterns of somatic DNA alterations, including disparate roles for MEN1 gene inactivation, indicate that markedly different molecular pathogenetic processes exist for clonal outgrowth in severe uremic hyperparathyroidism versus common parathyroid adenomas.

Lemos, F. B. C., J. N. M. Ijzermans, et al. (2003). "Differential Expression of Heme Oxygenase-1 and Vascular Endothelial Growth Factor in Cadaveric and Living Donor Kidneys after Ischemia-Reperfusion." J. Am. Soc. Nephrol. **14**(12): 3278-3287.

<http://www.jasn.org/cgi/content/abstract/14/12/3278>

ABSTRACT. The extent of graft damage after ischemia-reperfusion reflects the balance between deleterious events and protective factors. Heme oxygenase-1 (HO-1) and vascular endothelial growth factor (VEGF) may contribute to cytoprotection by their anti-inflammatory and antiapoptotic properties. For investigating whether HO-1 and VEGF play a role in the adaptive response to ischemia-reperfusion injury after renal transplantation, kidney biopsies were analyzed from living (n = 45) and cadaveric (n = 16) donors, obtained at three time points: at the end of cold storage T(-1), after warm ischemia T(0), and after reperfusion T(+1). The mRNA expression levels of HO-1, VEGF165, Bcl-2, Bax, and hypoxia inducible factor-1{alpha} were quantified by real-time reverse transcriptase-PCR, and the HO-1 and VEGF proteins were analyzed by immunohistochemistry. Cadaveric donor kidneys presented higher mRNA expression levels of hypoxia inducible factor-1{alpha}. In contrast, mRNA expression levels of HO-1, VEGF165, and Bcl-2 were significantly lower in kidneys from cadaveric donors. Overall, a significant correlation was observed between mRNA expression of Bcl-2 and VEGF165, between Bcl-2 and HO-1, and between HO-1 and VEGF165. Moreover, protein expression of HO-1 and VEGF was detected in the same anatomical kidney compartments (glomerulus, arteries, and distal tubules). Renal function at the first week posttransplantation (analyzed by serum creatinine levels) showed a significant correlation with both HO-1 and VEGF mRNA expression, reinforcing the protective role of both genes in the early events of transplantation. It is concluded that the lower expression of HO-1, VEGF165, and Bcl-2 in cadaveric donor kidneys can reflect a defective adaptation against ischemia-reperfusion injury that may affect their function in the short term.

Lyons, L. A., D. S. Biller, et al. (2004). "Feline Polycystic Kidney Disease Mutation Identified in PKD1." J. Am. Soc. Nephrol. **15**(10): 2548-2555.

<http://www.jasn.org/cgi/content/abstract/15/10/2548>

Autosomal dominant polycystic kidney disease (ADPKD) is a commonly inherited disorder in humans that causes the formation of fluid-filled renal cysts, often leading to renal failure. PKD1 mutations cause 85% of ADPKD. Feline PKD is autosomal dominant and has clinical presentations similar to humans. PKD affects [~]38% of Persian cats worldwide, which is [~]6% of cats, making it the most prominent inherited feline disease. Previous analyses have shown significant linkage between the PKD phenotype and microsatellite markers linked to the feline homolog for PKD1. In this report, the feline PKD1 gene was scanned for causative mutations and a C>A transversion was identified at c.10063 (human ref NM_000296) in exon 29, resulting in a stop mutation at position 3284, which suggests a loss of [~]25% of the C-terminus of the protein. The same mutation has not been identified in humans, although similar regions of the protein are truncated. The C>A transversion has been identified in the heterozygous state in 48 affected cats examined, including 41 Persians, a Siamese, and several other breeds that have been known to outcross with Persians. In addition, the mutation is segregating concordantly in all available PKD families. No unaffected cats have been identified with the mutation. No homozygous cats have been identified, supporting the suggestion that the mutation is embryonic lethal. These data suggest that the stop mutation causes feline PKD, providing a test to identify cats that will develop PKD and demonstrating that the domestic cat is an ideal model for human PKD.

Morigi, M., D. Macconi, et al. (2002). "Protein Overload-Induced NF- κ B Activation in Proximal Tubular Cells Requires H₂O₂ through a PKC-Dependent Pathway." *J. Am. Soc. Nephrol.* **13**(5): 1179-1189.

<http://www.jasn.org/cgi/content/abstract/13/5/1179>

ABSTRACT. Abnormal traffic of proteins through the glomerular capillary has an intrinsic toxicity that results in tubular dysfunction and interstitial inflammation. It has been previously shown that in porcine proximal tubular cells high concentrations of albumin activated NF- κ B, which is responsible for the enhanced synthesis of the inflammatory chemokine RANTES. This study investigates whether reactive oxygen species (ROS) served as second messengers in protein overload-induced NF- κ B activation. Human proximal tubular cells (HK-2) were incubated (5 to 60 min) with human albumin and IgG (1 to 30 mg/ml). Both proteins induced a rapid or significant increase in hydrogen peroxide (H₂O₂) production at 5 min and persisting at 60 min. This effect was dose-dependent. The contribution of H₂O₂ in regulating NF- κ B activation was evaluated by using the antioxidants dimethyl-thiourea and pyrrolidine dithiocarbamate in protein-overloaded HK-2 cells. Both agents, by preventing H₂O₂ generation, induced human albumin or IgG inhibited NF- κ B activation. Stimulation of HK-2 with exogenous H₂O₂ resulted in the activation of a NF- κ B subunit pattern similar to that obtained after protein challenge. Specific inhibitors of protein kinase C (PKC) activity significantly prevented H₂O₂ production and consequent NF- κ B activation, suggesting that ROS generation in HK-2 cells occurs downstream of PKC activation. Either antioxidants or PKC inhibitor almost completely abolished the upregulation of the monocyte chemoattractant protein-1 gene induced by excess albumin, as evaluated by real-time PCR, thus supporting a role for PKC and ROS as critical signals for the expression of NF- κ B-dependent inflammatory genes. To identify the enzymatic sources responsible for the increased H₂O₂ production, the effect of diphenyleneiodonium, an inhibitor of the membrane NADP(H) oxidase, was studied, as was the effect of rotenone, which blocks complex I of the mitochondrial respiratory chain. It was found that both agents significantly reduced the exaggerated H₂O₂ induced by protein overload. These data indicate that exposure to excess proteins in proximal tubular cells induces the formation of ROS, which are responsible for NF- κ B activation and consequent induction of NF- κ B-dependent inflammatory signals.

Sawitzki, B., B. Kieselbach, et al. (2004). "IFN- γ Regulation in Anti-CD4 Antibody-Induced T Cell

Unresponsiveness." *J. Am. Soc. Nephrol.* **15**(3): 695-703.

<http://www.jasn.org/cgi/content/abstract/15/3/695>

ABSTRACT. The anti-rat CD4 mAb RIB5/2 is very potent in inducing allospecific tolerance in vivo. It is interesting that the unresponsiveness is breakable by exogenous IL-2 applied during the induction phase of tolerance. The molecular mechanisms underlying anti-CD4 antibody-mediated inhibition of allospecific T cell activation and how this is antagonized by exogenous IL-2 were investigated. Anti-CD4 treatment, in vivo and in vitro, completely abrogated IL-2 production by alloreactive T cells. In contrast, anti-CD4-treated alloactivated T cells showed similar IFN- γ mRNA expression as untreated alloactivated T cells but did not secrete any protein. Thus, the anti-CD4 antibody cannot prevent IFN- γ mRNA expression but is interfering with posttranscriptional mechanisms that control IFN- γ production during alloactivation of T cells. Addition of IL-2 but not IL-15 to anti-CD4-treated alloactivated T cells restored IFN- γ protein production without leading to enhanced IFN- γ mRNA expression. Further investigations revealed a diminished activation of translation initiation factor eIF2 α in anti-CD4-treated T cells, which was restored by exogenous IL-2. As activated eIF2 α is essential for the translation of IFN- γ mRNA, the results may explain the reversibility of anti-CD4-induced unresponsiveness by exogenous IL-2. Furthermore, these results not only shed further light onto the molecular mechanisms of tolerance induction but also reveal the possible weaknesses of anti-CD4 antibody-induced unresponsiveness.

Schmid, H., A. Henger, et al. (2003). "Gene Expression Profiles of Podocyte-Associated Molecules as Diagnostic Markers in Acquired Proteinuric Diseases." *J. Am. Soc. Nephrol.* **14**(11): 2958-2966.

<http://www.jasn.org/cgi/content/abstract/14/11/2958>

ABSTRACT. For identifying potential diagnostic markers of proteinuric glomerulopathies, glomerular mRNA levels of molecules relevant for podocyte function (α -actinin-4, glomerular epithelial protein 1, Wilms tumor antigen 1, synaptopodin, dystroglycan, nephrin, podoplanin, and podocin) were determined by quantitative real-time RT-PCR from microdissected glomeruli. Biopsies from 83 patients with acquired proteinuric diseases were analyzed (minimal change disease [MCD; n = 13], benign nephrosclerosis [n = 16], membranous glomerulopathy [n = 31], focal and segmental glomerulosclerosis [FSGS; n = 9], and controls [n = 14]). Gene expression levels normalized to two different housekeeping transcripts (glyceraldehyde-3-phosphate-dehydrogenase and 18 S rRNA) did not allow a separation between proteinuric disease categories. However, a significant positive correlation between α -actinin-4, glomerular epithelial protein 1, synaptopodin, dystroglycan, Wilms tumor antigen 1, and nephrin was found in all analyzed glomeruli, whereas podocin mRNA expression did not correlate. Because varying amounts of housekeeper cDNA per glomerulus can confound expression ratios relevant for a subpopulation of cells, an "in silico" microdissection was performed using a podocyte-specific cDNA as a reference gene. Expression ratio of podocin to synaptopodin, the two genes with the most disparate expression, allowed a robust separation of FSGS from MCD and nephrosclerosis. Segregation of FSGS from MCD via this ratio was confirmed in an independent population of formaldehyde-fixed archival biopsies (MCD, n = 5; FSGS, n = 4) after glomerular laser capture microdissection. In addition, the expression marker was able to predict steroid responsiveness in diagnostically challenging cases of MCD versus FSGS (n = 6). As the above approach can be performed as an add-on diagnostic tool, these molecular diagnostic parameters could give novel information for the management of proteinuric diseases. E-mail: kretzler@medpoli.med.uni-muenchen.de

Smeets, P. H. E., R. A. M. H. van Aubel, et al. (2004). "Contribution of Multidrug Resistance Protein 2

(MRP2/ABCC2) to the Renal Excretion of p-aminohippurate (PAH) and Identification of MRP4 (ABCC4) as a Novel PAH Transporter." *J. Am. Soc. Nephrol.* **15**(11): 2828-2835.

<http://www.jasn.org/cgi/content/abstract/15/11/2828>

p-Aminohippurate (PAH) is the classical substrate used in the characterization of organic anion transport in renal proximal tubular cells. Although basolateral transporters for PAH uptake from blood into the cell have been well characterized, there is still little knowledge on the apical urinary efflux transporters. The multidrug resistance protein 2 (MRP2/ABCC2) is localized to the apical membrane and mediates ATP-dependent PAH transport, but its contribution to urinary PAH excretion is not known. In this report, we show that renal excretion of PAH in isolated perfused kidneys from wild-type and Mrp2-deficient (TR-) rats is not significantly different. Uptake of [¹⁴C]PAH in membrane vesicles expressing two different MRP2 clones isolated from Sf9 and MDCKII cells exhibited a low affinity for PAH (Sf9, 5 {+/-} 2 mM; MDCKII, 2.1 {+/-} 0.6 mM). Human MRP4 (ABCC4), which has recently been localized to the apical membrane, expressed in Sf9 cells had a much higher affinity for PAH (K_m = 160 {+/-} 50 {micro}M). Various inhibitors of MRP2-mediated PAH transport also inhibited MRP4. Probenecid stimulated MRP2 at low concentrations but had no effect on MRP4; but at high probenecid concentrations, both MRP2 and MRP4 were inhibited. Sulfapyrazole only stimulated MRP2, but inhibited MRP4. Real-time PCR and Western blot analysis showed that renal cortical expression of MRP4 is approximately fivefold higher as compared with MRP2. MRP4 is a novel PAH transporter that has higher affinity for PAH and is expressed more highly in kidney than MRP2, and may therefore be more important in renal PAH excretion.

Stambe, C., R. C. Atkins, et al. (2003). "Blockade of p38{alpha} MAPK Ameliorates Acute Inflammatory Renal Injury in Rat Anti-GBM Glomerulonephritis." *J. Am. Soc. Nephrol.* **14**(2): 338-351.

<http://www.jasn.org/cgi/content/abstract/14/2/338>

ABSTRACT. The p38 mitogen-activated protein kinase (MAPK) pathway is a pro-inflammatory signal transduction pathway. The aim of this study was to examine the role of this pathway in acute renal inflammation. Immunostaining localized components of the p38 MAPK pathway (p38{alpha}, p-p38, p-ATF-2) in normal glomeruli, to podocytes, and occasional endothelial cells. This study identified an eightfold increase in glomerular activation of p38 MAPK (phosphorylated p38, p-p38) within 3 h of the induction of rat anti-glomerular basement membrane (GBM) glomerulonephritis and localized p-p38 and p-ATF-2 to infiltrating neutrophils, with increased staining of podocytes and endothelial cells. The relevance of these findings to human acute inflammatory renal disease was determined by examination of biopsy specimens. In patients with post-infectious glomerulonephritis, there was an increased number of positive p-p38 glomerular cells, including p-p38 staining of infiltrating neutrophils, compared with normal human kidney. In rats, administration of a specific p38 MAPK inhibitor, NPC 31145, before induction of anti-GBM disease prevented a loss of renal function and substantially reduced proteinuria. The reduction in renal injury was attributed to a 55% reduction in glomerular neutrophil infiltration and a 68% reduction in platelet accumulation. This was associated with an abrogation of glomerular P-selectin immunostaining and inhibition of glomerular P-selectin gene expression. In summary, this study has localized the components of the p38 MAPK pathway to cells in normal and diseased rat and human kidney and identified a number of important mechanisms by which signaling through the p38 MAPK pathway induces inflammatory renal disease. Blockade of the p38 pathway may be a novel therapeutic strategy for the treatment of acute renal inflammation. E-mail: cosimo.stambe@med.monash.edu.au

Sullivan, L. P., D. P. Wallace, et al. (2002). "Sulfonylurea-Sensitive K⁺ Transport is Involved in Cl⁻

Secretion and Cyst Growth by Cultured ADPKD Cells." *J. Am. Soc. Nephrol.* **13**(11): 2619-2627.

<http://www.jasn.org/cgi/content/abstract/13/11/2619>

ABSTRACT. Transepithelial chloride and fluid secretion by many types of epithelia involves activation of a conductive K⁺ pathway that serves to support the electrochemical driving force for Cl⁻ secretion. This study sought to determine if such a pathway is involved in Cl⁻ and fluid secretion by the cystic epithelia in autosomal dominant polycystic kidney disease (ADPKD). Primary cultures of cells derived from the cysts of patients with ADPKD were used. Confluent monolayers of these cells, mounted in Ussing chambers, were stimulated to secrete Cl⁻ by application of the adenylyl cyclase agonist, forskolin. The effects of various K⁺ channel blockers on the increase in short-circuit current (I_{sc}) generated by active Cl⁻ secretion were determined. Charybdotoxin, an inhibitor of Ca²⁺-sensitive K⁺ channels exerted no effect. Similarly, the chromanole 293B, an inhibitor of cAMP-induced K⁺ conductance, exerted no effect on cAMP-dependent anion secretion. Glibenclamide, an inhibitor of ATP-sensitive K⁺ channels and the cystic fibrosis transmembrane conductance regulator (CFTR), modestly inhibited the forskolin-stimulated current when applied to the apical surface of the monolayers, suggesting a relatively weak effect on CFTR. Basolateral application of glibenclamide inhibited I_{sc} to a greater extent. This latter effect may be due to inhibition of a K⁺-conductive transport step. Glibenclamide exerted little effect on the I_{sc} of nonstimulated monolayers. Cyst growth in ADPKD is driven by cell proliferation and Cl⁻ and fluid secretion. The effect of glibenclamide on the growth of cysts formed within a collagen gel by cultured ADPKD cells was tested. Addition of glibenclamide to the media bathing the cysts inhibited their growth. Glibenclamide also blocked the formation of cysts when it was added to the media at the time the cells were seeded within the collagen gel. Glibenclamide was also found to inhibit the proliferation of ADPKD cells. RT-PCR analysis demonstrated that the ATP-sensitive K⁺ channel, Kir 6.2, is expressed in cultured ADPKD cells and in normal human kidney. These results suggest that ATP-sensitive K⁺ channel blockers should be investigated as possible therapeutic agents to inhibit cyst growth in ADPKD.

Takeji, M., N. Kawada, et al. (2004). "CCAAT/Enhancer-Binding Protein {delta} Contributes to Myofibroblast Transdifferentiation and Renal Disease Progression." *J. Am. Soc. Nephrol.* **15**(9): 2383-2390.

<http://www.jasn.org/cgi/content/abstract/15/9/2383>

ABSTRACT. Myofibroblasts are pivotal participants in pathologic processes in a wide variety of organs, such as lung, liver, and kidney, by producing several inflammatory cytokines and extracellular matrices. The mechanism by which transdifferentiation from original cell to myofibroblast occurs, however, is still unclear. The expression of smooth muscle {alpha}-actin (SM{alpha}A) is the most characteristic feature of myofibroblasts; therefore, it was speculated that any factors that promote SM{alpha}A expression might be the key to transdifferentiation to myofibroblasts and disease exacerbation. A transcription factor CCAAT/enhancer-binding protein {delta} (C/EBP{delta}) was identified and demonstrated to bind to sequences including the CArG motif from SM{alpha}A intron 1 and to increase transcriptional activity of this promoter. Expression of SM{alpha}A and C/EBP{delta} in the glomerular area was upregulated in rat anti-Thy1 glomerulonephritis and mouse Habu-venom glomerulonephritis, both of which are models of mesangioproliferative glomerulonephritis. In the latter model, C/EBP{delta} knockout mice demonstrated significantly less SM{alpha}A expression in the glomerular area on day 8 and less renal functional deterioration on day 14, compared with wild-type mice. These data suggest an important role for C/EBP{delta} in myofibroblast transdifferentiation and glomerulonephritis exacerbation.

Tanaka, T., T. Kuroiwa, et al. (2002). "Human Platelets Stimulate Mesangial Cells to Produce Monocyte Chemoattractant Protein-1 via the CD40/CD40 Ligand Pathway and May Amplify Glomerular Injury." J. Am. Soc. Nephrol. **13**(10): 2488-2496.

<http://www.jasn.org/cgi/content/abstract/13/10/2488>

ABSTRACT. Platelets are thought to play an important role in the initiation and the progression of a variety of glomerulonephritides. This study examined whether platelets induce production of monocyte chemoattractant protein-1 (MCP-1), a chemokine involved in leukocyte recruitment and glomerular injury, by cultured human mesangial cells (MC). To this end, platelets isolated from normal human donors were cocultured with MC at various ratios. MCP-1 synthesis was evaluated by quantitative real-time PCR and enzyme-linked immunosorbent assay. Platelets at 1:100 ratio (MC to platelets) induced an approximately 20-fold increase in mesangial MCP-1 mRNA and protein expression through an obligatory cell-to-cell contact-dependent mechanism. Importantly, blockade of the CD40/CD40 ligand (CD40L) pathway with neutralizing antibodies decreased MCP-1 production by approximately 60%. It was confirmed that CD40 was functionally expressed on MC. Gel-shift assays and inhibitors of phosphorylation were used to demonstrate that activation of p38 mitogen-activated protein kinase, protein tyrosine kinases, and nuclear factor- κ B activation were essential for MCP-1 production. These data indicate that platelet/MC contact stimulates the production of MCP-1 and may contribute to glomerular inflammatory responses by recruiting leukocytes from the peripheral blood. E-mail: tkuroiwa@med.gunma-u.ac.jp

Umekawa, T., Y. Hatanaka, et al. (2004). "Effect of Angiotensin II Receptor Blockage on Osteopontin Expression and Calcium Oxalate Crystal Deposition in Rat Kidneys." J. Am. Soc. Nephrol. **15**(3): 635-644.

<http://www.jasn.org/cgi/content/abstract/15/3/635>

ABSTRACT. Hyperoxaluria leads to calcium oxalate (CaOx) crystallization and development of tubulointerstitial lesions in the kidneys. Treatment of hyperoxaluric rats with angiotensin II (Ang II) type I receptor blocker (ARB) reduces lesion formation. Because Ang II mediates osteopontin (OPN) synthesis, which is involved in both macrophage recruitment and CaOx crystallization, it was hypothesized that ARB acts via OPN. Hyperoxaluria was induced in 10-wk-old male Sprague-Dawley rats, and they were treated with ARB candesartan. At the end of 4 wk, kidneys were examined for crystal deposits, ED-1-positive cells, and expression of OPN mRNA. PCR was used to quantify OPN, renin, and angiotensin-converting enzyme (ACE) mRNA in kidneys. RIA was used to determine renal, plasma, and urinary OPN; plasma renin; Ang II and ACE; and renal Ang II. For evaluating oxidative stress, malondialdehyde was measured. Urinary calcium, oxalate, creatinine, and albumin were also determined. Despite similar urinary calcium and oxalate levels, kidneys of hyperoxaluric rats on candesartan had fewer CaOx crystals, fewer ED-1-positive cells, reduced OPN expression, and reduced malondialdehyde than hyperoxaluric rats. Urinary albumin excretion and serum creatinine levels improved significantly on candesartan treatment. mRNA for OPN, renin, and ACE were significantly elevated in hyperoxaluric rats. OPN synthesis and production increased with hyperoxaluria but to a lesser extent in candesartan-treated hyperoxaluric rats. These results show for the first time that oxalate can activate the renal renin-angiotensin system and that oxalate-induced upregulation of OPN is in part mediated via renal renin-angiotensin system.

Wahab, N. A., B. S. Weston, et al. (2005). "Connective Tissue Growth Factor CCN2 Interacts with and Activates the Tyrosine Kinase Receptor TrkA." J. Am. Soc. Nephrol. **16**(2): 340-351.

<http://www.jasn.org/cgi/content/abstract/16/2/340>

Connective tissue growth factor (CTGF) is implicated as a factor promoting tissue fibrosis in several disorders, including diabetic nephropathy. However, the molecular mechanism(s) by which it functions is not known. CTGF rapidly activates several intracellular signaling molecules in human mesangial cells (HMC), including extracellular signal-related kinase 1/2, Jun NH2-terminal kinase, protein kinase B, CaMK II, protein kinase C{alpha}, and protein kinase C{delta}, suggesting that it functions via a signaling receptor. Treating HMC with CTGF stimulated tyrosine phosphorylation of proteins 75 to 80 and 140 to 180 kD within 10 min, and Western blot analysis of anti-phosphotyrosine immunoprecipitates identified the neurotrophin receptor TrkA (molecular weight approximately 140 kD). Cross-linking rCTGF to cell surface proteins with 3,3'-dithiobis(sulfosuccinimidylpropionate) revealed that complexes formed with TrkA and with the general neurotrophin co-receptor p75NTR. rCTGF stimulated phosphorylation of TrkA (tyr 490, 674/675). K252a, a known selective inhibitor of Trk, blocked this phosphorylation, CTGF-induced activation of signaling proteins, and CTGF-dependent induction of the transcription factor TGF- β -inducible early gene in HMC. It is concluded that TrkA serves as a tyrosine kinase receptor for CTGF.

J. Antimicrob. Chemother. (13)

Aubouy, A., S. Jafari, et al. (2003). "DHFR and DHPS genotypes of *Plasmodium falciparum* isolates from Gabon correlate with in vitro activity of pyrimethamine and cycloguanil, but not with sulfadoxine-pyrimethamine treatment efficacy." *J. Antimicrob. Chemother.* **52**(1): 43-49.

<http://jac.oupjournals.org/cgi/content/abstract/52/1/43>

Objectives: To assess the relationship between the presence of DHFR and DHPS mutations in *Plasmodium falciparum*, parasite in vitro resistance, and in vivo efficacy of sulfadoxine-pyrimethamine (SP) treatment. Patients and methods: Measurement of SP treatment efficacy in malaria-infected children in Gabon was combined with in vitro tests of susceptibility to pyrimethamine and cycloguanil, and molecular genotyping at several DHFR and DHPS loci of parasites isolated before treatment. DHFR was studied at codons 108, 51, and 59, whereas DHPS gene was typed at positions 436, 437, 540 and 581. Results: SP treatment was effective in 86% of children by day 28. Seventy-five percent of isolates were in vitro resistant to pyrimethamine and 65.5% to cycloguanil. No mutation was detected at codons 540 and 581 of the DHPS gene. Most isolates (71.8%) presented with the triple mutant DHFR genotype, whereas 64.3% combined at least three DHFR and one DHPS mutations. The increase in the number of DHFR mutations was associated with an increase in in vitro resistance to pyrimethamine and cycloguanil; three DHFR mutations conferred pyrimethamine and to a lesser extent cycloguanil resistance. Treatment failures only occurred with isolates presenting at least two DHFR mutations (S108N and C59R) and one DHPS mutation (S436A or A437G), but SP treatment of infections with such parasites gave treatment success in 82.0% of children. Conclusions: DHFR mutations that lead to high-level in vitro resistance to pyrimethamine plus 1-2 DHPS mutations are not sufficient to induce in vivo failure of SP treatment in young children from Gabon.

Boyd, D. A., P. Kibsey, et al. (2004). "Enterococcus faecium N03-0072 carries a new VanD-type vancomycin resistance determinant: characterization of the VanD5 operon." *J. Antimicrob.*

Chemother. **54**(3): 680-683.

<http://jac.oupjournals.org/cgi/content/abstract/54/3/680>

Objectives: To genotypically characterize the vancomycin resistance mechanism of *Enterococcus faecium* N03-0072, which was negative by PCR for the currently known van genotypes. Methods: PCR was used to amplify the entire vancomycin resistance operon and the complete nucleotide sequence was determined by dideoxy cycle sequencing. Results: Analysis revealed a VanD-type operon with 94% nucleotide identity to the VanD4 operon and 90% nucleotide identity to the VanD1/D3 operons. A set of universal primers was designed in order to identify all current vanD variants by PCR. Conclusions: *E. faecium* N03-0072 carries a new VanD-type operon, designated VanD5.

Fernandes, C. J., L. A. Fernandes, et al. (2005). "Cefoxitin resistance as a surrogate marker for the detection of methicillin-resistant *Staphylococcus aureus*." J. Antimicrob. Chemother. **55**(4): 506-510.

<http://jac.oupjournals.org/cgi/content/abstract/55/4/506>

Objectives: To evaluate the usefulness of cefoxitin when used as a surrogate marker for the detection of methicillin resistance. Patients and methods: Eight hundred and seventy-one strains of *Staphylococcus aureus*, collected from eight tertiary referral centres serving diverse socio-economic populations, were included in the study using NCCLS disc diffusion and the agar dilution methods. Results: Using cefoxitin and NCCLS criteria for disc diffusion, the sensitivity and specificity for recognizing methicillin resistance were both 100%. Similar results were obtained when the strains were tested by the agar dilution method. The cefoxitin MICs for methicillin-susceptible strains were ≤ 4 mg/L. Conclusions: Testing with cefoxitin as a surrogate marker for the detection of methicillin resistance was very accurate with both disc diffusion and agar dilution methods. Such testing clearly distinguished methicillin-resistant strains of *S. aureus* from methicillin-susceptible strains.

Hayes, J. R., D. D. Wagner, et al. (2005). "Distribution of streptogramin resistance determinants among *Enterococcus faecium* from a poultry production environment of the USA." J. Antimicrob. Chemother. **55**(1): 123-126.

<http://jac.oupjournals.org/cgi/content/abstract/55/1/123>

Objectives: The impact of agricultural use of antimicrobials on the present and future efficacy of therapeutic drugs in human medicine is a growing public concern. Quinupristin/dalfopristin has been approved to treat human disease caused by vancomycin-resistant *Enterococcus faecium* and is related to virginiamycin, a streptogramin complex that has long been used in USA agriculture poultry production. Methods: Streptogramin-resistant isolates of *E. faecium* from poultry production environments on the eastern seaboard were recovered without selection for streptogramin resistance and examined using ribotyping to evaluate clonal bias. Colony PCR screening for the previously described streptogramin resistance determinants *erm*(A), *erm*(B), *msr*(C), *vgb*(A), *vat*(D) and *vat*(E) was performed to determine the prevalence of streptogramin resistance mechanisms from these environments. Results: The collection of *E. faecium* isolates was unevenly distributed among 28 ribogroups and did not cluster geographically. The most prevalent ribogroups was composed of isolates that possessed diverse antimicrobial resistance profiles. Of the 127 isolates examined, 63% were resistant to quinupristin/dalfopristin. The resistance determinants *erm*(A) and *erm*(B) were observed among 6% and 10%, respectively, of

streptogramin-resistant isolates. *msr(C)* was detected in a single isolate that was resistant to macrolide and lincosamide antimicrobials. The streptogramin B hydrolase *vgb(A)* and the streptogramin A acetyltransferases genes *vat(D)* and *vat(E)* were not detected in any of the *E. faecium* isolates. Conclusions: These results indicate that there is widespread resistance to streptogramin antimicrobials among *E. faecium* throughout the poultry production region in this study and that the mechanisms of resistance to streptogramin antimicrobials within this population remain largely uncharacterized.

Ho, P. L., R. C. W. Wong, et al. (2004). "Application of a multiplex *pbp2b* and *pbp2x* PCR for prediction of penicillin resistance in *Streptococcus pneumoniae*." J. Antimicrob. Chemother. **53**(5): 890-891.

<http://jac.oupjournals.org>

Junker, L. M. and A. G. Hay (2004). "Effects of triclosan incorporation into ABS plastic on biofilm communities." J. Antimicrob. Chemother. **53**(6): 989-996.

<http://jac.oupjournals.org/cgi/content/abstract/53/6/989>

Objective: This study compared the attached biofilm populations on acrylonitrile-butadiene-styrene (ABS) plastic with and without the incorporation of the antimicrobial triclosan [5-chloro-2-(2,4-dichlorophenoxy) phenol] after 1-3 weeks of exposure to drinking water. Methods: Biofilms were cultivated on triclosan-incorporated (TP) and control plastics (CP) in continuous flow culture reactors with drinking water as the growth medium and inoculum. After 1-3 weeks of exposure, the plastics were removed and the biofilms aseptically harvested. The attached communities were examined with respect to direct cell counts, culturability, triclosan resistance and community composition. Results: Based on these analyses, no significant differences were observed between the populations attached to TP and CP surfaces. Results from both a bioavailability assay and gas chromatography mass spectrometry analyses, revealed that only trace amounts of triclosan desorbed from the plastic. The lack of biofilm community difference, coupled with this limited desorption of triclosan from the TP indicates that the ABS plastic studied was no more effective at controlling bacterial populations than the control plastic because the antimicrobial was not bioavailable. Conclusions: These results call into question the long-term utility of triclosan incorporation into ABS plastic and highlight the need for proof of efficacy regarding the antimicrobial properties of such materials.

Kern, M. B., T. Klemmensen, et al. (2002). "Susceptibility of Danish *Escherichia coli* strains isolated from urinary tract infections and bacteraemia, and distribution of *sul* genes conferring sulphonamide resistance." J. Antimicrob. Chemother. **50**(4): 513-516.

<http://jac.oupjournals.org/cgi/content/abstract/50/4/513>

Antibiotic resistance of urinary tract pathogens has increased worldwide. Our aim was to provide information regarding resistance patterns of *Escherichia coli* in urinary tract infections (UTIs) and *E. coli* bacteraemia in Denmark. The overall resistance ranged from: ampicillin 20-47%, mecillinam 0-7%, trimethoprim 10-28%, sulfamethizole 22-47% and nitrofurantoin 0-3%. In strains with sulfamethizole MICs > 2048 mg/L, 97% carried *sulI*, *sulII* or both genes, with *sulII* being the most common. Among the *sulI* gene-positive strains, 96% were *intl 1* gene positive.

Kugelberg, E., S. Lofmark, et al. (2005). "Reduction of the fitness burden of quinolone resistance in *Pseudomonas aeruginosa*." *J. Antimicrob. Chemother.* **55**(1): 22-30.

<http://jac.oupjournals.org/cgi/content/abstract/55/1/22>

Objectives: Quinolone resistance in the opportunistic pathogen *Pseudomonas aeruginosa* is commonly caused by mutations that alter the target molecules DNA gyrase/topoisomerase IV, or cause activation of various efflux systems. We have analysed the effect of quinolone resistance caused by DNA gyrase/topoisomerase IV mutations on bacterial fitness. **Methods:** Norfloxacin-resistant mutants were isolated and by DNA sequencing the mutations conferring resistance were identified. Mutant fitness was determined by measuring growth rates in vitro. Mutants with reduced growth rates were serially passaged to obtain growth-compensated mutants. The level of DNA supercoiling was determined by isolating plasmid DNA from the susceptible, resistant and compensated mutants and comparing the topoisomer distribution patterns by gel electrophoresis in the presence of chloroquine. **Results:** Low-level resistance (4-48 mg/L) was caused by single mutations in *gyrA* or *gyrB*. Among these strains, three out of eight mutants showed lower fitness, whereas high-level resistant (>256 mg/L) mutants with double mutations in *gyrA* and *parC*, *parE*, *nfxB* or unknown genes all showed a reduced fitness. Slow-growing resistant mutants with a *gyrA* mutation had decreased DNA supercoiling. After serial passage in laboratory medium, mutant fitness was increased by compensatory mutation(s) that restored supercoiling to normal levels. The compensatory mutation(s) was not located in any of the genes (*gyrAB*, *topA*, *parCE*, *hupB*, *fis*, *hupN*, *himAD* or *PA5348*) that were expected to affect supercoiling. **Conclusions:** Our results show that no cost' and compensatory mutations are common in quinolone-resistant *P. aeruginosa*.

Lee, R. E. B., T. T. Liu, et al. (2005). "Genome-wide expression profiling of the response to ciclopirox olamine in *Candida albicans*." *J. Antimicrob. Chemother.* **55**(5): 655-662.

<http://jac.oupjournals.org/cgi/content/abstract/55/5/655>

Objectives: The aim of this study was to identify changes in the gene expression profile of *Candida albicans* upon exposure to the hydroxypyridone anti-infective agent ciclopirox olamine in an effort to better understand its mechanism of action. **Methods:** *C. albicans* SC5314 was exposed to either medium alone or ciclopirox olamine at a concentration equivalent to the IC50 (0.24 mg/L) for 3 h. RNA was isolated and gene expression profiles were compared using DNA microarrays. Differential expression of select genes was confirmed by real-time reverse transcription (RT)-PCR. Mutants disrupted for *CDR2* and both *CDR1* and *CDR2*, as well as a clinical isolate overexpressing *CDR1* and *CDR2*, were examined for changes in susceptibility to ciclopirox olamine. **Results:** A total of 49 genes were found to be responsive to ciclopirox olamine, including 36 up-regulated genes and 13 down-regulated genes. These included genes involved in small molecule transport (*HGT11*, *HXT5*, *ENA22*, *PHO84*, *CDR4*), iron uptake (*FRE30*, *FET34*, *FTR1*, *FTR2*, *SIT1*) and cell stress (*SOD1*, *SOD22*, *CDR1*, *DDR48*). Mutants disrupted for *CDR2* and both *CDR1* and *CDR2*, as well as a clinical isolate overexpressing *CDR1* and *CDR2*, showed no change in susceptibility to ciclopirox olamine compared with the respective parent. **Conclusions:** Consistent with the hypothesis that ciclopirox olamine acts as an iron chelator, it induced changes in expression of many genes involved in iron uptake. Despite induction of the multidrug efflux pump genes *CDR1* and, to a lesser extent, *CDR2* by ciclopirox olamine, these genes do not affect susceptibility to this agent.

Merlino, J., J. Watson, et al. (2002). "Detection and expression of methicillin/oxacillin resistance in multidrug-resistant and non-multidrug-resistant *Staphylococcus aureus* in Central Sydney, Australia." *J. Antimicrob. Chemother.* **49**(5): 793-801.

<http://jac.oupjournals.org/cgi/content/abstract/49/5/793>

Ninety clinical *Staphylococcus aureus* isolates from separate patients were examined phenotypically and genotypically for susceptibility to methicillin/oxacillin. Thirty were methicillin/oxacillin susceptible and 60 were methicillin and oxacillin resistant (MRSA). The 60 MRSA isolates examined were subdivided into two groups according to their antibiotic profiles and comprised 30 non-multidrug-resistant (NMDR) isolates, resistant to less than two non- β -lactam antibiotics, and 30 multidrug-resistant (MDR) isolates, resistant to three or more non- β -lactam antibiotics. Phenotypic and genotypic analysis of methicillin/oxacillin showed that despite use of the guidelines published by the NCCLS for the testing of *S. aureus* susceptibility to methicillin/oxacillin, MIC values of some NMDR MRSA isolates fell below the NCCLS-recommended breakpoints. Etest strips failed to detect two NMDR MRSA isolates tested with oxacillin and four tested with methicillin. Lowering the NCCLS-recommended oxacillin screen agar concentration from 6 to 2 mg/L and temperature of incubation to 30°C, improved the specificity and sensitivity of NMDR MRSA detection from 87% to 100%. On PFGE analysis these NMDR MRSA strains were genotypically different. Genotypic tests, such as multiplex PCR for the *mecA/nuc* genes and DNA hybridization for the *mecA* gene, or phenotypic monoclonal antibody-based tests to detect penicillin-binding protein 2a (PBP2a) offer advantages for problematic isolates in detecting or confirming low-level phenotypic heterogeneous *mecA* expression of oxacillin and methicillin resistance in NMDR MRSA.

Plante, I., D. Centron, et al. (2003). "An integron cassette encoding erythromycin esterase, *ere(A)*, from *Providencia stuartii*." *J. Antimicrob. Chemother.* **51**(4): 787-790.

<http://jac.oupjournals.org/cgi/content/abstract/51/4/787>

We have mapped the variable region of the two class 1 integrons found in the multiresistant strain *Providencia stuartii* 1723. Integron 1 contains a new arrangement of gene cassettes, *aacA4-aadB-aadA1*, conferring resistance to all aminoglycosides used for clinical treatment. Integron 2 contains a variant of the gene cassette *ere(A)*, coding for an erythromycin esterase, whose nucleotide sequence shares 93.7% DNA identity with *ere(A)* from *Escherichia coli* BM2195 plasmid pIP1100.

Poulsen, A. B., R. Skov, et al. (2003). "Detection of methicillin resistance in coagulase-negative staphylococci and in staphylococci directly from simulated blood cultures using the EVIGENE MRSA Detection Kit." *J. Antimicrob. Chemother.* **51**(2): 419-421.

<http://jac.oupjournals.org/cgi/content/abstract/51/2/419>

The EVIGENE MRSA Detection Kit was evaluated on coagulase-negative staphylococci (CoNS) from agar plates and on staphylococci directly from positive spiked blood cultures. For the CoNS study, a total of 242 isolates were tested, and of these 237 gave valid test results. For the 237 valid tests, all gave correct *mecA* classification. For the blood culture procedure, a collection of 51 *mecA*-positive *Staphylococcus aureus*, 21 *mecA*-negative *S. aureus*, 31 *mecA*-positive CoNS and 28 *mecA*-negative CoNS were used for the simulated blood cultures. For the *S. aureus* strains, all gave valid test results and correct *mecA* classification. One of the MRSA isolates gave a very faint *nuc* signal, and another four isolates gave results close to the cut-off of the kit; however, these were still clearly positive when read by the naked eye. For the CoNS isolates, 51 of the 59 strains gave valid results. All of these 51 strains gave correct *mecA* status. Thus the EVIGENE MRSA Detection Kit can provide fast and accurate determination of methicillin resistance in CoNS. This preliminary study of the blood culture procedure indicates that it is possible to

achieve determination of methicillin resistance in staphylococci 8 h after positivity of the blood culture, making same-day detection of methicillin resistance possible.

Yan, J.-J., J.-J. Wu, et al. (2004). "Plasmid-mediated 16S rRNA methylases conferring high-level aminoglycoside resistance in *Escherichia coli* and *Klebsiella pneumoniae* isolates from two Taiwanese hospitals." *J. Antimicrob. Chemother.* **54**(6): 1007-1012.

<http://jac.oupjournals.org/cgi/content/abstract/54/6/1007>

Objectives: This study was conducted to investigate the occurrence of 16S rRNA methylases that confer high-level aminoglycoside resistance in *Klebsiella pneumoniae* and *Escherichia coli* isolates from two Taiwanese hospitals and the characteristics of these isolates. **Methods:** A total of 1624 *K. pneumoniae* and 2559 *E. coli* isolates consecutively collected over an 18 month period from a university hospital and seven *E. coli* and eight *K. pneumoniae* isolates that were resistant to amikacin from a district hospital were analysed. Two 16S rRNA methylase genes, *armA* and *rmtB*, were detected by PCR-based assays. β -Lactamase characteristics were determined by phenotypic and genotypic methods. **Results:** Overall, 28 *armA*-positive and seven *rmtB*-positive isolates were identified, and extended-spectrum β -lactamases (ESBLs) were detected in 33 (94.3%) isolates. The prevalence rates of *armA* and *rmtB* at the university hospital were 0.9% (n=15) and 0.3% (n=5) in *K. pneumoniae* and 0.4% (n=10) and 0.04% (n=1) in *E. coli*. CTX-M-3, CTX-M-14, SHV-5-like ESBLs, and CMY-2 were detected alone or in combination in 21, 6, 11, and 2, respectively, of the 28 *armA*-positive isolates. CTX-M-14 was detected in six of the seven *rmtB*-positive isolates. Fingerprinting of conjugative plasmids revealed the dissemination of closely related plasmids containing both *armA* and *bla*CTX-M-3. PFGE suggests that *armA* and *rmtB* spread by both horizontal transfer and clonal spread. **Conclusions:** This is the first report of the emergence of 16S rRNA methylases in Enterobacteriaceae in Taiwan. The spread of the multidrug-resistant isolates producing both ESBLs and 16S rRNA methylases may become a clinical problem.

J. Biochem. (Tokyo) (4)

Degawa, M., M. Namiki, et al. (2003). "Constitutive Expression of Cytochrome P450 Genes in Newly Established Rat Hepatic Cell Lines." *J. Biochem. (Tokyo)* **133**(6): 825-831.

<http://jb.oupjournals.org/cgi/content/abstract/133/6/825>

Two cultured cell lines, called Kan-R1 and Kan-R2, were established from rat hepatic cells by in vitro culture with a hepatocarcinogen, 3-methoxy-4-aminoazobenzene, and examined for the gene expression of cytochrome P450 (P450) isoforms, CYP1A1, CYP1A2, CYP2B1, CYP2B2, CYP3A1 and CYP3A2, by the RT-PCR method. It was revealed that all the P450 genes examined were expressed in both cell lines, although the two cell lines differed in cell size and colony-forming ability on a soft agar. The expression levels of the CYP1A2, CYP2B1, CYP2B2, CYP3A1, and CYP3A2 genes were lower than those in liver tissues, while that of CYP1A1 was higher in the cell lines. In both cell lines, cycloheximide, an inhibitor of protein synthesis, augmented the gene expression of the P450s except CYP2B1. These findings indicate that the newly established hepatic cell lines substantially express the P450 genes for CYP1A1, CYP1A2, CYP2B1, CYP2B2, CYP3A1, and CYP3A2, and that the constitutive gene expression of these

P450s, with the exception of CYP2B1, may be inhibited by negative transcription factors.

Omata, Y., H. Sakamoto, et al. (2004). "Purification and Characterization of Human Uroporphyrinogen III Synthase Expressed in *Escherichia coli*." *J. Biochem. (Tokyo)* **136**(2): 211-220.

<http://jb.oupjournals.org/cgi/content/abstract/136/2/211>

The side-chain asymmetry of physiological porphyrins is produced by the cooperative action of hydroxymethylbilane synthase and uroporphyrinogen (uro'gen) III synthase. Although the role of uro'gen III synthase is essential for the chemistry of porphyrin biosynthesis, many aspects, structural as well as mechanical, of uro'gen III synthase have yet to be studied. We report here an expression system in *Escherichia coli* and a purification procedure for human uro'gen III synthase. The enzyme in the lysate was unstable, but we found that glycerol prevents the activity loss in the lysate. The purified enzyme showed remarkable thermostability, particularly when kept in phosphate buffer containing DTT or EDTA, indicating that the enzyme activity may depend on its oxidation state. Examination of the relationship between the number of Cys residues that are accessible to 5,5'-dithiobis(2-nitrobenzoic acid) and the remaining activity during heat inactivation showed that a particular Cys residue is involved in activity loss. From the crystal structure of human uro'gen III synthase [Mathews et al. (2001) *EMBO J.* 20, 5832-5839], this Cys residue was considered to be Cys73, which is buried deep inside the enzyme, suggesting that Cys73 of human uro'gen III synthase plays an important role in enzyme activity.

Saito, H., K. Takeda, et al. (2003). "Germ Cell-Specific Expression of Microphthalmia-Associated Transcription Factor mRNA in Mouse Testis." *J. Biochem. (Tokyo)* **134**(1): 143-150.

<http://jb.oupjournals.org/cgi/content/abstract/134/1/143>

The gene coding for microphthalmia-associated transcription factor (Mitf) contains many promoters that could generate multiple Mitf isoforms with distinct amino-termini, such as ubiquitously expressed Mitf-A and Mitf-H. To gain further insight into Mitf isoform multiplicity and the regulation of the promoter usage of the Mitf gene, we have analyzed the function of the amino-terminal domains of Mitf isoforms and the expression of Mitf mRNA in mouse postnatal testis, which is characterized by spermatogenesis and by a cool temperature because of its unique location. Here we show that the amino-terminal domain of Mitf-A possesses a transactivation activity, as judged by yeast expression analysis. We also show the expression of Mitf-A and Mitf-D mRNAs in testis by PCR-based methods. Moreover, in situ hybridization analysis revealed that an Mitf mRNA, probably representing Mitf-A and/or Mitf-D, is expressed in germ cells, including spermatogonia, spermatocytes that undergo meiosis, and round spermatids with the haploid genome, but is undetectable in elongated spermatids with remodeled and condensed chromatin. Notably, Mitf mRNA is undetectable in somatic Leydig cells and peritubular cells. Therefore, multiple promoters may direct differential expression of the Mitf gene in the testis and contribute to functional diversity of Mitf isoforms.

Tsuruta, H. and Y. Aizono (2003). "Catalytic Efficiency and Some Structural Properties of Cold-Active Protein-Tyrosine-Phosphatase." *J. Biochem. (Tokyo)* **133**(2): 225-230.

<http://jb.oupjournals.org/cgi/content/abstract/133/2/225>

A procedure was established for expression and purification of abundant recombinant cold-active

protein-tyrosine-phosphatase (RCPTase), which showed identical enzymatic characteristics to the native enzyme (NCPTase). The purified RCPTase showed high catalytic activity at low temperature and maximal activity at 30{degrees}C. RCPTase has a thermodynamic characteristic in that its activation enthalpy was determined to be low, 4.3 kcal/mol, at temperatures below 19.3{degrees}C, where the Arrhenius relationship exhibited an inflection point, in comparison with 20.3 kcal/mol above 19.3{degrees}C. Also, the thermostability, ΔG_{water} , of the catalytic site in the RCPTase molecule was increased with a decrease in temperature. It was considered that cold-active protein-tyrosine-phosphatase could maintain its catalytic site in a stable conformation for eliciting high catalytic activity with low activation enthalpy at low temperature.

J. Biol. Chem. (223)

Abbas, T., M. Olivier, et al. (2002). "Differential Activation of p53 by the Various Adducts of Mitomycin C." J. Biol. Chem. **277**(43): 40513-40519.

<http://www.jbc.org/cgi/content/abstract/277/43/40513>

Mitomycin C (MC) is a cytotoxic chemotherapeutic agent that causes DNA damage in the form of DNA cross-links as well as a variety of DNA monoadducts and is known to induce p53. The various DNA adducts formed upon treatment of mouse mammary tumor cells with MC as well as 10-decarbamoyl MC (DMC) and 2,7-diaminomitosene (2,7-DAM), the major MC metabolite, have been elucidated. The cytotoxicity of DMC parallels closely that of MC in a number of rodent cell lines tested, whereas 2,7-DAM is relatively noncytotoxic. In this study, we investigate the ability of MC, DMC, and 2,7-DAM to activate p53 at equidose concentrations by treating tissue culture cell lines with the three mitomycins. Whereas MC and DMC induced p53 protein levels and increased the levels of p21 and Gadd45 mRNA, 2,7-DAM did not. Furthermore, MC and DMC, but not 2,7-DAM, were able to induce apoptosis efficiently in ML-1 cells. Therefore the 2,7-DAM monoadducts were unable to activate the p53 pathway. Interestingly, DMC was able to initiate apoptosis via a p53-independent pathway whereas MC was not. This is the first finding that adducts of a multiadduct type DNA-damaging agent are differentially recognized by DNA damage sensor pathways.

Abdollahi, A., D. Pisarcik, et al. (2003). "LOT1 (PLAGL1/ZAC1), the Candidate Tumor Suppressor Gene at Chromosome 6q24-25, Is Epigenetically Regulated in Cancer." J. Biol. Chem. **278**(8): 6041-6049.

<http://www.jbc.org/cgi/content/abstract/278/8/6041>

LOT1 is a zinc-finger nuclear transcription factor, which possesses anti-proliferative effects and is frequently silenced in ovarian and breast cancer cells. The LOT1 gene is localized at chromosome 6q24-25, a chromosomal region maternally imprinted and linked to growth retardation in several organs and progression of disease states such as transient neonatal diabetes mellitus. Toward understanding the molecular mechanism underlying the loss of LOT1 expression in cancer, we have characterized the genomic structure and analyzed its epigenetic regulation. Genome mapping of LOT1 in comparison with the other splice variants, namely ZAC1 and PLAGL1, revealed that its mRNA (~4.7 kb; GenBankTM accession number U72621) is

potentially spliced using six exons spanning at least 70 kb of the human genome. 5'-RACE (rapid amplification of cDNA ends) data indicate the presence of at least two transcription start sites. We found that in vitro methylation of the LOT1 promoter causes a significant loss in its ability to drive luciferase transcription. To determine the nature of in vivo methylation of LOT1, we used bisulfite-sequencing strategies on genomic DNA. We show that in the ovarian and breast cancer cell lines and/or tumors the 5'-CpG island of LOT1 is a differentially methylated region. In these cell lines the ratio of methylated to unmethylated CpG dinucleotides in this region ranged from 31 to 99% and the ovarian tumors have relatively higher cytosine methylation than normal tissues. Furthermore, we show that trichostatin A, a specific inhibitor of histone deacetylase, relieves transcriptional silencing of LOT1 mRNA in malignantly transformed cells. It appears that, unlike DNA methylation, histone deacetylation does not target the promoter, and rather it is indirect and may be elicited by a mechanism upstream of the LOT1 regulatory pathway. Taken together, the data suggest that expression of LOT1 is under the control of two epigenetic modifications and that, in the absence of loss of heterozygosity, the biallelic (two-hit) or maximal silencing of LOT1 requires both processes.

Adachi, H. and M. Tsujimoto (2002). "Characterization of the Human Gene Encoding the Scavenger Receptor Expressed by Endothelial Cell and Its Regulation by a Novel Transcription Factor, Endothelial Zinc Finger Protein-2." *J. Biol. Chem.* **277**(27): 24014-24021.

<http://www.jbc.org/cgi/content/abstract/277/27/24014>

The scavenger receptor expressed by endothelial cell (SREC), mediates the selective uptake of modified low density lipoprotein (LDL), such as acetylated LDL and oxidized LDL, into endothelial cells. The SREC gene spans 12 kilobase pairs and contains 11 exons. Analysis of full-length cDNA clones of SREC from a peripheral blood leukocyte cDNA library revealed that at least five alternatively spliced cDNAs were present, and two of them encoded soluble forms of SREC. The transcription start site of the SREC gene was mapped, and DNA sequence analysis revealed an Sp1 binding site in its proximal region. Deletion analysis of the 5'-flanking sequence revealed that sequence between base pairs [-]108 and [-]98 was critical for the promoter activity. This region contained half of an inverted repeat (IR) sequence with a triple nucleotide spacer (IR-3). A protected sequence between base pairs [-]268 and +17 was defined by in vitro DNase I footprinting analysis using human umbilical vein endothelial cell (HUVEC) nuclear extract. A novel transcription factor, endothelial zinc finger protein-2 (EZF-2), that binds to the 5'-flanking critical region of the SREC promoter activity was cloned from a HUVEC cDNA library employing a one-hybrid system. Whereas purified recombinant Sp1 alone produced similar protection in in vitro DNase I footprinting analysis, EZF-2 also bound to the 5'-flanking region SREC promoter. Co-transfection of SREC promoter and Sp1 or EZF-2 expression plasmids in HUVEC revealed that EZF-2 but not Sp1 increased SREC promoter activity. On the other hand, the mutation of either the Sp1 motif or IR-3 motif resulted in a decrease in the promoter activity. These results suggest that whereas Sp1 is the major nuclear protein bound to the regulatory region of the promoter, both EZF-2 and Sp1 are responsible for its regulation.

Agorio, A., C. Chalar, et al. (2003). "Alternative mRNAs Arising from Trans-splicing Code for Mitochondrial and Cytosolic Variants of Echinococcus granulosus Thioredoxin Glutathione Reductase." *J. Biol. Chem.* **278**(15): 12920-12928.

<http://www.jbc.org/cgi/content/abstract/278/15/12920>

Thioredoxin and glutathione systems are the major thiol-dependent redox systems in animal cells. They transfer via the reversible oxidoreduction of thiols the reducing equivalents of NADPH to numerous substrates and substrate reductases and constitute major defenses against oxidative

stress. In this study, we cloned from the helminth parasite *Echinococcus granulosus* two trans-spliced mRNA variants that encode thioredoxin glutathione reductases (TGR). These variants code for mitochondrial and cytosolic selenocysteine-containing isoforms that possess identical glutaredoxin (Grx) and thioredoxin reductase (TR) domains and differ exclusively in their N termini. Western blot analysis of subcellular fractions with specific anti-TGR antibodies showed that TGR is present in both compartments. The biochemical characterization of the native purified TGR suggests that the Grx and TR domains of the enzyme can function either coupled or independently of each other, because the Grx domain can accept electrons from either TR domains or the glutathione system and the TR domains can transfer electrons to either the fused Grx domain or to *E. granulosus* thioredoxin.

Ahmad, Z., M. Salim, et al. (2002). "Human Biliverdin Reductase Is a Leucine Zipper-like DNA-binding Protein and Functions in Transcriptional Activation of Heme Oxygenase-1 by Oxidative Stress." J. Biol. Chem. **277**(11): 9226-9232.

<http://www.jbc.org/cgi/content/abstract/277/11/9226>

Human biliverdin reductase (hBVR) is a serine/threonine kinase that catalyzes reduction of the heme oxygenase (HO) activity product, biliverdin, to bilirubin. A domain of biliverdin reductase (BVR) has primary structural features that resemble leucine zipper proteins. A heptad repeat of five leucines (L1-L5), a basic domain, and a conserved alanine characterize the domain. In hBVR, a lysine replaces L3. The secondary structure model of hBVR predicts an [alpha]-helix-turn-[beta]-sheet for this domain. hBVR translated by the rabbit reticulocyte lysate system appears on a nondenaturing gel as a single band with molecular mass of ~69 kDa. The protein on a denaturing gel separates into two anti-hBVR immunoreactive proteins of ~39.9 + 34.6 kDa. The dimeric form, but not purified hBVR, binds to a 100-mer DNA fragment corresponding to the mouse HO-1 (hsp32) promoter region encompassing two activator protein (AP-1) sites. The specificity of DNA binding is suggested by the following: (a) hBVR does not bind to the same DNA fragment with one or zero AP-1 sites; (b) a 56-bp random DNA with one AP-1 site does not form a complex with hBVR; (c) in vitro translated HO-1 does not interact with the 100-mer DNA fragment with two AP-1 sites; (d) mutation of Lys143, Leu150, or Leu157 blocks both the formation of the ~69-kDa specimens and hBVR DNA complex formation; and (e) purified preparations of hBVR or hHO-1 do not bind to DNA with two AP-1 sites. The potential significance of the AP-1 binding is suggested by the finding that the response of HO-1, in COS cells stably transfected with antisense hBVR, with 66% reduced BVR activity, to superoxide anion ([IMG]img001.gif" ALT="O₂">) formed by menadione is attenuated, whereas induction by heme is not affected. We propose a role for BVR in the signaling cascade for AP-1 complex activation necessary for HO-1 oxidative stress response.

Allaman-Pillet, N., J. Storling, et al. (2003). "Calcium- and Proteasome-dependent Degradation of the JNK Scaffold Protein Islet-brain 1." J. Biol. Chem. **278**(49): 48720-48726.

<http://www.jbc.org/cgi/content/abstract/278/49/48720>

In models of type 1 diabetes, cytokines induce pancreatic {beta}-cell death by apoptosis. This process seems to be facilitated by a reduction in the amount of the islet-brain 1/JNK interacting protein 1 (IB1/JIP1), a JNK-scaffold with an anti-apoptotic effect. A point mutation S59N at the N terminus of the scaffold, which segregates in diabetic patients, has the functional consequence of sensitizing cells to apoptotic stimuli. Neither the mechanisms leading to IB1/JIP1 down-regulation by cytokines nor the mechanisms leading to the decreased capacity of the S59N mutation to protect cells from apoptosis are understood. Here, we show that IB1/JIP1 stability is modulated by intracellular calcium. The effect of calcium depends upon JNK activation, which primes the

scaffold for ubiquitination-mediated degradation via the proteasome machinery. Furthermore, we observe that the S59N mutation decreases IB1/JIP1 stability by sensitizing IB1/JIP1 to calcium- and proteasome-dependent degradation. These data indicate that calcium influx initiated by cytokines mediates ubiquitination and degradation of IB1/JIP1 and may, therefore, provide a link between calcium influx and JNK-mediated apoptosis in pancreatic β -cells.

Arai, H., T. Furuya, et al. (2004). "Neurotoxic Effects of Lipopolysaccharide on Nigral Dopaminergic Neurons Are Mediated by Microglial Activation, Interleukin-1 β , and Expression of Caspase-11 in Mice." *J. Biol. Chem.* **279**(49): 51647-51653.

<http://www.jbc.org/cgi/content/abstract/279/49/51647>

The endotoxin lipopolysaccharide (LPS), a component of the Gram-negative bacterial cell wall, selectively induces degeneration of substantia nigral (SN) dopaminergic neurons via activation of microglial cells in rats and mice. Caspase-11 plays a crucial role in LPS-induced septic shock in mice. We examined the mechanism of LPS neurotoxicity on SN dopaminergic neurons in C57BL/6 mice and caspase-11 knockout mice. Mice were stereotactically injected with LPS into the SN on one side and vehicle into the SN of the other side. Immunohistochemistry, Western blotting analysis, enzyme-linked immunosorbent assay, and reverse transcriptase-PCR were performed to evaluate damage of SN dopaminergic neurons and activation of microglial cells. Intranigral injection of LPS at 1 or 3 $\mu\text{g}/\mu\text{l}$ /site decreased tyrosine hydroxylase-positive neurons and increased microglial cells in the SN compared with the contralateral side injected with vehicle at days 7 and 14 post-injection in C57BL/6 mice. Intranigral injection of LPS at 3 $\mu\text{g}/\mu\text{l}$ /site induced the expression of caspase-11 mRNA in the ventral midbrain at 6, 8, and 12 h post-injection, and the expression of caspase-11-positive cells in the SN at 8 and 12 h post-injection. Moreover, LPS at 3 $\mu\text{g}/\mu\text{l}$ /site increased interleukin-1 β content in the ventral midbrain at 12 and 24 h post-injection. LPS failed to elicit these responses in caspase-11 knockout mice. Our results indicate that the neurotoxic effects of LPS on nigral dopaminergic neurons are mediated by microglial activation, interleukin-1 β , and caspase-11 expression in mice.

Ayabe, T., H. Wulff, et al. (2002). "Modulation of Mouse Paneth Cell α -Defensin Secretion by mIKCa1, a Ca^{2+} -activated, Intermediate Conductance Potassium Channel." *J. Biol. Chem.* **277**(5): 3793-3800.

<http://www.jbc.org/cgi/content/abstract/277/5/3793>

Paneth cells in small intestinal crypts secrete microbicidal α -defensins in response to bacteria and bacterial antigens (Ayabe, T., Satchell, D. P., Wilson, C. L., Parks, W. C., Selsted, M. E., and Ouellette, A. J. (2000) *Nat. Immunol.* **1**, 113-138). We now report that the Ca^{2+} -activated K^{+} channel mIKCa1 modulates mouse Paneth cell secretion. mIKCa1 cDNA clones identified in a mouse small intestinal crypt library by hybridization to human IKCa1 cDNA probes were isolated, and DNA sequence analysis showed that they were identical to mIKCa1 cDNAs isolated from erythroid cells and liver. The genomic organization was found to be conserved between mouse and human IKCa1 as shown by comparisons of the respective cDNA and genomic sequences. Reverse transcriptase-PCR experiments using nested primers amplified mIKCa1 from the lower half of bisected crypts and from single Paneth cells, but not from the upper half of bisected crypts, villus epithelium, or undifferentiated crypt epithelial cells, suggesting a lineage-specific role for mIKCa1 in mouse small bowel epithelium. The cloned mIKCa1 channel was calcium-activated and was blocked by ten structurally diverse peptide and nonpeptide inhibitors with potencies spanning 9 orders of magnitude and indistinguishable from that of the human homologue. Consistent with channel blockade, charybdotoxin, clotrimazole, and the highly

selective IKCa1 inhibitors, TRAM-34 and TRAM-39, inhibited (~50%) Paneth cell secretion stimulated by bacteria or bacterial lipopolysaccharide, measured both as bactericidal activity and secreted cryptdin protein, but the inactive analog, TRAM-7, did not block secretion. These results demonstrate that mIKCa1 is modulator of Paneth cell [alpha]-defensin secretion and disclose an involvement in mucosal defense of the intestinal epithelium against ingested bacterial pathogens.

Barlic, J., D. H. McDermott, et al. (2004). "Interleukin (IL)-15 and IL-2 Reciprocally Regulate Expression of the Chemokine Receptor CX3CR1 through Selective NFAT1- and NFAT2-dependent Mechanisms." *J. Biol. Chem.* **279**(47): 48520-48534.

<http://www.jbc.org/cgi/content/abstract/279/47/48520>

We have recently reported that interleukin (IL)-15 and IL-2, which signal through IL-2R β γ , oppositely regulate expression of the proinflammatory chemokine receptor CX3CR1. Here we delineate molecular mechanisms responsible for this paradox. By using a luciferase reporter plasmid, we identified a 433-bp region spanning the major transcriptional start point of human CX3CR1 that, when expressed in human peripheral blood mononuclear cells (PBMCs), possessed strong constitutive promoter activity. IL-2 and IL-15 treatment increased and abolished this activity, respectively, mimicking their effects on endogenous CX3CR1. IL-2 and IL-15 have been reported to also have opposite effects on the immunoregulatory transcription factor NFAT (nuclear factor of activated T cells), and the 433-bp region contains a κ B-like NFAT site. The effects of IL-15 and IL-2 on both CX3CR1 reporter activity and endogenous CX3CR1 transcription in PBMCs were abolished by the NFAT inhibitors cyclosporin A and VIVIT. Moreover, mutation of the κ B-like NFAT sequence markedly attenuated IL-2 and IL-15 modulation of CX3CR1 promoter-reporter activity in PBMCs. Furthermore, chromatin immunoprecipitation revealed that IL-15 promoted specific recruitment of NFAT1 but not NFAT2 to the CX3CR1 promoter, whereas IL-2 had the converse effect. This appears to be relevant in vivo because mouse CX3CR1 mRNA was expressed in both PBMCs and splenocytes from NFAT1^{-/-} mice injected with recombinant IL-15 but was undetectable in cells from IL-15-injected NFAT1^{+/+} BALB/c mice; as predicted, IL-2 up-regulated cx3cr1 in both mouse strains to a similar extent. Thus, by pharmacologic, genetic, and biochemical criteria in vitro and in vivo, our results suggest that IL-15 and IL-2 oppositely regulate CX3CR1 gene expression by differentially recruiting NFAT1 and NFAT2 to a κ B-like NFAT site within the CX3CR1 promoter. We propose that expression of CX3CR1 and possibly other immunoregulatory genes may be determined in part by the balance of NFAT1 and NFAT2 activity in leukocytes.

Basu, S. S., M. J. Karbarz, et al. (2002). "Expression Cloning and Characterization of the C28 Acyltransferase of Lipid A Biosynthesis in Rhizobium leguminosarum." *J. Biol. Chem.* **277**(32): 28959-28971.

<http://www.jbc.org/cgi/content/abstract/277/32/28959>

An unusual feature of lipid A from plant endosymbionts of the Rhizobiaceae family is the presence of a 27-hydroxyoctacosanoic acid (C28) moiety. An enzyme that incorporates this acyl chain is present in extracts of Rhizobium leguminosarum, Rhizobium etli, and Sinorhizobium meliloti but not Escherichia coli. The enzyme transfers 27-hydroxyoctacosanate from a specialized acyl carrier protein (AcpXL) to the precursor Kdo2 ((3-deoxy-D-manno-octulosonic acid)₂-lipid IVA. We now report the identification of five hybrid cosmids that direct the overexpression of this activity by screening ~4000 lysates of individual colonies of an R. leguminosarum 3841 genomic DNA library in the host strain S. meliloti 1021. In these heterologous constructs, both the C28 acyltransferase and C28-AcpXL are overproduced. Sequencing of a 9-kb insert from cosmid pSSB-1, which is also present in the other cosmids,

shows that acpXL and the lipid A acyltransferase gene (lpxXL) are close to each other but not contiguous. Nine other open reading frames around lpxXL were also sequenced. Four of them encode orthologues of fatty acid and/or polyketide biosynthetic enzymes. AcpXL purified from *S. meliloti* expressing pSSB-1 is fully acylated, mainly with 27-hydroxyoctacosanoate. Expression of lpxXL in *E. coli* behind a T7 promoter results in overproduction in vitro of the expected R. leguminosarum acyltransferase, which is C28-AcpXL-dependent and utilizes (3-deoxy-D-manno-octulosonic acid)-lipid IVA as the acceptor. These findings confirm that lpxXL is the structural gene for the C28 acyltransferase. LpxXL is distantly related to the lauroyltransferase (LpxL) of *E. coli* lipid A biosynthesis, but highly significant LpxXL orthologues are present in *Agrobacterium tumefaciens*, *Brucella melitensis*, and all sequenced strains of *Rhizobium*, consistent with the occurrence of long secondary acyl chains in the lipid A molecules of these organisms.

Beaulieu, N., S. Morin, et al. (2002). "An Essential Role for DNA Methyltransferase DNMT3B in Cancer Cell Survival." *J. Biol. Chem.* **277**(31): 28176-28181.

<http://www.jbc.org/cgi/content/abstract/277/31/28176>

Abnormal methylation and associated silencing of tumor suppressor genes is a common feature of many types of cancers. The observation of persistent methylation in human cancer cells lacking the maintenance methyltransferase DNMT1 suggests the involvement of other DNA methyltransferases in gene silencing in cancer. To test this hypothesis, we have evaluated methylation and gene expression in cancer cells specifically depleted of DNMT3A or DNMT3B, de novo methyltransferases that are expressed in adult tissues. Here we have shown that depletion of DNMT3B, but not DNMT3A, induced apoptosis of human cancer cells but not normal cells. DNMT3B depletion reactivated methylation-silenced gene expression but did not induce global or juxtacentromeric satellite demethylation as did specific depletion of DNMT1. Furthermore, the effect of DNMT3B depletion was rescued by exogenous expression of either of the splice variants DNMT3B2 or DNMT3B3 but not DNMT1. These results indicate that DNMT3B has significant site selectivity that is distinct from DNMT1, regulates aberrant gene silencing, and is essential for cancer cell survival.

Beekman, J. M., J. E. Bakema, et al. (2004). "Modulation of Fc{gamma}RI (CD64) Ligand Binding by Blocking Peptides of Periplakin." *J. Biol. Chem.* **279**(32): 33875-33881.

<http://www.jbc.org/cgi/content/abstract/279/32/33875>

Fc{gamma}RI requires both the intracellular domain of the {alpha}-chain and associated leukocyte Fc receptor (FcR) {gamma}-chains for its biological function. We recently found the C terminus of periplakin to selectively interact with the cytoplasmic domain of the Fc{gamma}RI {alpha}-chain. It thereby enhances the capacity of Fc{gamma}RI to bind, internalize, and present antigens on MHC class II. Here, we characterized the domains involved in Fc{gamma}RI-periplakin interaction using truncated and alanine-substituted Fc{gamma}RI mutants and randomly mutagenized periplakin. This allowed us to design TAT peptides that selectively interfered with endogenous Fc{gamma}RI-periplakin interactions. The addition of these peptides to Fc{gamma}RI-expressing cells modulated Fc{gamma}RI ligand binding, as assessed by erythrocyte-antibody-rosetting. These data support a dominant-negative role of C-terminal periplakin for Fc{gamma}RI biological activity and implicate periplakin as a novel regulator of Fc{gamma}RI in immune cells.

Benezra, M., N. Chevallier, et al. (2003). "BRCA1 Augments Transcription by the NF- κ B Transcription Factor by Binding to the Rel Domain of the p65/RelA Subunit." *J. Biol. Chem.* **278**(29): 26333-26341.

<http://www.jbc.org/cgi/content/abstract/278/29/26333>

BRCA1 is a tumor suppressor gene mutated in cases of hereditary breast and ovarian cancer. BRCA1 protein is involved in apoptosis and growth/tumor suppression. In this study, we present evidence that p65/RelA, one of the two subunits of the transcription factor NF- κ B, binds to the BRCA1 protein. Treatment of 293T cells with the cytokine tumor necrosis factor- α induces an interaction between endogenous p65/RelA and BRCA1. GST-protein affinity assay experiments reveal that the Rel homology domain of the p65/RelA subunit of NF- κ B interacts with multiple sites within the N-terminal region of BRCA1. Transient transfection of BRCA1 significantly enhances the ability of the tumor necrosis factor- α or interleukin-1 β to activate transcription from the promoters of NF- κ B target genes. Mutation of the NF- κ B-binding sites in the NF- κ B reporter blocks the effect of BRCA1 on transcription. Also the ability of BRCA1 to activate NF- κ B target genes is inhibited by a super-stable inhibitor of NF- κ B and by the chemical inhibitor SN-50. These data indicate that BRCA1 acts as a co-activator with NF- κ B. In addition, we show that cells infected with an adenovirus expressing BRCA1 up-regulate the endogenous expression of NF- κ B target genes Fas and interferon- β . Together, this information suggests that BRCA1 may play a role in cell life-death decisions following cell stress by modulation of the activity of NF- κ B.

Benlloch, M., A. Ortega, et al. (2005). "Acceleration of Glutathione Efflux and Inhibition of γ -Glutamyltranspeptidase Sensitize Metastatic B16 Melanoma Cells to Endothelium-induced Cytotoxicity." *J. Biol. Chem.* **280**(8): 6950-6959.

<http://www.jbc.org/cgi/content/abstract/280/8/6950>

Highly metastatic B16 melanoma (B16M)-F10 cells, as compared with the low metastatic B16M-F1 line, have higher GSH content and preferentially overexpress BCL-2. In addition to its anti-apoptotic properties, BCL-2 inhibits efflux of GSH from B16M-F10 cells and thereby may facilitate metastatic cell resistance against endothelium-induced oxidative/nitrosative stress. Thus, we investigated in B16M-F10 cells which molecular mechanisms channel GSH release and whether their modulation may influence metastatic activity. GSH efflux was abolished in multidrug resistance protein 1 knock-out (MRP-/-1) B16M-F10 transfected with the Bcl-2 gene or in MRP-/-1 B16M-F10 cells incubated with L-methionine, which indicates that GSH release from B16M-F10 cells is channeled through MRP1 and a BCL-2-dependent system (likely related to an L-methionine-sensitive GSH carrier previously detected in hepatocytes). The BCL-2-dependent system was identified as the cystic fibrosis transmembrane conductance regulator, since monoclonal antibodies against this ion channel or H-89 (a protein kinase A-selective inhibitor)-induced inhibition of cystic fibrosis transmembrane conductance regulator gene expression completely blocked the BCL-2-sensitive GSH release. By using a perfusion system that mimics in vivo conditions, we found that GSH depletion in metastatic cells can be achieved by using Bcl-2 antisense oligodeoxynucleotide- and verapamil (an MRP1 activator)-induced acceleration of GSH efflux, in combination with acivicin-induced inhibition of γ -glutamyltranspeptidase (which limits GSH synthesis by preventing cysteine generation from extracellular GSH). When applied under in vivo conditions, this strategy increased tumor cytotoxicity (up to $\sim$ 90%) during B16M-F10 cell adhesion to the hepatic sinusoidal endothelium.

Bennin, D. A., A. S. A. Don, et al. (2002). "Cyclin G2 Associates with Protein Phosphatase 2A Catalytic and Regulatory B' Subunits in Active Complexes and Induces Nuclear Aberrations and a G1/S

Phase Cell Cycle Arrest." *J. Biol. Chem.* **277**(30): 27449-27467.

<http://www.jbc.org/cgi/content/abstract/277/30/27449>

Cyclin G2, together with cyclin G1 and cyclin I, defines a novel cyclin family expressed in terminally differentiated tissues including brain and muscle. Cyclin G2 expression is up-regulated as cells undergo cell cycle arrest or apoptosis in response to inhibitory stimuli independent of p53 (Horne, M., Donaldson, K., Goolsby, G., Tran, D., Mulheisen, M., Hell, J. and Wahl, A. (1997) *J. Biol. Chem.* **272**, 12650-12661). We tested the hypothesis that cyclin G2 may be a negative regulator of cell cycle progression and found that ectopic expression of cyclin G2 induces the formation of aberrant nuclei and cell cycle arrest in HEK293 and Chinese hamster ovary cells. Cyclin G2 is primarily partitioned to a detergent-resistant compartment, suggesting an association with cytoskeletal elements. We determined that cyclin G2 and its homolog cyclin G1 directly interact with the catalytic subunit of protein phosphatase 2A (PP2A). An okadaic acid-sensitive (<2 nM) phosphatase activity coprecipitates with endogenous and ectopic cyclin G2. We found that cyclin G2 also associates with various PP2A B' regulatory subunits, as previously shown for cyclin G1. The PP2A/A subunit is not detectable in cyclin G2-PP2A-B'-C complexes. Notably, cyclin G2 colocalizes with both PP2A/C and B' subunits in detergent-resistant cellular compartments, suggesting that these complexes form in living cells. The ability of cyclin G2 to inhibit cell cycle progression correlates with its ability to bind PP2A/B' and C subunits. Together, our findings suggest that cyclin G2-PP2A complexes inhibit cell cycle progression.

Besteiro, S., M. Biran, et al. (2002). "Succinate Secreted by *Trypanosoma brucei* Is Produced by a Novel and Unique Glycosomal Enzyme, NADH-dependent Fumarate Reductase." *J. Biol. Chem.* **277**(41): 38001-38012.

<http://www.jbc.org/cgi/content/abstract/277/41/38001>

In all trypanosomatids, including *Trypanosoma brucei*, glycolysis takes place in peroxisome-like organelles called glycosomes. These are closed compartments wherein the energy and redox (NAD⁺/NADH) balances need to be maintained. We have characterized a *T. brucei* gene called FRDg encoding a protein 35% identical to *Saccharomyces cerevisiae* fumarate reductases. Microsequencing of FRDg purified from glycosome preparations, immunofluorescence, and Western blot analyses clearly identified this enzyme as a glycosomal protein that is only expressed in the procyclic form of *T. brucei* but is present in all the other trypanosomatids studied, i.e. *Trypanosoma congolense*, *Crithidia fasciculata* and *Leishmania amazonensis*. The specific inactivation of FRDg gene expression by RNA interference showed that FRDg is responsible for the NADH-dependent fumarate reductase activity detected in glycosomal fractions and that at least 60% of the succinate secreted by the *T. brucei* procyclic form (in the presence of D-glucose as the sole carbon source) is produced in the glycosome by FRDg. We conclude that FRDg plays a key role in the energy metabolism by participating in the maintenance of the glycosomal NAD⁺/NADH balance. We have also detected a significant pyruvate kinase activity in the cytosol of the *T. brucei* procyclic cells that was not observed previously. Consequently, we propose a revised model of glucose metabolism in procyclic trypanosomes that may also be valid for all other trypanosomatids except the *T. brucei* bloodstream form. Interestingly, H. Gest has hypothesized previously (Gest, H. (1980) *FEMS Microbiol. Lett.* **7**, 73-77) that a soluble NADH-dependent fumarate reductase has been present in primitive organisms and evolved into the present day fumarate reductases, which are quinol-dependent. FRDg may have the characteristics of such an ancestral enzyme and is the only NADH-dependent fumarate reductase characterized to date.

Bierl, C., B. Voetsch, et al. (2004). "Determinants of Human Plasma Glutathione Peroxidase (GPx-3)

Expression." *J. Biol. Chem.* **279**(26): 26839-26845.

<http://www.jbc.org/cgi/content/abstract/279/26/26839>

Plasma glutathione peroxidase (GPx-3) is a selenocysteine-containing protein with antioxidant properties. GPx-3 deficiency has been associated with cardiovascular disease and stroke. The regulation of GPx-3 expression remains largely uncharacterized, however, and we studied its transcriptional and translational determinants in a cultured cell system. In transient transfections of a renal cell line (Caki-2), the published sequence cloned upstream of a luciferase reporter gene produced minimal activity (relative luminescence (RL) = 0.6 {+/-} 0.4). Rapid amplification of cDNA ends was used to identify a novel transcription start site that is located 233 bp downstream (3') of the published site and that produced a >25-fold increase in transcriptional activity (RL = 16.8 {+/-} 1.9; $p < 0.0001$). Analysis of the novel GPx-3 promoter identified Sp-1- and hypoxia-inducible factor-1-binding sites, as well as the redox-sensitive metal response element and antioxidant response element. Hypoxia was identified as a strong transcriptional regulator of GPx-3 expression, in part through the presence of the hypoxia-inducible factor-1-binding site, leading to an almost 3-fold increase in expression levels after 24 h compared with normoxic conditions (normalized RL = 3.5 {+/-} 0.3 versus 1.2 {+/-} 0.1; $p < 0.001$). We also investigated the role of the translational cofactors tRNA^{Sec}, SECIS-binding protein-2, and SelD (selenophosphate synthetase D) in GPx-3 protein expression. tRNA^{Sec} and SelD significantly enhanced GPx-3 expression, whereas SECIS-binding protein-2 showed a trend toward increased expression. These results demonstrate the presence of a novel functional transcription start site for the human GPx-3 gene with a promoter regulated by hypoxia, and identify unique translational determinants of GPx-3 expression.

Billich, A., F. Bornancin, et al. (2003). "Phosphorylation of the Immunomodulatory Drug FTY720 by Sphingosine Kinases." *J. Biol. Chem.* **278**(48): 47408-47415.

<http://www.jbc.org/cgi/content/abstract/278/48/47408>

The immunomodulatory drug FTY720 is phosphorylated in vivo, and the resulting FTY720 phosphate as a ligand for sphingosine-1-phosphate receptors is responsible for the unique biological effects of the compound. So far, phosphorylation of FTY720 by murine sphingosine kinase (SPHK) 1a had been documented. We found that, while FTY720 is also phosphorylated by human SPHK1, the human type 2 isoform phosphorylates the drug 30-fold more efficiently, because of a lower K_m of FTY720 for SPHK2. Similarly, murine SPHK2 was more efficient than SPHK1a. Among splice variants of the human SPHKs, an N-terminally extended SPHK2 isoform was even more active than SPHK2 itself. Further SPHK superfamily members, namely ceramide kinase and a "SPHK-like" protein, failed to phosphorylate sphingosine and FTY720. Thus, only SPHK1 and 2 appear to be capable of phosphorylating FTY720. Using selective assay conditions, SPHK1 and 2 activities in murine tissues were measured. While activity of SPHK2 toward sphingosine was generally lower than of SPHK1, FTY720 phosphorylation was higher under conditions favoring SPHK2. In human endothelial cells, while activity of SPHK1 toward sphingosine was 2-fold higher than of SPHK2, FTY720 phosphorylation was 7-fold faster under SPHK2 assay conditions. Finally, FTY720 was poorly phosphorylated in human blood as compared with rodent blood, in line with the low activity of SPHK1 and in particular of SPHK2 in human blood. To conclude, both SPHK1 and 2 are capable of phosphorylating FTY720, but SPHK2 is quantitatively more important than SPHK1.

Boraston, A. B., E. Kwan, et al. (2003). "Recognition and Hydrolysis of Noncrystalline Cellulose." *J. Biol. Chem.* **278**(8): 6120-6127.

<http://www.jbc.org/cgi/content/abstract/278/8/6120>

Cellulase Cel5A from alkalophilic *Bacillus* sp. 1139 contains a family 17 carbohydrate-binding module (BspCBM17) and a family 28 CBM (BspCBM28) in tandem. The two modules have significantly similar amino acid sequences, but amino acid residues essential for binding are not conserved. BspCBM28 was obtained as a discrete polypeptide by engineering the *cel5A* gene. BspCBM17 could not be obtained as a discrete polypeptide, so a family 17 CBM from endoglucanase Cel5A of *Clostridium cellulovorans*, CcCBM17, was used to compare the binding characteristics of the two families of CBM. Both CcCBM17 and BspCBM28 recognized two classes of binding sites on amorphous cellulose: a high affinity site ($K_a \sim 1 \times 10^6 \text{ M}^{-1}$) and a low affinity site ($K_a \sim 2 \times 10^4 \text{ M}^{-1}$). They did not compete for binding to the high affinity sites, suggesting that they bound at different sites on the cellulose. A polypeptide, BspCBM17/CBM28, comprising the tandem CBMs from Cel5A, bound to amorphous cellulose with a significantly higher affinity than the sum of the affinities of CcCBM17 and BspCBM28, indicating cooperativity between the linked CBMs. Cel5A mutants were constructed that were defective in one or both of the CBMs. The mutants differed from the wild-type enzyme in the amounts and sizes of the soluble products produced from amorphous cellulose. This suggests that either the CBMs can modify the action of the catalytic module of Cel5A or that they target the enzyme to areas of the cellulose that differ in susceptibility to hydrolysis.

Bozinovski, S., J. E. Jones, et al. (2002). "Granulocyte/Macrophage-Colony-stimulating Factor (GM-CSF) Regulates Lung Innate Immunity to Lipopolysaccharide through Akt/Erk Activation of NFkappa B and AP-1 in Vivo." *J. Biol. Chem.* **277**(45): 42808-42814.

<http://www.jbc.org/cgi/content/abstract/277/45/42808>

The lung innate immune response to lipopolysaccharide (LPS) coordinates cellular inflammation, mediator, and protease release essential for host defense but deleterious in asthma, chronic obstructive pulmonary disease, and cystic fibrosis. In vitro, LPS signals to the transcription factors NF[kappa]B via TLR4, MyD88, and IL-1R-associated kinase (IRAK), to AP-1 by mitogen-activated protein (MAP) kinases, and via an alternate route in IRAK-deficient mice, but the in vivo lung signaling pathway(s) are not understood. We investigated the role of Akt and Erk1/2 as LPS intensely stimulates granulocyte/macrophage-colony-stimulating factor (GM-CSF) release, and neutralizing GM-CSF profoundly suppressed LPS-induced inflammation, suppressed expression and activity of lung proteases, significantly reduced GM-CSF and tumor necrosis factor [alpha] (TNF[alpha]) mRNA expression, and dampened nuclear localization of both NF[kappa]B (p50/65) and AP-1. LPS markedly activated Akt and Erk1/2, but not p38, in a GM-CSF-dependent manner in direct temporal association with NF[kappa]B and AP-1 activation. Pharmacological inhibition of Akt or Erk activation in LPS-treated tracheal explants ex vivo inhibited the release of GM-CSF. These data implicate GM-CSF-dependent activation of Akt in the amplification of this response and demonstrate the role of Erks rather than p38 in lung LPS inflammatory responses. Inhibition of GM-CSF may be of therapeutic benefit in inflammatory diseases in which LPS contributes to lung damage.

Bream, J. H., D. L. Hodge, et al. (2004). "A Distal Region in the Interferon- γ Gene Is a Site of Epigenetic Remodeling and Transcriptional Regulation by Interleukin-2." *J. Biol. Chem.* **279**(39): 41249-41257.

<http://www.jbc.org/cgi/content/abstract/279/39/41249>

Interferon- γ (IFN- γ) is a multifunctional cytokine that defines the development of

Th1 cells and is critical for host defense against intracellular pathogens. IL-2 is another key immunoregulatory cytokine that is involved in T helper differentiation and is known to induce IFN- γ expression in natural killer (NK) and T cells. Despite concerted efforts to identify the one or more transcriptional control mechanisms by which IL-2 induces IFN- γ mRNA expression, no such genomic regulatory regions have been described. We have identified a DNase I hypersensitivity site [~]3.5-4.0 kb upstream of the transcriptional start site. Using chromatin immunoprecipitation assays we found constitutive histone H3 acetylation in this distal region in primary human NK cells, which is enhanced by IL-2 treatment. This distal region is also preferentially acetylated on histones H3 and H4 in primary Th1 cells as compared with Th2 cells. Within this distal region we found a Stat5-like motif, and in vitro DNA binding assays as well as in vivo chromosomal immunoprecipitation assays showed IL-2-induced binding of both Stat5a and Stat5b to this distal element in the IFNG gene. We examined the function of this Stat5-binding motif by transfecting human peripheral blood mononuclear cells with -3.6 kb of IFNG-luciferase constructs and found that phorbol 12-myristate 13-acetate/ionomycin-induced transcription was augmented by IL-2 treatment. The effect of IL-2 was lost when the Stat5 motif was disrupted. These data led us to conclude that this distal region serves as both a target of chromatin remodeling in the IFNG locus as well as an IL-2-induced transcriptional enhancer that binds Stat5 proteins.

Brown, A. J., S. M. Goldsworthy, et al. (2003). "The Orphan G Protein-coupled Receptors GPR41 and GPR43 Are Activated by Propionate and Other Short Chain Carboxylic Acids." J. Biol. Chem. **278**(13): 11312-11319.

<http://www.jbc.org/cgi/content/abstract/278/13/11312>

GPR41 and GPR43 are related members of a homologous family of orphan G protein-coupled receptors that are tandemly encoded at a single chromosomal locus in both humans and mice. We identified the acetate anion as an agonist of human GPR43 during routine ligand bank screening in yeast. This activity was confirmed after transient transfection of GPR43 into mammalian cells using Ca^{2+} mobilization and [^{35}S]guanosine 5'-O-(3-thiotriphosphate) binding assays and by coexpression with GIRK G protein-regulated potassium channels in *Xenopus laevis* oocytes. Other short chain carboxylic acid anions such as formate, propionate, butyrate, and pentanoate also had agonist activity. GPR41 is related to GPR43 (52% similarity; 43% identity) and was activated by similar ligands but with differing specificity for carbon chain length, with pentanoate being the most potent agonist. A third family member, GPR42, is most likely a recent gene duplication of GPR41 and may be a pseudogene. GPR41 was expressed primarily in adipose tissue, whereas the highest levels of GPR43 were found in immune cells. The identity of the cognate physiological ligands for these receptors is not clear, although propionate is known to occur in vivo at high concentrations under certain pathophysiological conditions.

Budagian, V., E. Bulanova, et al. (2003). "Signaling through P2X7 Receptor in Human T Cells Involves p56lck, MAP Kinases, and Transcription Factors AP-1 and NF-kappa B." J. Biol. Chem. **278**(3): 1549-1560.

<http://www.jbc.org/cgi/content/abstract/278/3/1549>

ATP-gated ion channel P2X receptors are expressed on the surface of most immune cells and can trigger multiple cellular responses, such as membrane permeabilization, cytokine production, and cell proliferation or apoptosis. Despite broad distribution and pleiotropic activities, signaling pathways downstream of these ionotropic receptors are still poorly understood. Here, we describe intracellular signaling events in Jurkat cells treated with millimolar concentrations of extracellular ATP. Within minutes, ATP treatment resulted in the phosphorylation and activation of p56lck

kinase, extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase but not p38 kinase. These effects were wholly dependent upon the presence of extracellular Ca^{2+} ions in the culture medium. Nevertheless, calmodulin antagonist calmidazolium and CaM kinase inhibitor KN-93 both had no effect on the activation of p56lck and ERK, whereas a pretreatment of Jurkat cells with MAP kinase kinase inhibitor P098059 was able to abrogate phosphorylation of ERK. Further, expression of c-Jun and c-Fos proteins and activator protein (AP-1) DNA binding activity were enhanced in a time-dependent manner. In contrast, DNA binding activity of NF-[kappa]B was reduced. ATP failed to stimulate the phosphorylation of ERK and c-Jun N-terminal kinase and activation of AP-1 in the p56lck-deficient isogenic T cell line JCaM1, suggesting a critical role for p56lck kinase in downstream signaling. Regarding the biological significance of the ATP-induced signaling events we show that although extracellular ATP was able to stimulate proliferation of both Jurkat and JCaM1 cells, an increase in interleukin-2 transcription was observed only in Jurkat cells. The nucleotide selectivity and pharmacological profile data supported the evidence that the ATP-induced effects in Jurkat cells were mediated through the P2X7 receptor. Taken together, these results demonstrate the ability of extracellular ATP to activate multiple downstream signaling events in a human T-lymphoblastoid cell line.

Cabral, A., D. F. Fischer, et al. (2003). "Distinct Functional Interactions of Human Skn-1 Isoforms with Ese-1 during Keratinocyte Terminal Differentiation." *J. Biol. Chem.* **278**(20): 17792-17799.

<http://www.jbc.org/cgi/content/abstract/278/20/17792>

Among the three major POU proteins expressed in human skin, Oct-1, Tst-1/Oct-6, and Skn-1/Oct-11, only the latter induced SPRR2A, a marker of keratinocyte terminal differentiation. In this study, we have identified three Skn-1 isoforms, which encode proteins with various N termini, generated by alternative promoter usage. These isotypes showed distinct expression patterns in various skin samples, internal squamous epithelia, and cultured human keratinocytes. Skn-1a and Skn-1d1 bound the SPRR2A octamer site with comparable affinity and functioned as transcriptional activators. Skn-1d2 did not affect SPRR2A expression. Skn-1a, the largest protein, functionally cooperated with Ese-1/Elf-3, an epithelial-specific transcription factor, previously implicated in SPRR2A induction. This cooperativity, which depended on an N-terminal pointed-like domain in Skn-1a, was not found for Skn-1d1. Actually, Skn-1d1 counteracted the cooperativity between Skn-1a and Ese-1. Apparently, the human Skn-1 locus encodes multifunctional protein isotypes, subjected to biochemical cross-talk, which are likely to play a major role in the fine-tuning of keratinocyte terminal differentiation.

Cabral, W. A., A. Fertala, et al. (2002). "Procollagen with Skipping of alpha 1(I) Exon 41 Has Lower Binding Affinity for alpha 1(I) C-telopeptide, Impaired in Vitro Fibrillogenesis, and Altered Fibril Morphology." *J. Biol. Chem.* **277**(6): 4215-4222.

<http://www.jbc.org/cgi/content/abstract/277/6/4215>

Previous in vitro data on type I collagen self-assembly into fibrils suggested that the amino acid 776-796 region of the [alpha]1(I) chain is crucial for fibril formation because it serves as the recognition site for the telopeptide of a docking collagen monomer. We used a natural collagen mutation with a deletion of amino acids 766-801 to confirm the importance of this region for collagen fibril formation. The proband has type III osteogenesis imperfecta and is heterozygous for a COL1A1 IVS 41 A+4 [right-arrow] C substitution. The intronic mutation causes splicing of exon 41, confirmed by sequencing of normal and shorter reverse transcriptase-PCR products. Reverse transcriptase-PCR using RNA from proband dermal fibroblasts and clonal cell lines showed the mutant cDNA was about 15% of total [alpha]1(I) cDNA. The mutant transcript is translated; structurally abnormal [alpha] chains are demonstrated in the cell layer of proband

fibroblasts by SDS-urea-PAGE. The proportion of mutant chains in the secreted procollagen was determined to be 10% by resistance to digestion with MMP-1, since chains lacking exon 41 are missing the vertebral collagenase cleavage site. Secreted proband collagen was used for analysis of kinetics of binding of $[\alpha]1(I)$ C-telopeptide using an optical biosensor. Telopeptide had slower association and faster dissociation from proband than from normal collagen. Purified proband pC-collagen was used to study fibril formation. The presence of the mutant molecules decreases the rate of fibril formation. The fibrils formed in the presence of 10-15% mutant molecules have strikingly increased length compared with normal collagen, but are well organized, as demonstrated by D-periodicity. These results suggest that some collagen molecules containing the mutant chain are incorporated into fibrils and that the absence of the telopeptide binding region from even a small portion of the monomers interferes with fibril growth. Both abnormal fibrils and slower remodeling may contribute to the severe phenotype.

Cabral, W. A., E. Makareeva, et al. (2005). "Mutations near amino end of $\alpha 1(I)$ collagen cause combined OI/EDS by interference with N-propeptide processing." *J. Biol. Chem.*: M414698200.

<http://www.jbc.org/cgi/content/abstract/M414698200v1>

Patients with OI/EDS form a distinct subset of osteogenesis imperfecta patients. In addition to skeletal fragility, they have characteristics of Ehlers-Danlos syndrome. We identified 7 children with types III or IV OI, plus severe large and small joint laxity and early progressive scoliosis. In each child with OI/EDS, we identified a mutation in the first 90 residues of the helical region of $[\alpha]1(I)$ collagen. These mutations prevent or delay removal of the procollagen N-propeptide by purified N-proteinase (ADAMTS-2) in vitro and in pericellular assays. The mutant pN-collagen which results is efficiently incorporated into matrix by cultured fibroblasts and osteoblasts and is prominently present in newly incorporated and immaturely crosslinked collagen. Dermal collagen fibrils have significantly reduced cross-sectional diameters, corroborating incorporation of pN-collagen into fibrils in vivo. Differential scanning calorimetry revealed that these mutant collagens are less stable than the corresponding procollagens, which is not seen with other type I collagen helical mutations. These mutations disrupt a distinct folding region of high thermal stability in the first 90 residues at the amino end of type I collagen and alter the secondary structure of the adjacent N-proteinase cleavage site. Thus, these OI/EDS collagen mutations are directly responsible for the bone fragility of OI and indirectly responsible for EDS symptoms, by interference with N-propeptide removal.

Cabral, W. A., M. V. Merts, et al. (2003). "Type I Collagen Triplet Duplication Mutation in Lethal Osteogenesis Imperfecta Shifts Register of α Chains throughout the Helix and Disrupts Incorporation of Mutant Helices into Fibrils and Extracellular Matrix." *J. Biol. Chem.* **278**(12): 10006-10012.

<http://www.jbc.org/cgi/content/abstract/278/12/10006>

The majority of collagen mutations causing osteogenesis imperfecta (OI) are glycine substitutions that disrupt formation of the triple helix. A rare type of collagen mutation consists of a duplication or deletion of one or two Gly-X-Y triplets. These mutations shift the register of collagen chains with respect to each other in the helix but do not interrupt the triplet sequence, yet they have severe clinical consequences. We investigated the effect of shifting the register of the collagen helix by a single Gly-X-Y triplet on collagen assembly, stability, and incorporation into fibrils and matrix. These studies utilized a triplet duplication in COL1A1 exon 44 that occurred in the cDNA and gDNA of two siblings with lethal OI. The normal allele encodes three identical Gly-Ala-Hyp triplets at aa 868-876, whereas the mutant allele encodes four. The register shift delays helix formation, causing overmodification. Differential scanning calorimetry yielded a decrease in T_m of

2 {degrees}C for helices with one mutant chain and a 6 {degrees}C decrease in helices with two mutant chains. An in vitro binary co-processing assay of N-proteinase cleavage demonstrated that procollagen with the triplet duplication has slower N-propeptide cleavage than in normal controls or procollagen with pro[alpha]1(I) G832S, G898S, or G997S substitutions, showing that the register shift persists through the entire helix. The register shift disrupts incorporation of mutant collagen into fibrils and matrix. Proband fibrils formed inefficiently in vitro and contained only normal helices and helices with a single mutant chain. Helices with two mutant chains and a significant portion of helices with one mutant chain did not form fibrils. In matrix deposited by proband fibroblasts, mutant chains were abundant in the immaturely cross-linked fraction but constituted a minor fraction of maturely cross-linked chains. The profound effects of shifting the collagen triplet register on chain interactions in the helix and on fibril formation correlate with the severe clinical consequences.

Carrillo, C. D., E. Taboada, et al. (2004). "Genome-wide Expression Analyses of *Campylobacter jejuni* NCTC11168 Reveals Coordinate Regulation of Motility and Virulence by *flhA*." J. Biol. Chem. **279**(19): 20327-20338.

<http://www.jbc.org/cgi/content/abstract/279/19/20327>

We examined two variants of the genome-sequenced strain, *Campylobacter jejuni* NCTC11168, which show marked differences in their virulence properties including colonization of poultry, invasion of Caco-2 cells, and motility. Transcript profiles obtained from whole genome DNA microarrays and proteome analyses demonstrated that these differences are reflected in late flagellar structural components and in virulence factors including those involved in flagellar glycosylation and cytolethal distending toxin production. We identified putative {sigma}28 and {sigma}54 promoters for many of the affected genes and found that greater differences in expression were observed for {sigma}28-controlled genes. Inactivation of the gene encoding {sigma}28, *flhA*, resulted in an unexpected increase in transcripts with {sigma}54 promoters, as well as decreased transcription of {sigma}28-regulated genes. This was unlike the transcription profile observed for the attenuated *C. jejuni* variant, suggesting that the reduced virulence of this organism was not entirely due to impaired function of {sigma}28. However, inactivation of *flhA*, an important component of the flagellar export apparatus, resulted in expression patterns similar to that of the attenuated variant. These findings indicate that the flagellar regulatory system plays an important role in campylobacter pathogenesis and that *flhA* is a key element involved in the coordinate regulation of late flagellar genes and of virulence factors in *C. jejuni*.

Cecchetto, G., S. Amillis, et al. (2004). "The AzgA Purine Transporter of *Aspergillus nidulans*: CHARACTERIZATION OF A PROTEIN BELONGING TO A NEW PHYLOGENETIC CLUSTER." J. Biol. Chem. **279**(5): 3132-3141.

<http://www.jbc.org/cgi/content/abstract/279/5/3132>

The *azgA* gene of *Aspergillus nidulans* encodes a hypoxanthine-adenine-guanine transporter. It has been cloned by a novel transposon methodology. The null phenotype of *azgA* was defined by a number of mutations, including a large deletion. In mycelia, the *azgA* gene is, like other genes of purine catabolism, induced by uric acid and repressed by ammonium. Its transcription depends on the pathway-specific UaY zinc binuclear cluster protein and the broad domain AreA GATA factor. AzgA is not closely related to any other characterized membrane protein, but many close homologues of unknown function are present in fungi, plants, and prokaryotes but not metazoa. Two of three data bases and the phylogeny presented in this article places proteins of this family in a cluster clearly separated (but perhaps phylogenetically related) from the NAT family that includes other eukaryotic and prokaryotic nucleobase transporters. Thus AzgA is the first

characterized member of this family or subfamily of membrane proteins.

Chang, W.-T. and A. M. Huang (2004). "{alpha}-Pal/NRF-1 Regulates the Promoter of the Human Integrin-associated Protein/CD47 Gene." J. Biol. Chem. **279**(15): 14542-14550.

<http://www.jbc.org/cgi/content/abstract/279/15/14542>

Integrin-associated protein (IAP or CD47) is expressed in a variety of tissues, including the nervous system and immune system. To understand how cells control the expression of the IAP gene, we cloned the 5'-proximal region of the human IAP gene and investigated IAP promoter activity by transient transfection. RT-PCR confirmed the expression of IAP transcripts in human neuroblastoma IMR-32 and hepatoma HepG2 cells. Deletion analysis identified a core promoter of the human IAP gene located between nucleotide positions -232 and -12 relative to the translation initiation codon in these two cell lines. Site-directed mutagenesis and gel electrophoretic mobility shift assay identified a {alpha}-Pal/NRF-1 binding element within the IAP core promoter. Supershift assays using the {alpha}-Pal/NRF-1 antiserum confirmed the binding of this transcription factor on the {alpha}-Pal/NRF-1 site. Overexpression of the DNA binding domain of {alpha}-Pal/NRF-1 in cells enhanced DNA-{alpha}-Pal/NRF-1 binding in vitro. Furthermore, overexpression of full-length {alpha}-Pal/NRF-1 significantly enhanced IAP promoter activity while overexpression of dominant-negative mutant reduced promoter activity both in the cultured human cell lines and primary mouse cortical cells. These results revealed that {alpha}-Pal/NRF-1 is an essential transcription factor in the regulation of human IAP gene expression.

Cheng, D., T. C. Nelson, et al. (2003). "Identification of Acyl Coenzyme A:Monoacylglycerol Acyltransferase 3, an Intestinal Specific Enzyme Implicated in Dietary Fat Absorption." J. Biol. Chem. **278**(16): 13611-13614.

<http://www.jbc.org/cgi/content/abstract/278/16/13611>

Acyl coenzyme A:monoacylglycerol acyltransferase (MGAT) catalyzes the synthesis of diacylglycerol using 2-monoacylglycerol and fatty acyl coenzyme A. This enzymatic reaction is believed to be an essential and rate-limiting step for the absorption of fat in the small intestine. Although the first MGAT-encoding cDNA, designated MGAT1, has been recently isolated, it is not expressed in the small intestine and hence cannot account for the high intestinal MGAT enzyme activity that is important for the physiology of fat absorption. In the current study, we report the identification of a novel MGAT, designated MGAT3, and present evidence that it fulfills the criteria to be the elusive intestinal MGAT. MGAT3 encodes a ~36-kDa transmembrane protein that is highly homologous to MGAT1 and -2. In humans, expression of MGAT3 is restricted to gastrointestinal tract with the highest level found in the ileum. At the cellular level, recombinant MGAT3 is localized to the endoplasmic reticulum. Recombinant MGAT3 enzyme activity produced in insect Sf9 cells selectively acylates 2-monoacylglycerol with higher efficiency than other stereoisomers. The molecular identification of MGAT3 will facilitate the evaluation of using intestinal MGAT as a potential point of intervention for antiobesity therapies.

Cheng, S., H. Afif, et al. (2004). "Activation of Peroxisome Proliferator-activated Receptor {gamma} Inhibits Interleukin-1{beta}-induced Membrane-associated Prostaglandin E2 Synthase-1 Expression in Human Synovial Fibroblasts by Interfering with Egr-1." J. Biol. Chem. **279**(21): 22057-22065.

<http://www.jbc.org/cgi/content/abstract/279/21/22057>

Membrane-associated prostaglandin (PG) E2 synthase-1 (mPGES-1) catalyzes the conversion of PGH2 to PGE2, which contributes to many biological processes. Peroxisome proliferator-activated receptor γ (PPAR γ) is a ligand-activated transcription factor and plays an important role in growth, differentiation, and inflammation in different tissues. Here, we examined the effect of PPAR γ ligands on interleukin-1 β (IL-1 β)-induced mPGES-1 expression in human synovial fibroblasts. PPAR γ ligands 15-deoxy- $\Delta^{12,14}$ prostaglandin J2 (15d-PGJ2) and the thiazolidinedione troglitazone (TRO), but not PPAR α ligand Wy14643, dose-dependently suppressed IL-1 β -induced PGE2 production, as well as mPGES-1 protein and mRNA expression. 15d-PGJ2 and TRO suppressed IL-1 β -induced activation of the mPGES-1 promoter. Overexpression of wild-type PPAR γ further enhanced, whereas overexpression of a dominant negative PPAR γ alleviated, the suppressive effect of both PPAR γ ligands. Furthermore, pretreatment with an antagonist of PPAR γ , GW9662, relieves the suppressive effect of PPAR γ ligands on mPGES-1 protein expression, suggesting that the inhibition of mPGES-1 expression is mediated by PPAR γ . We demonstrated that PPAR γ ligands suppressed Egr-1-mediated induction of the activities of the mPGES-1 promoter and of a synthetic reporter construct containing three tandem repeats of an Egr-1 binding site. The suppressive effect of PPAR γ ligands was enhanced in the presence of a PPAR γ expression plasmid. Electrophoretic mobility shift and supershift assays for Egr-1 binding sites in the mPGES-1 promoter showed that both 15d-PGJ2 and TRO suppressed IL-1 β -induced DNA-binding activity of Egr-1. These data define mPGES-1 and Egr-1 as novel targets of PPAR γ and suggest that inhibition of mPGES-1 gene transcription may be one of the mechanisms by which PPAR γ regulates inflammatory responses.

Chiang, M.-C., Y.-C. Lee, et al. (2005). "cAMP-response Element-binding Protein Contributes to Suppression of the A2A Adenosine Receptor Promoter by Mutant Huntingtin with Expanded Polyglutamine Residues." *J. Biol. Chem.* **280**(14): 14331-14340.

<http://www.jbc.org/cgi/content/abstract/280/14/14331>

Huntington's disease is a neurodegenerative disease resulting from a CAG (glutamine) trinucleotide expansion in exon 1 of the Huntingtin (Htt) gene. The role of the striatum-enriched A2A adenosine receptor (A2A-R) in Huntington's disease has attracted much attention lately. In the present study, we found that expression of mutant Htt with expanded poly(Q) significantly reduced the transcript levels of the endogenous A2A-R in PC12 cells and primary striatal neurons. Cotransfection of various promoter constructs of the A2A-R gene and an expression construct of poly(Q)-expanded Htt revealed that the Htt mutant suppressed the core promoter activity of the A2A-R gene. Stimulation of the A2A-R using CGS21680 forskolin, and a constitutively active cAMP-response element-binding protein (CREB) mutant elevated the reduced promoter activity of the A2A-R gene by mutant Htt. Moreover, the effect of CGS was blocked by an A2A-R-selective antagonist (CSC), two inhibitors of protein kinase A, and two dominant negative mutants of (CREB). The protein kinase A/CREB pathway therefore is involved in regulating A2A-R promoter activity. Consistently, an atypical CRE site (TCCAGG) is located in the core promoter region of the A2A-R gene. Electrophoretic gel mobility shift assay and mutational inactivation further demonstrated the functional binding of CREB to the core promoter region and showed that expression of poly(Q)-expanded Htt abolished the binding of CREB to this site. Stimulation of the A2A-R restored the reduced CREB binding caused by the mutant and concurrently reduced mutant Htt aggregation. Collectively, the poly(Q)-expanded mutant Htt suppressed expression of the A2A-R by inhibiting its core promoter at least partially by preventing CREB binding.

Chipuk, J. E., L. V. Stewart, et al. (2002). "Identification and Characterization of A Novel Rat Ov-Serpin Family Member, Trespin." *J. Biol. Chem.* **277**(29): 26412-26421.

<http://www.jbc.org/cgi/content/abstract/277/29/26412>

Serpins are responsible for regulating a variety of proteolytic processes through a unique irreversible suicide substrate mechanism. To discover novel genes regulated by transforming growth factor- β 1 (TGF- β 1), we performed differential display reverse transcriptase-PCR analysis of NRP-152 rat prostatic epithelial cells and cloned a novel rat serpin that is transcriptionally down-regulated by TGF- β and hence named trespin (TGF- β -repressible serine proteinase inhibitor (trespin). Trespin is a 397-amino acid member of the ov-serpin clade with a calculated molecular mass of 45.2 kDa and 72% amino acid sequence homology to human bomapin; however, trespin exhibits different tissue expression, cellular localization, and proteinase specificity compared with bomapin. Trespin mRNA is expressed in many tissues, including brain, heart, kidney, liver, lung, prostate, skin, spleen, and stomach. FLAG-trespin expressed in HEK293 cells is localized predominantly in the cytoplasm and is not constitutively secreted. The presence of an arginine at the P1 position of trespin's reactive site loop suggests that trespin inhibits trypsin-like proteinases. Accordingly, in vitro transcribed and translated trespin forms detergent-stable and thermostable complexes with plasmin and elastase but not subtilisin A, trypsin, chymotrypsin, thrombin, or papain. Trespin interacts with plasmin at a near 1:1 stoichiometry, and immunopurified mammal-expressed trespin inhibits plasmin in a dose-dependent manner. These data suggest that trespin is a novel and functional member of the rat ov-serpin family.

Collin, M. and V. A. Fischetti (2004). "A Novel Secreted Endoglycosidase from *Enterococcus faecalis* with Activity on Human Immunoglobulin G and Ribonuclease B." *J. Biol. Chem.* **279**(21): 22558-22570.

<http://www.jbc.org/cgi/content/abstract/279/21/22558>

The human pathogen *Enterococcus faecalis* can degrade the N-linked glycans of human RNase B to acquire nutrients, but no gene or protein has been associated with this activity. We identified an 88-kDa secreted protein, endoglycosidase (Endo) E, which is most likely responsible for this activity. EndoE, encoded by *ndoE*, consists of an α -domain with a family 18 glycosyl hydrolase motif and a β -domain similar to family 20 glycosyl hydrolases. Phylogenetic analysis of EndoE indicates that the α -domain is related to human chitinases, and the β -domain is related to bacterial and human hexosaminidases. Recombinant expression of full-length EndoE or EndoE α , site-directed mutagenesis of the catalytic residues, mass spectroscopy, and homology modeling shows that EndoE α hydrolyzes the glycan on human RNase B, whereas EndoE β hydrolyzes the conserved glycan on IgG. Denaturation experiments indicate that the chitinase activity on RNase B is not dependent on the tertiary structure, although it is on IgG. The *ndoE* gene and secreted EndoE are present in most *E. faecalis* but not in *Enterococcus faecium* isolates. Correspondingly, *E. faecalis*, but not *E. faecium*, degrades the glycan on RNase B during growth. Thus, we have identified a secreted enzyme from *E. faecalis*, EndoE, which by two distinct activities hydrolyzes the glycans on RNase B and IgG. Both activities could be important for the molecular pathogenesis and persistence of *E. faecalis* during human infections.

Dai, E., H. Guan, et al. (2003). "Serp-1, a Viral Anti-inflammatory Serpin, Regulates Cellular Serine Proteinase and Serpin Responses to Vascular Injury." *J. Biol. Chem.* **278**(20): 18563-18572.

<http://www.jbc.org/cgi/content/abstract/278/20/18563>

Complex DNA viruses have tapped into cellular serpin responses that act as key regulatory steps in coagulation and inflammatory cascades. Serp-1 is one such viral serpin that effectively protects virus-infected tissues from host inflammatory responses. When given as purified protein, Serp-1 markedly inhibits vascular monocyte invasion and plaque growth in animal models. We have investigated mechanisms of viral serpin inhibition of vascular inflammatory responses. In vascular injury models, Serp-1 altered early cellular plasminogen activator (tissue plasminogen activator), inhibitor (PAI-1), and receptor (urokinase-type plasminogen activator) expression ($p < 0.01$). Serp-1, but not a reactive center loop mutant, up-regulated PAI-1 serpin expression in human endothelial cells. Treatment of endothelial cells with antibody to urokinase-type plasminogen activator and vitronectin blocked Serp-1-induced changes. Significantly, Serp-1 blocked intimal hyperplasia ($p < 0.0001$) after aortic allograft transplant ($p < 0.0001$) in PAI-1-deficient mice. Serp-1 also blocked plaque growth after aortic isograft transplant and after wire-induced injury ($p < 0.05$) in PAI-1-deficient mice indicating that increase in PAI-1 expression is not required for Serp-1 to block vasculopathy development. Serp-1 did not inhibit plaque growth in uPAR-deficient mice after aortic allograft transplant. We conclude that the poxviral serpin, Serp-1, attenuates vascular inflammatory responses to injury through a pathway mediated by native uPA receptors and vitronectin.

Danielli, A., F. C. Kafatos, et al. (2003). "Cloning and Characterization of Four *Anopheles gambiae* Serpin Isoforms, Differentially Induced in the Midgut by *Plasmodium berghei* Invasion." *J. Biol. Chem.* **278**(6): 4184-4193.

<http://www.jbc.org/cgi/content/abstract/278/6/4184>

The genomic locus SRPN10 of the malaria vector *Anopheles gambiae* codes for four alternatively spliced serine protease inhibitors of the serpin superfamily. The four 40- to 42-kDa isoforms differ only at their C terminus, which bears the reactive site loop, and exhibit protein sequence similarity with other insect serpins and mammalian serpins of the ovalbumin family. Inhibition experiments with recombinant purified SRPN10 serpins reveal distinct and specific inhibitory activity of three isoforms toward different proteases. All isoforms are mainly expressed in the midgut but also in pericardial cells and hemocytes of the mosquito. The cellular localization of SRPN10 serpins is nucleocytoplasmic in pericardial cells, in hemocytes and in a hemocyte-like mosquito cell line, but in the gut the proteins are mostly localized in the nucleus. Although the transcript levels of all SRPN10 isoforms are marginally affected by bacterial challenge, the transcripts of two isoforms (KRAL and RCM) are induced in female mosquitoes in response to midgut invasion by *Plasmodium berghei* ookinetes. The KRAL and RCM SRPN10 isoforms represent new potential markers to study the ookinete midgut invasion process in anopheline mosquitoes.

Dave, V., T. Childs, et al. (2004). "Nuclear Factor of Activated T Cells Regulates Transcription of the Surfactant Protein D Gene (Sftpd) via Direct Interaction with Thyroid Transcription Factor-1 in Lung Epithelial Cells." *J. Biol. Chem.* **279**(33): 34578-34588.

<http://www.jbc.org/cgi/content/abstract/279/33/34578>

Surfactant protein D (SP-D) plays critical roles in host defense, surfactant homeostasis, and pulmonary immunomodulation. Here, we identify a role of nuclear factor of activated T cells (NFATs) in regulation of murine SP-D gene (Sftpd) transcription. An NFAT-dependent enhancer modulated by NFATs or calcineurin and sensitive to cyclosporin was identified in the Sftpd promoter. Ionomycin and phorbol 12-myristate 13-acetate further increased the activity of this

enhancer, whereas VIVIT, a potent NFAT inhibitor peptide, selectively interfered with the calcineurin-NFAT interaction and abolished enhancer function. Gel supershift and DNase I protection assays identified DNA elements that bind NFAT in the Sftpd promoter. Calcineurin and NFATc3 proteins were detected in the embryonic and adult mouse lung epithelium, and the mRNA expression profiles of the NFATs were similar in immortalized mouse lung epithelial cells and alveolar epithelial type II cells. NFATc3 and TTF-1 activated the Sftpd promoter, synergized transcription, co-immunoprecipitated from mouse lung epithelial cells, and physically interacted in vitro. Components of the calcineurin/NFAT pathway were identified in respiratory epithelial cells of the lung that potentially augment rapid assembly of a multiprotein transcription complex on Sftpd promoter inducing SP-D expression.

Deroo, T., T. Denayer, et al. (2004). "Global Inhibition of Lef1/Tcf-dependent Wnt Signaling at Its Nuclear End Point Abrogates Development in Transgenic Xenopus Embryos." J. Biol. Chem. **279**(49): 50670-50675.

<http://www.jbc.org/cgi/content/abstract/279/49/50670>

Analysis of canonical Wnt signaling during vertebrate development by means of knock-out or transgenic approaches is often hampered by functional redundancy as well as pathway bifurcations downstream of the manipulated components. We report the design of an optimized chimera capable of blocking transcriptional activation of Lef1/Tcf- β -catenin target genes, thus enabling intervention with the canonical Wnt pathway at its nuclear end point. This construct was made hormone-inducible, both functionally and transcriptionally, and was transgenically integrated in Xenopus embryos. Down-regulation of target genes was clearly observed upon treatment of these embryos with dexamethasone. In addition, exposure of variously aged transgenic embryos to dexamethasone caused complex phenotypes with many new but also several recognizable features stemming from inhibition of canonical Wnt signaling. At least in some tissues, a significant reduction in cell proliferation and an increase in programmed cell death appeared to underlie these phenotypes. Our inducible transgenic system can serve a broad range of experimental settings designed to unveil new functional aspects of Lef1/Tcf- β -catenin signaling during vertebrate embryogenesis.

Dettwiler, S., C. Aringhieri, et al. (2004). "Distinct Sequence Motifs within the 68-kDa Subunit of Cleavage Factor Im Mediate RNA Binding, Protein-Protein Interactions, and Subcellular Localization." J. Biol. Chem. **279**(34): 35788-35797.

<http://www.jbc.org/cgi/content/abstract/279/34/35788>

Cleavage factor Im (CF Im) is required for the first step in pre-mRNA 3'-end processing and can be reconstituted in vitro from its heterologously expressed 25- and 68-kDa subunits. The binding of CF Im to the pre-mRNA is one of the earliest steps in the assembly of the cleavage and polyadenylation machinery and facilitates the recruitment of other processing factors. We identified regions in the subunits of CF Im involved in RNA binding, protein-protein interactions, and subcellular localization. CF Im68 has a modular domain organization consisting of an N-terminal RNA recognition motif and a C-terminal alternating charge domain. However, the RNA recognition motif of CF Im68 on its own is not sufficient to bind RNA but is necessary for association with the 25-kDa subunit. RNA binding appears to require a CF Im68/25 heterodimer. Whereas multiple protein interactions with other 3'-end-processing factors are detected with CF Im25, CF Im68 interacts with SRp20, 9G8, and hTra2 β , members of the SR family of splicing factors, via its C-terminal alternating charge domain. This domain is also required for targeting CF Im68 to the nucleus. However, CF Im68 does not concentrate in splicing speckles but in foci that partially colocalize with paraspeckles, a subnuclear component in which other proteins involved in

transcriptional control and RNA processing have been found.

Di Carlo, A., R. De Mori, et al. (2004). "Hypoxia Inhibits Myogenic Differentiation through Accelerated MyoD Degradation." *J. Biol. Chem.* **279**(16): 16332-16338.

<http://www.jbc.org/cgi/content/abstract/279/16/16332>

Cells undergo a variety of biological responses when placed in hypoxic conditions, including alterations in metabolic state and growth rate. Here we investigated the effect of hypoxia on the ability of myogenic cells to differentiate in culture. Exposure of myoblasts to hypoxia strongly inhibited multinucleated myotube formation and the expression of differentiation markers. We showed that hypoxia reversibly inhibited MyoD, Myf5, and myogenin expression. One key step in skeletal muscle differentiation involves the up-regulation of the cell cycle-dependent kinase inhibitors p21 and p27 as well as the product of the retinoblastoma gene (pRb). Myoblasts cultured under hypoxic conditions in differentiation medium failed to up-regulate both p21 and pRb despite the G1 cell cycle arrest, as evidenced by p27 accumulation and pRb hypophosphorylation. Hypoxia-dependent inhibition of differentiation was associated with MyoD degradation by the ubiquitin-proteasome pathway. MyoD overexpression in C2C12 myoblasts overrode the differentiation block imposed by hypoxic conditions. Thus, hypoxia by inducing MyoD degradation blocked accumulation of early myogenic differentiation markers such as myogenin and p21 and pRb, preventing both permanent cell cycle withdraw and terminal differentiation. Our study revealed a novel anti-differentiation effect exerted by hypoxia in myogenic cells and identified MyoD degradation as a relevant target of hypoxia.

Dinadayala, P., A. Lemassu, et al. (2004). "Revisiting the Structure of the Anti-neoplastic Glucans of Mycobacterium bovis Bacille Calmette-Guerin: STRUCTURAL ANALYSIS OF THE EXTRACELLULAR AND BOILING WATER EXTRACT-DERIVED GLUCANS OF THE VACCINE SUBSTRAINS." *J. Biol. Chem.* **279**(13): 12369-12378.

<http://www.jbc.org/cgi/content/abstract/279/13/12369>

The attenuated strain of Mycobacterium bovis Bacille Calmette-Guerin (BCG), used worldwide to prevent tuberculosis and leprosy, is also clinically used as an immunotherapeutic agent against superficial bladder cancer. An anti-tumor polysaccharide has been isolated from the boiling water extract of the Tice substrain of BCG and tentatively characterized as consisting primarily of repeating units of 6-linked-glucosyl residues. Mycobacterium tuberculosis and other mycobacterial species produce a glycogen-like {alpha}-glucan composed of repeating units of 4-linked glucosyl residues substituted at some 6 positions by short oligoglucosyl units that also exhibits an anti-tumor activity. Therefore, the impression prevails that mycobacteria synthesize different types of anti-neoplastic glucans or, alternatively, the BCG substrains are singular in producing a unique type of glucan that may confer to them their immunotherapeutic property. The present study addresses this question through the comparative analysis of {alpha}-glucans purified from the extracellular materials and boiling water extracts of three vaccine substrains. The polysaccharides were purified, and their structural features were established by mono- and two-dimensional NMR spectroscopy and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of the enzymatic and chemical degradation products of the purified compounds. The glucans isolated by the two methods from the three substrains of BCG were shown to exhibit identical structural features shared with the glycogen-like {alpha}-glucan of M. tuberculosis and other mycobacteria. Incidentally, we observed an occasional release of dextrans from Sephadex columns that may explain the reported occurrence of 6-substituted {alpha}-glucans in mycobacteria.

Dolgachev, V., M. S. Farooqui, et al. (2004). "De Novo Ceramide Accumulation Due to Inhibition of Its Conversion to Complex Sphingolipids in Apoptotic Photosensitized Cells." *J. Biol. Chem.* **279**(22): 23238-23249.

<http://www.jbc.org/cgi/content/abstract/279/22/23238>

The oxidative stress induced by photodynamic therapy (PDT) with the photosensitizer phthalocyanine 4 is accompanied by increases in ceramide mass. To assess the regulation of de novo sphingolipid metabolism during PDT-induced apoptosis, Jurkat human T lymphoma and Chinese hamster ovary cells were labeled with [14C]serine, a substrate of serine palmitoyltransferase (SPT), the enzyme catalyzing the initial step in the sphingolipid biosynthesis. A substantial elevation in [14C]ceramide with a concomitant decrease in [14C]sphingomyelin was detected. The labeling of [14C]ceramide was completely abrogated by the SPT inhibitor ISP-1. In addition, ISP-1 partly suppressed PDT-induced apoptosis. Pulse-chase experiments showed that the contribution of sphingomyelin degradation to PDT-initiated increase in de novo ceramide was absent or minor. PDT had no effect on either mRNA amounts of the SPT subunits LCB1 and LCB2, LCB1 protein expression, or SPT activity in Jurkat cells. Moreover in Chinese hamster ovary cells LCB1 protein underwent substantial photodestruction, and SPT activity was profoundly inhibited after treatment. We next examined whether PDT affects conversion of ceramide to complex sphingolipids. Sphingomyelin synthase, as well as glucosylceramide synthase, was inactivated by PDT in both cell lines in a dose-dependent manner. These results are the first to show that in the absence of SPT up-regulation PDT induces accumulation of de novo ceramide by inhibiting its conversion to complex sphingolipids.

Dorsam, G. and E. J. Goetzl (2002). "Vasoactive Intestinal Peptide Receptor-1 (VPAC-1) Is a Novel Gene Target of the Hemolymphopoietic Transcription Factor Ikaros." *J. Biol. Chem.* **277**(16): 13488-13493.

<http://www.jbc.org/cgi/content/abstract/277/16/13488>

Vasoactive intestinal peptide and its G-protein-coupled receptors, VPAC-1 and VPAC-2, are highly expressed in the immune system and modulate diverse T cell functions. The human VPAC-1 5'-flanking region (1.4 kb) contains four high affinity Ikaros (IK) consensus sequences. Ikaros native protein from T cell nuclear extracts and IK-1 and IK-2 recombinant proteins recognized an IK high affinity binding motif in the VPAC-1 promoter in electrophoretic mobility shift assays by a sequence-specific mechanism, and anti-IK antibodies supershifted this complex. Stable NIH-3T3 clones overexpressing IK-1 or IK-2 isoforms were generated to investigate Ikaros regulation of endogenous VPAC-1 expression as assessed by quantifying VPAC-1 mRNA and protein. By traditional and fluorometric-based kinetic reverse transcription-PCR and 125I-labeled vasoactive intestinal peptide binding, both IK-1 and IK-2 suppressed endogenous VPAC-1 expression in NIH-3T3 clones by a range of 50-93%. When a series of nested deletions of the VPAC-1 luciferase reporter construct were transiently transfected into IK-2 clones there was up to a 41% decrease in transcriptional activity compared with vector control. Two major IK-2 binding domains also were identified at [-]1076 to [-]623 bp and at [-]222 to [-]35 bp, respectively. As both Ikaros and its novel target VPAC-1 are highly expressed in T cells, this system may be a dominant determinant of the VPAC-1 expression in immune responses.

Dunty, J. M. and M. D. Schaller (2002). "The N Termini of Focal Adhesion Kinase Family Members Regulate Substrate Phosphorylation, Localization, and Cell Morphology." *J. Biol. Chem.* **277**(47):

45644-45654.

<http://www.jbc.org/cgi/content/abstract/277/47/45644>

The focal adhesion kinase (FAK) and cell adhesion kinase [beta] (CAK[beta], PYK2, CADTK, RAFTK) are highly homologous FAK family members, yet clearly have unique roles in the cell. Comparative analyses of FAK and CAK[beta] have revealed intriguing differences in their activities. These differences were investigated further through the characterization of a set of FAK/CAK[beta] chimeric kinases. CAK[beta] exhibited greater catalytic activity than FAK in vitro, providing a molecular basis for differential substrate phosphorylation by FAK and CAK[beta] in vivo. Furthermore, the N terminus may regulate catalytic activity since chimeras containing the FAK N terminus and CAK[beta] catalytic domain exhibited a striking high level of catalytic activity and substrate phosphorylation. Unexpectedly, a modulatory role for the N termini in subcellular localization was also revealed. Chimeras containing the FAK N terminus and CAK[beta] C terminus localized to focal adhesions, whereas chimeras containing the N and C termini of CAK[beta] did not. Finally, prominent changes in cell morphology were induced upon expression of chimeras containing the CAK[beta] N terminus, which were not associated with apoptotic cell death, cell cycle progression delay, or changes in Rho activity. These results demonstrate novel regulatory roles for the N terminus of FAK family kinases.

Elfering, S. L., T. M. Sarkela, et al. (2002). "Biochemistry of Mitochondrial Nitric-oxide Synthase." J. Biol. Chem. **277**(41): 38079-38086.

<http://www.jbc.org/cgi/content/abstract/277/41/38079>

We reported that the generation of nitric oxide by mitochondria is catalyzed by a constitutive, mitochondrial nitric-oxide synthase (mtNOS). Given that this production may establish the basis for a novel regulatory pathway of energy metabolism, oxygen consumption, and oxygen free radical production, it becomes imperative to identify unequivocally and characterize this enzyme to provide a basis for its regulation. The mitochondrial localization of mtNOS was supported by following the hepatic distribution of mtNOS, immunoblotting submitochondrial fractions, and immunohistochemistry of liver tissues. mtNOS was identified as brain NOS[alpha] by various methods (mass spectrometry of proteolytic fragments, amino acid analysis, molecular weight, pl, and analysis of PCR fragments), excluding the occurrence of a novel isoform or other splice variants. Distribution of mtNOS transcript indicated its occurrence in liver, brain, heart, muscle, kidney, lung, testis, and spleen. In contrast to brain NOS, mtNOS has two post-translational modifications: acylation with myristic acid and phosphorylation at the C terminus. The former modification is a reversible and post-translational process, which may serve for subcellular targeting or membrane anchoring. The latter modification could be linked to enzymatic regulation. These results are discussed in terms of the role that nitric oxide may have in cellular bioenergetics.

Ezoe, S., I. Matsumura, et al. (2005). "GATA Transcription Factors Inhibit Cytokine-dependent Growth and Survival of a Hematopoietic Cell Line through the Inhibition of STAT3 Activity." J. Biol. Chem. **280**(13): 13163-13170.

<http://www.jbc.org/cgi/content/abstract/280/13/13163>

Although GATA-1 and GATA-2 were shown to be essential for the development of hematopoietic cells by gene targeting experiments, they were also reported to inhibit the growth of hematopoietic cells. Therefore, in this study, we examined the effects of GATA-1 and GATA-2 on

cytokine signals. A tamoxifen-inducible form of GATA-1 (GATA-1/ERT) showed a minor inhibitory effect on interleukin-3 (IL-3)-dependent growth of an IL-3-dependent cell line Ba/F3. On the other hand, it drastically inhibited TPO-dependent growth and gp130-mediated growth/survival of Ba/F3. Similarly, an estradiol-inducible form of GATA-2 (GATA-2/ER) disrupted thrombopoietin (TPO)-dependent growth and gp130-mediated growth/survival of Ba/F3. As for this mechanism, we found that both GATA-1 and GATA-2 directly bound to STAT3 both in vitro and in vivo and inhibited its DNA-binding activity in gel shift assays and chromatin immunoprecipitation assays, whereas they hardly affected STAT5 activity. In addition, endogenous GATA-1 was found to interact with STAT3 in normal megakaryocytes, suggesting that GATA-1 may inhibit STAT3 activity in normal hematopoietic cells. Furthermore, we found that GATA-1 suppressed STAT3 activity through its N-zinc finger domain. Together, these results suggest that, besides the roles as transcription factors, GATA family proteins modulate cytokine signals through protein-protein interactions, thereby regulating the growth and survival of hematopoietic cells.

Fujii, I., Y. Yasuoka, et al. (2004). "Hydrolytic Polyketide Shortening by Ayg1p, a Novel Enzyme Involved in Fungal Melanin Biosynthesis." *J. Biol. Chem.* **279**(43): 44613-44620.

<http://www.jbc.org/cgi/content/abstract/279/43/44613>

The pentaketide 1,3,6,8-tetrahydroxynaphthalene (T4HN) is a key precursor of 1,8-dihydroxynaphthalene-melanin, an important virulence factor in pathogenic fungi, where T4HN is believed to be the direct product of pentaketide synthases. We showed recently the involvement of a novel protein, Ayg1p, in the formation of T4HN from the heptaketide precursor YWA1 in *Aspergillus fumigatus*. To investigate the mechanism of its enzymatic function, Ayg1p was purified from an *Aspergillus oryzae* strain that overexpressed the *ayg1* gene. The Ayg1p converted the naphthopyrone YWA1 to T4HN with a release of the acetoacetic acid. Although Ayg1p does not show significant homology with known enzymes, a serine protease-type hydrolytic motif is present in its sequence, and serine-specific inhibitors strongly inhibited the activity. To identify its catalytic residues, site-directed Ayg1p mutants were expressed in *Escherichia coli*, and their enzyme activities were examined. The single substitution mutations S257A, D352A, and H380A resulted in a complete loss of enzyme activity in Ayg1p. These results indicated that the catalytic triad Asp352-His380-Ser257 constituted the active-site of Ayg1p. From a Dixon plot analysis, 2-acetyl-1,3,6,8-tetrahydroxynaphthalene was found to be a strong mixed-type inhibitor, suggesting the involvement of an acyl-enzyme intermediate. These studies support the mechanism in which the Ser257 at the active site functions as a nucleophile to attack the YWA1 side-chain 1'-carbonyl and cleave the carbon-carbon bond between the naphthalene ring and the side chain. Acetoacetic acid is subsequently released from the Ser257-O-acetoacetylated Ayg1p by hydrolysis. An enzyme with activity similar to Ayg1p in melanin biosynthesis has not been reported in any other organism.

Fukuchi, M., A. Tabuchi, et al. (2004). "Activity-dependent Transcriptional Activation and mRNA Stabilization for Cumulative Expression of Pituitary Adenylate Cyclase-activating Polypeptide mRNA Controlled by Calcium and cAMP Signals in Neurons." *J. Biol. Chem.* **279**(46): 47856-47865.

<http://www.jbc.org/cgi/content/abstract/279/46/47856>

Although it has been established that an activity-dependent gene transcription is induced by the calcium (Ca²⁺) signals in neurons, it is unclear how the specific mRNA moieties are transiently accumulated in response to synaptic transmission which evokes multiple intracellular signals including Ca²⁺ and cAMP ones. The expression of pituitary adenylate cyclase activating polypeptide (PACAP), a neuropeptide, is controlled by Ca²⁺ signals evoked via membrane

depolarization in neurons, and, in cultured rat cortical neuronal cells, we found that the Ca^{2+} signal-mediated activation of the PACAP gene promoter was critically controlled by a single cAMP-response element (CRE) located at around -200, to which the CRE-binding protein predominantly bound. The Ca^{2+} signal-induced expression of PACAP mRNA was enhanced by forskolin, which evokes cAMP signals. In support, the PACAP gene promoter was synergistically enhanced by Ca^{2+} and cAMP signals through the CRE, accompanying a prolonged activation of extracellular signal-related protein kinase 1/2 and CRE-binding protein. On the other hand, sole administration of forskolin markedly reduced the cellular content of PACAP mRNA, which was restored by the addition of Ca^{2+} signals. We found that the stability of PACAP mRNA was increased in response to Ca^{2+} signals but not that of activity-regulated cytoskeleton-associated protein (Arc) mRNA, indicating an activity-dependent stabilization of specific mRNA species in neurons, which can antagonize the regulation mediated by cAMP signals. Thus, the transcriptional activation and mRNA stabilization are coordinately regulated by Ca^{2+} and cAMP signals for the cumulative expression of PACAP mRNA in neurons.

Furusato, M., N. Sueyoshi, et al. (2002). "Molecular Cloning and Characterization of Sphingolipid Ceramide N-Deacylase from a Marine Bacterium, *Shewanella alga* G8." *J. Biol. Chem.* **277**(19): 17300-17307.

<http://www.jbc.org/cgi/content/abstract/277/19/17300>

Recently, lyso-sphingolipids have been identified as ligands for several orphan G protein-coupled receptors, although the molecular mechanism for their generation has yet to be clarified. Here, we report the molecular cloning of the enzyme, which catalyzes the generation of lyso-sphingolipids from various sphingolipids (sphingolipid ceramide N-deacylase). The 75-kDa enzyme was purified from the marine bacterium, *Shewanella alga* G8, and its gene was cloned from a G8 genomic library using sequences of the purified enzyme. The cloned enzyme was composed of 992 amino acids, including a signal sequence of 35 residues, and its molecular weight was estimated to be 109,843. Significant sequence similarities were found with an unknown protein of *Streptomyces fradiae* Y59 and a *Lumbricus terrestris* lectin but not other known functional proteins. The 106-kDa recombinant enzyme expressed in *Escherichia coli* hydrolyzed various glycosphingolipids and sphingomyelin, although it seems to be much less active than the native 75-kDa enzyme. In vitro translation using wheat germ extract revealed the activity of a 75-kDa deletion mutant lacking a C terminus to be much stronger than that of the full-length enzyme, suggesting that C-terminal processing is necessary for full activity.

Gardner, A. F., C. M. Joyce, et al. (2004). "Comparative Kinetics of Nucleotide Analog Incorporation by Vent DNA Polymerase." *J. Biol. Chem.* **279**(12): 11834-11842.

<http://www.jbc.org/cgi/content/abstract/279/12/11834>

Comparative kinetic and structural analyses of a variety of polymerases have revealed both common and divergent elements of nucleotide discrimination. Although the parameters for dNTP incorporation by the hyperthermophilic archaeal Family B Vent DNA polymerase are similar to those previously derived for Family A and B DNA polymerases, parameters for analog incorporation reveal alternative strategies for discrimination by this enzyme. Discrimination against ribonucleotides was characterized by a decrease in the affinity of NTP binding and a lower rate of phosphoryl transfer, whereas discrimination against ddNTPs was almost exclusively due to a slower rate of phosphodiester bond formation. Unlike Family A DNA polymerases, incorporation of 9-[(2-hydroxyethoxy)methyl]X triphosphates (where X is adenine, cytosine, guanine, or thymine; acyNTPs) by Vent DNA polymerase was enhanced over ddNTPs via a 50-fold increase in phosphoryl transfer rate. Furthermore, a mutant with increased propensity for

nucleotide analog incorporation (VentA488L DNA polymerase) had unaltered dNTP incorporation while displaying enhanced nucleotide analog binding affinity and rates of phosphoryl transfer. Based on kinetic data and available structural information from other DNA polymerases, we propose active site models for dNTP, ddNTP, and acyNTP selection by hyperthermophilic archaeal DNA polymerases to rationalize structural and functional differences between polymerases.

Gelebart, P., T. Kovacs, et al. (2002). "Expression of Endomembrane Calcium Pumps in Colon and Gastric Cancer Cells. INDUCTION OF SERCA3 EXPRESSION DURING DIFFERENTIATION." J. Biol. Chem. **277**(29): 26310-26320.

<http://www.jbc.org/cgi/content/abstract/277/29/26310>

Calcium mobilization from the endoplasmic reticulum (ER) into the cytosol is a key component of several signaling networks controlling tumor cell growth, differentiation, or apoptosis. Sarco/endoplasmic reticulum calcium transport ATPases (SERCA-type calcium pumps), enzymes that accumulate calcium in the ER, play an important role in these phenomena. We report that SERCA3 expression is significantly reduced or lost in colon carcinomas when compared with normal colonic epithelial cells, which express this enzyme at a high level. To study the involvement of SERCA enzymes in differentiation, in this work differentiation of colon and gastric cancer cell lines was initiated, and the change in the expression of SERCA isoenzymes as well as intracellular calcium levels were investigated. Treatment of the tumor cells with butyrate or other established differentiation inducing agents resulted in a marked and specific induction of the expression of SERCA3, whereas the expression of the ubiquitous SERCA2 enzymes did not change significantly or was reduced. A similar marked increase in SERCA3 expression was found during spontaneous differentiation of post-confluent Caco-2 cells, and this closely correlated with the induction of other known markers of differentiation. Analysis of the expression of the SERCA3 alternative splice isoforms revealed induction of all three known iso-SERCA3 variants (3a, 3b, and 3c). Butyrate treatment of the KATO-III gastric cancer cells led to higher resting cytosolic calcium concentrations and, in accordance with the lower calcium affinity of SERCA3, to diminished ER calcium content. These data taken together indicate a defect in SERCA3 expression in colon cancers as compared with normal colonic epithelium, show that the calcium homeostasis of the endoplasmic reticulum may be remodeled during cellular differentiation, and indicate that SERCA3 constitutes an interesting new differentiation marker that may prove useful for the analysis of the phenotype of gastrointestinal adenocarcinomas.

Gencic, S. and D. A. Grahame (2003). "Nickel in Subunit beta of the Acetyl-CoA Decarbonylase/Synthase Multienzyme Complex in Methanogens. CATALYTIC PROPERTIES AND EVIDENCE FOR A BINUCLEAR Ni-Ni SITE." J. Biol. Chem. **278**(8): 6101-6110.

<http://www.jbc.org/cgi/content/abstract/278/8/6101>

The acetyl-CoA decarbonylase/synthase (ACDS) complex catalyzes the central reaction of acetyl C-C bond cleavage in methanogens growing on acetate and is also responsible for synthesis of acetyl units during growth on C-1 substrates. The ACDS [beta] subunit contains nickel and an Fe/S center and reacts with acetyl-CoA forming an acetyl-enzyme intermediate presumably directly involved in acetyl C-C bond activation. To investigate the role of nickel in this process two forms of the *Methanosarcina thermophila* [beta] subunit were overexpressed in anaerobically grown *Escherichia coli*. Both contained an Fe/S center but lacked nickel and were inactive in acetyl-enzyme formation in redox-dependent acetyltransferase assays. However, high activity developed during incubation with NiCl₂. The native and nickel-reconstituted proteins both contained iron and nickel in a 2:1 ratio, with insignificant levels of other metals, including copper.

Binding of nickel elicited marked changes in the UV-visible spectrum, with intense charge transfer bands indicating multiple thiolate ligation to nickel. The kinetics of nickel incorporation matched the time course for enzyme activation. Other divalent metal ions could not substitute for nickel in yielding catalytic activity. Acetyl-CoA was formed in reactions with CoA, CO, and methylcobalamin, directly demonstrating C-C bond activation by the [beta] subunit in the absence of other ACDS subunits. Nickel was indispensable in this process too and was needed to form a characteristic EPR-detectable enzyme-carbonyl adduct in reactions with CO. In contrast to enzyme activation, EPR signal formation did not require addition of reducing agent, indicating indirect catalytic involvement of the paramagnetic species. Site-directed mutagenesis indicated that Cys-278 and Cys-280 coordinate nickel, with Cys-189 essential for Fe/S cluster formation. The results are consistent with an Ni₂[Fe₄S₄] arrangement at the active site. A mechanism for C-C bond activation is proposed that includes a specific role for the Fe₄S₄ center and accounts for the absolute requirement for nickel.

Gomez, M. F., A. S. Stevenson, et al. (2002). "Opposing Actions of Inositol 1,4,5-Trisphosphate and Ryanodine Receptors on Nuclear Factor of Activated T-cells Regulation in Smooth Muscle." J. Biol. Chem. **277**(40): 37756-37764.

<http://www.jbc.org/cgi/content/abstract/277/40/37756>

The nuclear factor of activated T-cells (NFAT), originally identified in T-cells, has since been shown to play a role in mediating Ca²⁺-dependent gene transcription in diverse cell types outside of the immune system. We have previously shown that nuclear accumulation of NFATc3 is induced in ileal smooth muscle by platelet-derived growth factor in a manner that depends on Ca²⁺ influx through L-type, voltage-dependent Ca²⁺ channels. Here we show that NFATc3 is also the predominant NFAT isoform expressed in cerebral artery smooth muscle and is induced to accumulate in the nucleus by UTP and other Gq/11-coupled receptor agonists. This induction is mediated by calcineurin and is dependent on sarcoplasmic reticulum Ca²⁺ release through inositol 1,4,5-trisphosphate receptors and extracellular Ca²⁺ influx through L-type, voltage-dependent Ca²⁺ channels. Consistent with results obtained in ileal smooth muscle, depolarization-induced Ca²⁺ influx fails to induce NFAT nuclear accumulation in cerebral arteries. We also provide evidence that Ca²⁺ release by ryanodine receptors in the form of Ca²⁺ sparks may exert an inhibitory influence on UTP-induced NFATc3 nuclear accumulation and further suggest that UTP may act, in part, by inhibiting Ca²⁺ sparks. These results are consistent with a multifactorial regulation of NFAT nuclear accumulation in smooth muscle that is likely to involve several intracellular signaling pathways, including local effects of sarcoplasmic reticulum Ca²⁺ release and effects attributable to global elevations in intracellular Ca²⁺.

Graham, D. E., H. Xu, et al. (2002). "Identification of Coenzyme M Biosynthetic Phosphosulfolactate Synthase. A NEW FAMILY OF SULFONATE-BIOSYNTHESIZING ENZYMES." J. Biol. Chem. **277**(16): 13421-13429.

<http://www.jbc.org/cgi/content/abstract/277/16/13421>

The hyperthermophilic euryarchaeon *Methanococcus jannaschii* uses coenzyme M (2-mercaptoethanesulfonic acid) as the terminal methyl carrier in methanogenesis. We describe an enzyme from that organism, (2R)-phospho-3-sulfolactate synthase (ComA), that catalyzes the first step in coenzyme M biosynthesis. ComA catalyzed the stereospecific Michael addition of sulfite to phosphoenolpyruvate over a broad range of temperature and pH conditions. Substrate and product analogs moderately inhibited activity. This enzyme has no significant sequence similarity to previously characterized enzymes; however, its Mg²⁺-dependent enzyme reaction mechanism may be analogous to one proposed for enolase. A diverse group of microbes and

plants have homologs of ComA that could have been recruited for sulfolactate or sulfolipid biosyntheses.

Greenwood, I. A., L. J. Miller, et al. (2002). "The Large Conductance Potassium Channel beta -Subunit Can Interact with and Modulate the Functional Properties of a Calcium-activated Chloride Channel, CLCA1." J. Biol. Chem. **277**(25): 22119-22122.

<http://www.jbc.org/cgi/content/abstract/277/25/22119>

We have recently compared the biophysical and pharmacological properties of native Ca^{2+} -activated Cl^- currents in murine portal vein with mCLCA1 channels cloned from murine portal vein myocytes (Britton, F. C., Ohya, S., Horowitz, B., and Greenwood, I. A. (2002) *J. Physiol. (Lond.)* 539, 107-117). These channels shared a similar relative permeability to various anions, but the expressed channel current lacked the marked time dependence of the native current. In addition, the expressed channel showed a lower Ca^{2+} sensitivity than the native channel. As non-pore-forming regulatory [beta]-subunits alter the kinetics and increase the Ca^{2+} sensitivity of Ca^{2+} -dependent K^+ channels (BK channels) we investigated whether co-expression of [beta]-subunits with CLCA1 would alter the kinetics/ Ca^{2+} sensitivity of mCLCA1. Internal dialysis of human embryonic kidney cells stably expressing CLCA1 with 500 nM Ca^{2+} evoked a significantly larger current when the [beta]-subunit KCNMB1 was co-expressed. In a small number of co-transfected cells marked time dependence to the activation kinetics was observed. Interaction studies using the mammalian two-hybrid technique demonstrated a physical association between CLCA1 and KCNMB1 when co-expressed in human embryonic kidney cells. These data suggest that activation of CLCA1 can be modified by accessory subunits.

Gronlund, H., T. Bergman, et al. (2003). "Formation of Disulfide Bonds and Homodimers of the Major Cat Allergen Fel d 1 Equivalent to the Natural Allergen by Expression in Escherichia coli." J. Biol. Chem. **278**(41): 40144-40151.

<http://www.jbc.org/cgi/content/abstract/278/41/40144>

Dander from the domestic cat (*Felis domesticus*) is one of the most common causes of IgE-mediated allergy. Attempts to produce tetrameric folded major allergen Fel d 1 by recombinant methods with structural features similar to the natural allergen have been only partially successful. In this study, a recombinant folded Fel d 1 with molecular and biological properties similar to the natural counterpart was produced. A synthetic gene coding for direct fusion of the Fel d 1 chain 2 N-terminally to chain 1 was constructed by overlapping oligonucleotides in PCR. *Escherichia coli* expression resulted in a non-covalently associated homodimer with an apparent molecular mass of 30 kDa defined by size exclusion chromatography. Furthermore, each 19,177-Da subunit displayed a disulfide pattern identical to that found in the natural Fel d 1, i.e. Cys3(1) Cys73(2), Cys44(1)-Cys48(2), Cys70(1)-Cys7(2), as determined by electrospray mass spectrometry after tryptic digestion. Circular dichroism analysis showed identical folds of natural and recombinant Fel d 1. Furthermore, recombinant Fel d 1 reacted specifically with serum IgE, inducing expression of CD203c on basophils and lymphoproliferative responses in cat-allergic patients. The results show that the overall fold and immunological properties of the recombinant Fel d 1 are very similar to those of natural Fel d 1. Moreover, the recombinant Fel d 1 construct provides a tool for defining the three-dimensional structure of Fel d 1 and represents a reagent for diagnosis and allergen-specific immunotherapy of cat allergy.

Gross, I., D. J. Morrison, et al. (2003). "The Receptor Tyrosine Kinase Regulator Sprouty1 Is a Target of the Tumor Suppressor WT1 and Important for Kidney Development." J. Biol. Chem. **278**(42): 41420-41430.

<http://www.jbc.org/cgi/content/abstract/278/42/41420>

WT1 encodes a transcription factor involved in kidney development and tumorigenesis. Using representational difference analysis, we identified a new set of WT1 targets, including a homologue of the Drosophila receptor tyrosine kinase regulator, sprouty. Sprouty1 was up-regulated in cell lines expressing wild-type but not mutant WT1. WT1 bound to the endogenous sprouty1 promoter in vivo and directly regulated sprouty1 through an early growth response gene-1 binding site. Expression of Sprouty1 and WT1 overlapped in the developing metanephric mesenchyme, and Sprouty1, like WT1, plays a key role in the early steps of glomerulus formation. Disruption of Sprouty1 expression in embryonic kidney explants by antisense oligonucleotides reduced condensation of the metanephric mesenchyme, leading to a decreased number of glomeruli. In addition, sprouty1 was expressed in the ureteric tree and antisense-treated ureteric trees had cystic lumens. Therefore, sprouty1 represents a physiologically relevant target gene of WT1 during kidney development.

Gu, B. J., R. Sluyter, et al. (2004). "An Arg307 to Gln Polymorphism within the ATP-binding Site Causes Loss of Function of the Human P2X7 Receptor." J. Biol. Chem. **279**(30): 31287-31295.

<http://www.jbc.org/cgi/content/abstract/279/30/31287>

The P2X7 receptor is a ligand-gated channel that is highly expressed on mononuclear cells of the immune system and that mediates ATP-induced apoptosis. Wide variations in the function of the P2X receptor have been observed, explained in part by loss-of-function polymorphisms that change Glu496 to Ala (E496A) and Ile568 to Asn (I568N). In this study, a third polymorphism, which substitutes an uncharged glutamine for the highly positively charged Arg307 (R307Q), has been found in heterozygous dosage in 12 of 420 subjects studied. P2X7 function was measured by ATP-induced fluxes of Rb⁺, Ba²⁺, and ethidium⁺ into peripheral blood monocytes or various lymphocyte subsets and was either absent or markedly decreased. Transfection experiments showed that P2X7 carrying the R307Q mutation lacked either channel or pore function despite robust protein synthesis and surface expression of the receptor. The monoclonal antibody (clone L4) that binds to the extracellular domain of wild type P2X7 and blocks P2X7 function failed to bind to the R307Q mutant receptor. Differentiation of monocytes to macrophages up-regulated P2X7 function in cells heterozygous for the R307Q to a value 10-40% of that for wild type macrophages. However, macrophages from a subject who was double heterozygous for R307Q/I568N remained totally non-functional for P2X7, and lymphocytes from the same subject also lacked ATP-stimulated phospholipase D activity. These data identify a third loss-of-function polymorphism affecting the human P2X7 receptor, and since the affected Arg307 is homologous to those amino acids essential for ATP binding to P2X1 and P2X2, it is likely that this polymorphism abolishes the binding of ATP to the extracellular domain of P2X7.

He, H., F. Soncin, et al. (2003). "Elevated Expression of Heat Shock Factor (HSF) 2A Stimulates HSF1-induced Transcription during Stress." J. Biol. Chem. **278**(37): 35465-35475.

<http://www.jbc.org/cgi/content/abstract/278/37/35465>

Heat shock factor 2 (HSF2) belongs to a family of structurally related transcription factors, which share the property of binding to heat shock elements in the promoters of hsp molecular

chaperone genes. However, unlike HSF1, which is essential for hsp gene transcription, the cellular functions of HSF2 are not well known. Here we show that human HSF2, although an ineffective activator of the hsp70 promoter in vitro and in vivo in the absence of stress, participates in the activation of the hsp70 promoter by heat shock. HSF2 was not, however, activated by heat shock in cells deficient in functional HSF1, suggesting a requirement for HSF1 in HSF2-mediated transcriptional enhancement. In addition, HSF2 regulation involves differential activity of two isoforms, HSF2A and HSF2B, which arise from alternative splicing of a common hsf2 gene. Under basal conditions, both HSF2 isoforms are ineffective in activating the hsp70 transcription. However, heat shock differentially activates HSF2A in vivo. This phenomenon appears to be physiologically significant, as human myeloprogenitor cells differentiating along the erythroid lineage express HSF2A de novo and undergo a large increase in capacity to activate the hsp70 promoter. Our experiments further show that HSF1 is physically associated with HSF2 in the cell and that such binding is enhanced by heat shock. Our data suggest a mechanism involving the formation of heterocomplexes between HSF1 and HSF2 with enhanced activity to activate the hsp70 promoter when compared with HSF1 or HSF2 homotrimers.

Hellberg, A., J. Poole, et al. (2002). "Molecular Basis of the Globoside-deficient Pk Blood Group Phenotype. IDENTIFICATION OF FOUR INACTIVATING MUTATIONS IN THE UDP-N-ACETYLGALACTOSAMINE: GLOBOTRIAOSYL CERAMIDE 3-beta -N-ACETYLGALACTOSAMINYLTRANSFERASE GENE." *J. Biol. Chem.* **277**(33): 29455-29459.

<http://www.jbc.org/cgi/content/abstract/277/33/29455>

The biochemistry and molecular genetics underlying the related carbohydrate blood group antigens P, Pk, and LKE in the GLOB collection and P1 in the P blood group system are complex and not fully understood. Individuals with the rare but clinically important erythrocyte phenotypes P1k and P2k lack the capability to synthesize P antigen identified as globoside, the cellular receptor for Parvo-B19 virus and some P-fimbriated *Escherichia coli*. As in the ABO system, naturally occurring antibodies, anti-P of the IgM and IgG class with hemolytic and cytotoxic capacity, are formed. To define the molecular basis of the Pk phenotype we analyzed the full coding region of a candidate gene reported in 1998 as a member of the 3-[beta]-galactosyltransferase family but later shown to possess UDP-N-acetylgalactosamine:globotriaosylceramide 3-[beta]-N-acetylgalactosaminyltransferase or globoside synthase activity. Homozygosity for different nonsense mutations (C202 [right-arrow] T and 538insA) resulting in premature stop codons was found in blood samples from two individuals of the P2k phenotype. Two individuals with P1k and P2k phenotypes were homozygous for missense mutations causing amino acid substitutions (E266A or G271R) in a highly conserved region of the enzymatically active carboxyl-terminal domain in the transferase. We conclude that crucial mutations in the globoside synthase gene cause the Pk phenotype.

Hermoso, M., C. M. Satterwhite, et al. (2002). "CIC-3 Is a Fundamental Molecular Component of Volume-sensitive Outwardly Rectifying Cl⁻ Channels and Volume Regulation in HeLa Cells and *Xenopus laevis* Oocytes." *J. Biol. Chem.* **277**(42): 40066-40074.

<http://www.jbc.org/cgi/content/abstract/277/42/40066>

Volume-sensitive osmolyte and anion channels (VSOACs) are activated upon cell swelling in most vertebrate cells. Native VSOACs are believed to be a major pathway for regulatory volume decrease (RVD) through efflux of chloride and organic osmolytes. CIC-3 has been proposed to encode native VSOACs in *Xenopus laevis* oocytes and in some mammalian cells, including cardiac and vascular smooth muscle cells. The relationship between the CIC-3 chloride channel, the native volume-sensitive osmolyte and anion channel (VSOAC) currents, and cell volume

regulation in HeLa cells and *X. laevis* oocytes was investigated using CIC-3 antisense. In situ hybridization in HeLa cells, semiquantitative and real-time PCR, and immunoblot studies in HeLa cells and *X. laevis* oocytes demonstrated the presence of CIC-3 mRNA and protein, respectively. Exposing both cell types to hypotonic solutions induced cell swelling and activated native VSOACs. Transient transfection of HeLa cells with CIC-3 antisense oligonucleotide or *X. laevis* oocytes injected with antisense cRNA abolished the native CIC-3 mRNA transcript and protein and significantly reduced the density of native VSOACs activated by hypotonically induced cell swelling. In addition, antisense against native CIC-3 significantly impaired the ability of HeLa cells and *X. laevis* oocytes to regulate their volume. These results suggest that CIC-3 is an important molecular component underlying VSOACs and the RVD process in HeLa cells and *X. laevis* oocytes.

Hidaka, K., J. J. Caffrey, et al. (2002). "An Adjacent Pair of Human NUDT Genes on Chromosome X Are Preferentially Expressed in Testis and Encode Two New Isoforms of Diphosphoinositol Polyphosphate Phosphohydrolase." *J. Biol. Chem.* **277**(36): 32730-32738.

<http://www.jbc.org/cgi/content/abstract/277/36/32730>

Combinatorial expression of the various isoforms of diphosphoinositol synthases and phosphohydrolases determines the rates of phosphorylation/dephosphorylation cycles that have been functionally linked to vesicle trafficking, stress responses, DNA repair, and apoptosis. We now describe two new 19-kDa diphosphoinositol polyphosphate phosphohydrolases (DIPPs), named types 3[alpha] and 3[beta], which possess the canonical Nudix-type catalytic motif flanked on either side by short Gly-rich sequences. The two enzymes differ only in that Pro-89 in the [alpha] form is replaced by Arg-89 in the [beta] form, making the latter ~2-fold more active in vitro. Another Nudix substrate, diadenosine hexaphosphate, was hydrolyzed less efficiently ($k_{cat}/K_m = 0.2 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$) compared with diphosphoinositol polyphosphates ($k_{cat}/K_m = 2\text{-}40 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$). Catalytic activity in vivo was established by individual overexpression of the human (h) DIPP3 isoforms in HEK293 cells, which reduced cellular levels of diphosphoinositol polyphosphates by 40-50%. The hDIPP3 mRNA is preferentially expressed in testis, accompanied by relatively weak expression in the brain, contrasting with hDIPP1 and hDIPP2 which are widely expressed. The hDIPP3 genes (NUDT10 encodes hDIPP3[alpha]; NUDT11 encodes hDIPP3[beta]) are only 152 kbp apart at p11.22 on chromosome X and probably arose by duplication. Transcription of both genes is inactivated on one of the X chromosomes of human females to maintain appropriate gene dosage. The hDIPP3 pair add tissue-specific diversity to the molecular mechanisms regulating diphosphoinositol polyphosphate turnover.

Hijarrubia, M. J., J. F. Aparicio, et al. (2003). "Domain Structure Characterization of the Multifunctional alpha -Aminoadipate Reductase from *Penicillium chrysogenum* by Limited Proteolysis. ACTIVATION OF alpha -AMINOADIPATE DOES NOT REQUIRE THE PEPTIDYL CARRIER PROTEIN BOX OR THE REDUCTION DOMAIN." *J. Biol. Chem.* **278**(10): 8250-8256.

<http://www.jbc.org/cgi/content/abstract/278/10/8250>

The [alpha]-aminoadipate reductase ([alpha]-AAR) of *Penicillium chrysogenum*, an enzyme that activates the [alpha]-aminoadipic acid by forming an [alpha]-aminoadipyl adenylate and reduces the activated intermediate to [alpha]-aminoadipic semialdehyde, was purified to homogeneity by immunoaffinity techniques, and the kinetics for [alpha]-aminoadipic acid, ATP, and NADPH were determined. Sequencing of the N-terminal end confirmed the 10 first amino acids deduced from the nucleotide sequence. Its domain structure has been investigated using limited proteolysis and active site labeling. Trypsin and elastase were used to cleave the multienzyme, and the location of fragments within the primary structure was established by N-terminal sequence analysis. Initial

proteolysis generated two fragments: an N-terminal fragment housing the adenylation and the peptidyl carrier protein (PCP) domains (116 kDa) and a second fragment containing most of the reductive domain (28 kDa). Under harsher conditions the adenylation domain (about 64 kDa) and the PCP domain (30 kDa) become separated. Time-dependent acylation of [alpha]-AAR and of fragments containing the adenylation domain with tritiated [alpha]-aminoadipate occurred in vitro in the absence of NADPH. Addition of NADPH to the labeled [alpha]-AAR released most of the radioactive substrate. A fragment containing the adenylation domain was labeled even in absence of the PCP box. The labeling of this fragment (lacking PCP) was always weaker than that observed in the di-domain (adenylating and PCP) fragment suggesting that the PCP domain plays a role in the stability of the acyl intermediate. Low intensity direct acylation of the PCP box has also been observed. A domain structure of this multienzyme is proposed.

Ho, R. H., B. F. Leake, et al. (2004). "Ethnicity-dependent Polymorphism in Na⁺-taurocholate Cotransporting Polypeptide (SLC10A1) Reveals a Domain Critical for Bile Acid Substrate Recognition." *J. Biol. Chem.* **279**(8): 7213-7222.

<http://www.jbc.org/cgi/content/abstract/279/8/7213>

The key transporter responsible for hepatic uptake of bile acids from portal circulation is Na⁺-taurocholate cotransporting polypeptide (NTCP, SLC10A1). This transporter is thought to be critical for the maintenance of enterohepatic recirculation of bile acids and hepatocyte function. Therefore, functionally relevant polymorphisms in this transporter would be predicted to have an important impact on bile acid homeostasis/liver function. However, little is known regarding genetic heterogeneity in NTCP. In this study, we demonstrate the presence of multiple single nucleotide polymorphisms in NTCP in populations of European, African, Chinese, and Hispanic Americans. Specifically four nonsynonymous single nucleotide polymorphisms associated with a significant loss of transport function were identified. Cell surface biotinylation experiments indicated that the altered transport activity of T668C (Ile223 [-> Thr), a variant seen only in African Americans, was due at least in part to decreased plasma membrane expression. Similar expression patterns were observed when the variant alleles were expressed in HepG2 cells, and plasma membrane expression was assessed using immunofluorescence confocal microscopy. Interestingly the C800T (Ser267 [-> Phe) variant, seen only in Chinese Americans, exhibited a near complete loss of function for bile acid uptake yet fully normal transport function for the non-bile acid substrate estrone sulfate, suggesting this position may be part of a region in the transporter critical and specific for bile acid substrate recognition. Accordingly, our study indicates functionally important polymorphisms in NTCP exist and that the likelihood of being carriers of such polymorphisms is dependent on ethnicity.

Hoffmann, C., M. Pop, et al. (2004). "The Yersinia pseudotuberculosis Cytotoxic Necrotizing Factor (CNFY) Selectively Activates RhoA." *J. Biol. Chem.* **279**(16): 16026-16032.

<http://www.jbc.org/cgi/content/abstract/279/16/16026>

The cytotoxic necrotizing factors (CNF)1 and CNF2 from pathogenic Escherichia coli strains activate RhoA, Rac1, and Cdc42 by deamidation of Gln63 (RhoA) or Gln61 (Rac and Cdc42). Recently, a novel cytotoxic necrotizing factor termed CNFY was identified in Yersinia pseudotuberculosis strains (Lockman, H. A., Gillespie, R. A., Baker, B. D., and Shakhnovich, E. (2002) Infect. Immun. 70, 2708-2714). We amplified the cnfy gene from genomic DNA of Y. pseudotuberculosis, cloned and expressed the recombinant protein, and studied its activity. Recombinant GST-CNFY induced morphological changes in HeLa cells and caused an upward shift of RhoA in SDS-PAGE, as is known for GST-CNF1 and GST-CNF2. Mass spectrometric analysis of GST-CNFY-treated RhoA confirmed deamidation at Glu63. Treatment of RhoA, Rac1,

and Cdc42 with GST-CNFY decreased their GTPase activities, indicating that all of these Rho proteins could serve as substrates for GST-CNFY in vitro. In contrast, RhoA, but not Rac or Cdc42, was the substrate of GST-CNFY in culture cells. GST-CNFY caused marked stress fiber formation in HeLa cells after 2 h. In contrast to GST-CNF1, formation of filopodia or lamellipodia was not induced with GST-CNFY. Accordingly, effector pull-down experiments with lysates of toxin-treated cells revealed strong activation of RhoA but no activation of Rac1 or Cdc42 after 6 h of GST-CNFY-treatment. Moreover, in rat hippocampal neurons, GST-CNFY results in the retraction of neurites, indicating RhoA activation. In contrast, no activation of Rac or Cdc42 was found. Altogether, our data suggest that CNFY from *Y. pseudotuberculosis* is a strong, selective activator of RhoA, which can be used as a powerful tool for constitutive RhoA activation without concomitant activation of Rac1 or Cdc42.

Hoffmann, L., S. Maury, et al. (2003). "Purification, Cloning, and Properties of an Acyltransferase Controlling Shikimate and Quinate Ester Intermediates in Phenylpropanoid Metabolism." J. Biol. Chem. **278**(1): 95-103.

<http://www.jbc.org/cgi/content/abstract/278/1/95>

A protein hydrolyzing hydroxycinnamoyl-CoA esters has been purified from tobacco stem extracts by a series of high pressure liquid chromatography steps. The determination of its N-terminal amino acid sequence allowed design of primers permitting the corresponding cDNA to be cloned by PCR. Sequence analysis revealed that the tobacco gene belongs to a plant acyltransferase gene family, the members of which have various functions. The tobacco cDNA was expressed in bacterial cells as a recombinant protein fused to glutathione S-transferase. The fusion protein was affinity-purified and cleaved to yield the recombinant enzyme for use in the study of catalytic properties. The enzyme catalyzed the synthesis of shikimate and quinate esters shown recently to be substrates of the cytochrome P450 3-hydroxylase involved in phenylpropanoid biosynthesis. The enzyme has been named hydroxycinnamoyl-CoA: shikimate/quinat hydroxycinnamoyltransferase. We show that p-coumaroyl-CoA and caffeoyl-CoA are the best acyl group donors and that the acyl group is transferred more efficiently to shikimate than to quinate. The enzyme also catalyzed the reverse reaction, i.e. the formation of caffeoyl-CoA from chlorogenate (5-O-caffeoyl quinate ester). Thus, hydroxycinnamoyl-CoA:shikimate/quinat hydroxycinnamoyltransferase appears to control the biosynthesis and turnover of major plant phenolic compounds such as lignin and chlorogenic acid.

Holmborn, K., J. Ledin, et al. (2004). "Heparan Sulfate Synthesized by Mouse Embryonic Stem Cells Deficient in NDST1 and NDST2 Is 6-O-Sulfated but Contains No N-Sulfate Groups." J. Biol. Chem. **279**(41): 42355-42358.

<http://www.jbc.org/cgi/content/abstract/279/41/42355>

Heparan sulfate structure differs significantly between various cell types and during different developmental stages. The diversity is created during biosynthesis by sulfotransferases, which add sulfate groups to the growing chain, and a C5-epimerase, which converts selected glucuronic acid residues to iduronic acid. All these modifications are believed to depend on initial glucosamine N-sulfation carried out by the enzyme glucosaminyl N-deacetylase/N-sulfotransferase (NDST). Here we report that heparan sulfate synthesized by mouse embryonic stem cells deficient in NDST1 and NDST2 completely lacks N-sulfation but still contains 6-O-sulfate groups, demonstrating that 6-O-sulfation can occur without prior N-sulfation. Reverse transcriptase-PCR analysis indicates that all three identified 6-O-sulfotransferases are expressed by the cells, 6-O-sulfotransferase-1 being the dominating form. The 6-O-sulfated polysaccharide lacking N-sulfate groups also contains N-unsubstituted glucosamine units, raising questions

about how these units are generated.

Horibata, Y., K. Sakaguchi, et al. (2004). "Unique Catabolic Pathway of Glycosphingolipids in a Hydrozoan, *Hydra magnipapillata*, Involving Endoglycoceramidase." *J. Biol. Chem.* **279**(32): 33379-33389.

<http://www.jbc.org/cgi/content/abstract/279/32/33379>

Endoglycoceramidase (EGCase; EC 3.2.1.123) is an enzyme capable of cleaving the glycosidic linkage between oligosaccharides and ceramides of various glycosphingolipids. We detected strong EGCase activity in animals belonging to Cnidaria, Mollusca, and Annelida and cloned the enzyme from a hydra, *Hydra magnipapillata*. The hydra EGCase, consisting of 517 amino acid residues, showed 19.2% and 50.2% identity to the *Rhodococcus* and jellyfish EGCases, respectively. The recombinant hydra enzyme, expressed in CHOP (Chinese hamster ovary cells expressing polyoma LT antigen) cells, hydrolyzed [¹⁴C]GM1a to produce [¹⁴C]ceramide with a pH optimum at 3.0-3.5. Whole mount in situ hybridization and immunocytochemical analysis revealed that EGCase was widely expressed in the endodermal layer, especially in digestive cells. GM1a injected into the gastric cavity was incorporated and then directly catabolized by EGCase to produce GM1a-oligosaccharide and ceramide, which were further degraded by exoglycosidases and ceramidase, respectively. However, hydra exoglycosidases did not hydrolyze GM1a directly. These results indicate that the EGCase is indispensable for the catabolic processing of dietary glycosphingolipids in hydra, demonstrating the unique catabolic pathway for glycosphingolipids in the animal.

Huynh, T. T., V. T. Huynh, et al. (2003). "Gene Knockdown of γ -Glutamylcysteine Synthetase by RNAi in the Parasitic Protozoa *Trypanosoma brucei* Demonstrates That It Is an Essential Enzyme." *J. Biol. Chem.* **278**(41): 39794-39800.

<http://www.jbc.org/cgi/content/abstract/278/41/39794>

The parasitic protozoa *Trypanosoma brucei* utilizes a novel cofactor (trypanothione, T(SH)₂), which is a conjugate of GSH and spermidine, to maintain cellular redox balance. γ -Glutamylcysteine synthetase (γ -GCS) catalyzes the first step in the biosynthesis of GSH. To evaluate the importance of thiol metabolism to the parasite, RNAi methods were used to knock down gene expression of γ -GCS in procyclic *T. brucei* cells. Induction of γ -GCS RNAi with tetracycline led to cell death within 4-6 days post-induction. Cell death was preceded by the depletion of the γ -GCS protein and RNA and by the loss of the cellular pools of GSH and T(SH)₂. The addition of GSH (80 μ M) to cell cultures rescued the RNAi cell death phenotype and restored the intracellular thiol pools to wild-type levels. Treatment of cells with buthionine sulfoximine (BSO), an enzyme-activated inhibitor of γ -GCS, also resulted in cell death. However, the toxicity of the inhibitor was not reversed by GSH, suggesting that BSO has more than one cellular target. BSO depletes intracellular thiols to a similar extent as γ -GCS RNAi; however, addition of GSH did not restore the pools of GSH and T(SH)₂. These data suggest that BSO also acts to inhibit the transport of GSH or its peptide metabolites into the cell. The ability of BSO to inhibit both synthesis and transport of GSH likely makes it a more effective cytotoxic agent than an inhibitor with a single mode of action. Finally the potential for the T(SH)₂ biosynthetic enzymes to be regulated in response to reduced thiol levels was studied. The expression levels of ornithine decarboxylase and of S-adenosylmethionine decarboxylase, two essential enzymes in spermidine biosynthesis, remained constant in induced γ -GCS RNAi cell lines.

Inacio, A., A. L. Silva, et al. (2004). "Nonsense Mutations in Close Proximity to the Initiation Codon Fail to Trigger Full Nonsense-mediated mRNA Decay." *J. Biol. Chem.* **279**(31): 32170-32180.

<http://www.jbc.org/cgi/content/abstract/279/31/32170>

Nonsense-mediated mRNA decay (NMD) is a surveillance mechanism that degrades mRNAs containing premature translation termination codons. In mammalian cells, a termination codon is ordinarily recognized as "premature" if it is located greater than 50-54 nucleotides 5' to the final exon-exon junction. We have described a set of naturally occurring human β -globin gene mutations that apparently contradict this rule. The corresponding β -thalassemia genes contain nonsense mutations within exon 1, and yet their encoded mRNAs accumulate to levels approaching wild-type β -globin (β WT) mRNA. In the present report we demonstrate that the stabilities of these mRNAs with nonsense mutations in exon 1 are intermediate between β WT mRNA and β -globin mRNA carrying a prototype NMD-sensitive mutation in exon 2 (codon 39 nonsense; β 39). Functional analyses of these mRNAs with 5'-proximal nonsense mutations demonstrate that their relative resistance to NMD does not reflect abnormal RNA splicing or translation re-initiation and is independent of promoter identity and erythroid specificity. Instead, the proximity of the nonsense codon to the translation initiation AUG constitutes a major determinant of NMD. Positioning a termination mutation at the 5' terminus of the coding region blunts mRNA destabilization, and this effect is dominant to the "50-54 nt boundary rule." These observations impact on current models of NMD.

Inagaki, T., S. Suzuki, et al. (2003). "The Retinoic Acid-responsive Proline-rich Protein Is Identified in Promyeloleukemic HL-60 Cells." *J. Biol. Chem.* **278**(51): 51685-51692.

<http://www.jbc.org/cgi/content/abstract/278/51/51685>

To identify new genes that retinoic acid activates, we employed an mRNA differential display technique and screened for genes that are differentially expressed in promyeloleukemic HL-60 cells incubated in the presence of all-trans-retinoic acid (ATRA) compared with the absence of ATRA. We cloned the coding region of a retinoic acid-induced gene from a human thymus library, which was the mRNA encoding the 666-amino acid human homologue of mouse proline-rich protein 76. We have designated it RARP1 (retinoic acid response proline-rich protein 1). Transcription of an $\sim$ 2.4-kbp mRNA occurred mainly in organs with immune functions, such as thymus, spleen, and peripheral leukocytes. Cycloheximide blocked the ATRA-induced expression. In megakaryocyte-like human erythroleukemia HEL cells, the amount of RARP1 mRNA was high, but it was low in human T-lymphoblastoid Jurkat cells. A specific antibody against RARP1 recognized a 110-kDa protein, which accumulates after incubation of HL-60 cells with ATRA. In immunohistochemical experiments, strong RARP1 staining was observed in the megakaryocytes of bone marrow and spleen, and heterogeneous stain was seen in thymus. Transcriptional studies showed that RARP1 expression impaired the transactivation through activator protein1 and serum response element in all cell lines we checked, whereas it did not affect the transactivation through cAMP-response element in the same cell lines. Further analysis demonstrated that proline-rich regions of RARP1 are the functional regions regulated for suppression of activator protein1 transactivation. These data suggest that ATRA-inducible RARP1 selectively affects signal transduction and may contribute to myeloid and megakaryocytic differentiation.

Ishisaki, A., H. Hayashi, et al. (2003). "Human Umbilical Vein Endothelium-derived Cells Retain Potential

to Differentiate into Smooth Muscle-like Cells." J. Biol. Chem. **278**(2): 1303-1309.

<http://www.jbc.org/cgi/content/abstract/278/2/1303>

Mouse embryonic stem-derived cells were recently shown to differentiate into endothelial and smooth muscle cells. In the present study, we investigated whether human umbilical vein endothelium-derived cells retain the potential to differentiate into smooth muscle cells. Examination of biochemical markers, including basic calponin, SM22[alpha], prostaglandin E synthase, von Willebrand factor, and PECAM-1, as well as cell contractility, showed that whereas endothelium-derived cells cultured with fibroblast growth factor can be characterized as endothelial cells, when deprived of fibroblast growth factor, a significant fraction differentiates into smooth muscle-like cells. Reapplication of fibroblast growth factor reversed this differentiation. Activin A was up-regulated in fibroblast growth factor-deprived, endothelium-derived cells; moreover, the inhibitory effects of exogenous follistatin and overexpressed Smad7 on smooth muscle-like differentiation confirmed that the differentiation was driven by activin A signaling. These findings indicate that when deprived of fibroblast growth factor, human umbilical vein endothelium-derived cells are capable of differentiating into smooth muscle-like cells through activin A-induced, Smad-dependent signaling, and that maintenance of the endothelial cell phenotype and differentiation into smooth muscle-like cells are reciprocally controlled by fibroblast growth factor-1 and activin A.

Iwasaki, H., K. Chiba, et al. (2002). "Molecular Characterization of the Starfish Inositol 1,4,5-Trisphosphate Receptor and Its Role during Oocyte Maturation and Fertilization." J. Biol. Chem. **277**(4): 2763-2772.

<http://www.jbc.org/cgi/content/abstract/277/4/2763>

The release of calcium ions (Ca²⁺) from their intracellular stores is essential for the fertilization of oocytes of various species. The calcium pools can be induced to release Ca²⁺ via two main types of calcium channel receptor: the inositol 1,4,5-trisphosphate receptor (IP3R) and the ryanodine receptor. Starfish oocytes have often been used to study intracellular calcium mobilization during oocyte maturation and fertilization, but how the intracellular calcium channels contribute to intracellular calcium mobilization has never been understood fully, because these molecules have not been identified and no specific inhibitors of these channels have ever been found. In this study, we utilized a novel IP3R antagonist, the "IP3 sponge," to investigate the role of IP3 during fertilization of the starfish oocyte. The IP3 sponge strongly and specifically competed with endogenous IP3R for binding to IP3. By injecting IP3 sponge into starfish oocyte, the increase in intracellular calcium and formation of the fertilization envelope were both dramatically blocked, although oocyte maturation was not blocked. To investigate the role of IP3R in the starfish oocyte more precisely, we cloned IP3R from the ovary of starfish, and the predicted amino acid sequence indicated that the starfish IP3R has 58-68% identity to mammalian IP3R types 1, 2, and 3. We then raised antibodies that recognize starfish IP3R, and use of the antibodies to perform immunoblot analysis revealed that the level of expression of IP3R remained unchanged throughout oocyte maturation. An immunocytochemical study, however, revealed that the distribution of starfish IP3R changes during oocyte maturation.

Jackers, P., G. Szalai, et al. (2004). "Ets-dependent Regulation of Target Gene Expression during Megakaryopoiesis." J. Biol. Chem. **279**(50): 52183-52190.

<http://www.jbc.org/cgi/content/abstract/279/50/52183>

Megakaryopoiesis is the process by which hematopoietic stem cells in the bone marrow differentiate into mature megakaryocytes. The expression of megakaryocytic genes during megakaryopoiesis is controlled by specific transcription factors. Fli-1 and GATA-1 transcription factors are required for development of megakaryocytes and promoter analysis has defined *in vitro* functional binding sites for these factors in several megakaryocytic genes, including GPIIb, GPIX, and C-MPL. Herein, we utilize chromatin immunoprecipitation to examine the presence of Ets-1, Fli-1, and GATA-1 on these promoters *in vivo*. Fli-1 and Ets-1 occupy the promoters of GPIIb, GPIX, and C-MPL genes in both Meg-01 and CMK11-5 cells. Whereas GPIIb is expressed in both Meg-01 and CMK11-5 cells, GPIX and C-MPL are only expressed in the more differentiated CMK11-5 cells. Thus, *in vivo* occupancy by an Ets factor is not sufficient to promote transcription of some megakaryocytic genes. GATA-1 and Fli-1 are both expressed in CMK11-5 cells and co-occupy the GPIX and C-MPL promoters. Transcription of all three megakaryocytic genes is correlated with the presence of acetylated histone H3 and phosphorylated RNA polymerase II on their promoters. We also show that exogenous expression of GATA-1 in Meg-01 cells leads to the expression of endogenous *c-mpl* and *gpiX* mRNA. Whereas GPIIb, GPIX, and C-MPL are direct target genes for Fli-1, both Fli-1 and GATA-1 are required for formation of an active transcriptional complex on the C-MPL and GPIX promoters *in vivo*. In contrast, GPIIb expression appears to be independent of GATA-1 in Meg-01 cells.

Jackson-Hayes, L., S. Song, et al. (2003). "A Thyroid Hormone Response Unit Formed between the Promoter and First Intron of the Carnitine Palmitoyltransferase- α Gene Mediates the Liver-specific Induction by Thyroid Hormone." *J. Biol. Chem.* **278**(10): 7964-7972.

<http://www.jbc.org/cgi/content/abstract/278/10/7964>

Carnitine palmitoyltransferase-I (CPT-I) catalyzes the rate-controlling step of fatty acid oxidation. CPT-I converts long-chain fatty acyl-CoAs to acylcarnitines for translocation across the mitochondrial membrane. The mRNA levels and enzyme activity of the liver isoform, CPT-I[α], are greatly increased in the liver of hyperthyroid animals. Thyroid hormone (T3) stimulates CPT-I[α] transcription far more robustly in the liver than in non-hepatic tissues. We have shown that the thyroid hormone receptor (TR) binds to a thyroid hormone response element (TRE) located in the CPT-I[α] promoter. In addition, elements in the first intron participate in the T3 induction of CPT-I[α] gene expression, but the CPT-I[α] intron alone cannot confer a T3 response. We found that deletion of sequences in the first intron between +653 and +744 decreased the T3 induction of CPT-I[α]. Upstream stimulatory factor (USF) and CCAAT enhancer binding proteins (C/EBPs) bind to elements within this region, and these factors are required for the T3 response. The binding of TR and C/EBP to the CPT-I[α] gene *in vivo* was shown by the chromatin immunoprecipitation assay. We determined that TR can physically interact with USF-1, USF-2, and C/EBP[α]. Transgenic mice were created that carry CPT-I[α]-luciferase transgenes with or without the first intron of the CPT-I[α] gene. In these mouse lines, the first intron is required for T3 induction as well as high levels of hepatic expression. Our data indicate that the T3 stimulates CPT-I[α] gene expression in the liver through a T3 response unit consisting of the TRE in the promoter and additional factors, C/EBP and USF, bound in the first intron.

Jackson, M., J. W. Baird, et al. (2002). "Cloning and Characterization of Ehox, a Novel Homeobox Gene Essential for Embryonic Stem Cell Differentiation." *J. Biol. Chem.* **277**(41): 38683-38692.

<http://www.jbc.org/cgi/content/abstract/277/41/38683>

We report here the identification and characterization of a novel paired-like homeobox-containing gene (Ehox). This gene, identified in embryonic stem (ES) cells, is differentially expressed during

in vitro ES cell differentiation. We have assessed Ebox function using the ES cell in vitro differentiation system. This has involved molecular and biological analyses of the effects of sense or antisense Ebox expression (using episomal vectors) on ES cell differentiation. Analysis of antisense Ebox-expressing ES cells indicates that they are unable to express marker genes associated with hematopoietic, endothelial, or cardiac differentiation following removal of leukemia inhibitory factor. In contrast, overexpression of Ebox using the sense construct accelerated the appearance of these differentiation markers. ES cell self-renewal and differentiation assays reveal that inhibition of Ebox activity results in the maintenance of a stem cell phenotype in limiting concentrations of leukemia inhibitory factor and the almost complete impairment of the cardiomyocyte differentiation capacity of these cells. We therefore conclude that Ebox is a novel homeobox-containing gene that is essential for the earliest stages of murine ES cell differentiation.

Jeanson, L. and J.-F. Mouscadet (2002). "Ku Represses the HIV-1 Transcription. IDENTIFICATION OF A PUTATIVE Ku BINDING SITE HOMOLOGOUS TO THE MOUSE MAMMARY TUMOR VIRUS NRE1 SEQUENCE IN THE HIV-1 LONG TERMINAL REPEAT." *J. Biol. Chem.* **277**(7): 4918-4924.

<http://www.jbc.org/cgi/content/abstract/277/7/4918>

Ku has been implicated in nuclear processes, including DNA break repair, transcription, V(D)J recombination, and telomere maintenance. Its mode of action involves two distinct mechanisms: one in which a nonspecific binding occurs to DNA ends and a second that involves a specific binding to negative regulatory elements involved in transcription repression. Such elements were identified in mouse mammary tumor virus and human T cell leukemia virus retroviruses. The purpose of this study was to investigate a role for Ku in the regulation of human immunodeficiency virus (HIV)-1 transcription. First, HIV-1 LTR activity was studied in CHO-K1 cells and in CHO-derived xrs-6 cells, which are devoid of Ku80. LTR-driven expression of a reporter gene was significantly increased in xrs-6 cells. This enhancement was suppressed after re-expression of Ku80. Second, transcription of HIV-1 was followed in U1 human cells that were depleted in Ku by using a Ku80 antisense RNA. Ku depletion led to an increase of both HIV-1 mRNA synthesis and viral production compared with the parent cells. These results demonstrate that Ku acts as a transcriptional repressor of HIV-1 expression. Finally, a putative Ku-specific binding site was identified within the negative regulatory region of the HIV-1 long terminal repeat, which may account for this repression of transcription.

Jenkins, R. H., G. J. Thomas, et al. (2004). "Myofibroblastic Differentiation Leads to Hyaluronan Accumulation through Reduced Hyaluronan Turnover." *J. Biol. Chem.* **279**(40): 41453-41460.

<http://www.jbc.org/cgi/content/abstract/279/40/41453>

During the initiation and progression of fibrosis there is extensive differentiation of cells to a myofibroblastic phenotype. Because the synthesis of hyaluronan (HA) was recently linked to oncogenic epithelial-mesenchymal transformation, the present study investigated whether increased HA synthesis was also associated with myofibroblastic differentiation. HA synthesis and size were measured by incorporation of [³H]glucosamine, ion exchange, and size exclusion chromatography. Hyaluronan synthase (HAS) or hyaluronidase (HYAL) mRNA levels were assessed by reverse transcription-PCR. HYAL was detected by immunoblotting and the degradation of [³H]HA. Between 2- and 3-fold more HA appeared in the conditioned medium and became associated with the cells upon myofibroblastic differentiation. Inhibition of HAS and examination of HAS mRNA expression demonstrated that this was not the result of increased synthesis of HA or the induction of HAS 2. After differentiation, however, myofibroblasts

metabolized exogenously supplied [3H]HA at a slower rate than fibroblasts and expressed lower levels of both HYAL 1 and HYAL 2 mRNA. Immunoblotting revealed more HYAL 1 and 2 in the myofibroblast conditioned medium. After acidification, however, there was no difference in HA degradation. This suggests that much of the released HYAL is inactive and that the observed differences in HA degradation are caused by cell-associated rather than secreted activity. This was confirmed by immunohistochemical staining for HYAL 1 and HYAL 2. This finding indicates the potential importance of the HYAL enzymes in controlling fibrotic progression and contrasts HA synthesis as a mediator of oncogenic transformation with that of HA degradation controlling fibrogenic differentiation.

Jhandier, M. N., E. A. Kruglov, et al. (2005). "Portal fibroblasts regulate the proliferation of bile duct epithelia via expression of NTPDase2." J. Biol. Chem.: M412371200.

<http://www.jbc.org/cgi/content/abstract/M412371200v1>

Bile duct epithelia are the target of a number "cholangiopathies" characterized by disordered bile ductular proliferation. While mechanisms for bile ductular proliferation are unknown, recent evidence suggests that extracellular nucleotides regulate cell proliferation via activation of P2Y receptors. Portal fibroblasts may regulate bile duct epithelial P2Y receptors via expression of the ecto-nucleotidase NTPDase2. Thus, we tested the hypothesis that portal fibroblasts regulate bile duct epithelial proliferation via expression of NTPDase2. We generated a novel co-culture model of Mz-ChA-1 human cholangiocarcinoma cells and primary portal fibroblasts. Cell proliferation was measured by bromodeoxyuridine uptake. NTPDase2 expression was assessed by immunofluorescence and quantitative real-time RT-PCR. NTPDase2 expression in portal fibroblasts was blocked using siRNA. NTPDase2 overexpression in portal myofibroblasts isolated from bile duct ligated rats was achieved by cDNA transfection. Co-culture of Mz-ChA-1 cells with PF decreased their proliferation to 26% of control. Similar decreases in Mz-ChA-1 proliferation were induced by the soluble ecto-nucleotidase apyrase and the P2 receptor inhibitor suramin. The proliferation of Mz-ChA-1 cells returned to baseline when NTPDase2 expression in portal fibroblasts was inhibited using NTPDase2-specific siRNA. Untransfected portal myofibroblasts lacking NTPDase2 had no effect on Mz-ChA-1 proliferation, yet portal myofibroblasts transfected with NTPDase2 cDNA inhibited Mz-ChA-1 proliferation. We conclude that, portal fibroblasts inhibit bile ductular proliferation via expression of NTPDase2 and blockade of P2Y activation. Loss of NTPDase2 may mediate the bile ductular proliferation typical of obstructive cholestasis. This novel crosstalk signaling pathway may mediate pathologic alterations in bile ductular proliferation in other cholangiopathic conditions.

Jiang, H., R. S. Peterson, et al. (2002). "A Requirement for the CD44 Cytoplasmic Domain for Hyaluronan Binding, Pericellular Matrix Assembly, and Receptor-mediated Endocytosis in COS-7 Cells." J. Biol. Chem. **277**(12): 10531-10538.

<http://www.jbc.org/cgi/content/abstract/277/12/10531>

CD44-negative COS-7 cells were transfected with expression constructs for CD44H (the predominant CD44 isoform), CD44E (epithelial isoform), or truncation mutant derivatives lacking the carboxyl-terminal 67 amino acids of the cytoplasmic domain, CD44H[Delta]67 and CD44E[Delta]67. The truncation mutant CD44H[Delta]67 is identical to a naturally occurring alternatively spliced "short tail" CD44 isoform (CD44st), which incorporates exon 19 in place of exon 20. CD44st lacks intracellular signaling motifs as well as protein domains necessary for interaction with cytoskeletal components. Transfection of COS-7 cells with each construct yielded equivalent levels of mRNA expression, whereas no CD44 expression was observed in parental, nontransfected COS-7 cells. Western analysis and immunostaining of COS-7 transfectants

confirmed CD44 protein expression of the truncation mutant derivatives. COS-7 cells transfected with CD44H or CD44E gained the capacity to bind fluorescein-conjugated HA (fl-HA) and assemble HA-dependent pericellular matrices in the presence of exogenously added HA and proteoglycan. In addition, the CD44H- and CD44E-transfected cells were able to internalize surface-bound fl-HA. COS-7 cells transfected with the vector alone or with either of the mutant CD44 isoforms, CD44H[Delta]67 or CD44E[Delta]67, did not exhibit the capacity to assemble pericellular matrices or to bind and internalize the fl-HA. Cotransfection of CD44[Delta]67 mutants together with CD44H reduced the size of the HA-dependent pericellular matrices. Transfection of bovine articular chondrocytes with CD44[Delta]67 also inhibited pericellular matrix assembly. Collectively, these results indicate an obligatory requirement for the CD44 receptor cytoplasmic domain for ligand (HA) binding, formation and retention of the pericellular matrix, as well as CD44-mediated endocytosis of HA. In addition, the results suggest a potential regulatory role for the differentially expressed alternatively spliced short tail CD44 isoform.

Johnson, D. E. and C. C. Richardson (2003). "A Covalent Linkage between the Gene 5 DNA Polymerase of Bacteriophage T7 and Escherichia coli Thioredoxin, the Processivity Factor: FATE OF THIOREDOXIN DURING DNA SYNTHESIS." *J. Biol. Chem.* **278**(26): 23762-23772.

<http://www.jbc.org/cgi/content/abstract/278/26/23762>

Gene 5 protein (gp5) of bacteriophage T7 is a non-processive DNA polymerase, which acquires high processivity by binding to Escherichia coli thioredoxin. The gene 5 protein-thioredoxin complex (gp5/trx) polymerizes thousands of nucleotides before dissociating from a primer-template. We have engineered a disulfide linkage between the gene 5 protein and thioredoxin within the binding surface of the two proteins. The polymerase activity of the covalently linked complex (gp5-S-S-trx) is similar to that of gp5/trx on poly(dA)/oligo(dT). However, gp5-S-S-trx has only one third the polymerase activity of gp5/trx on single-stranded M13 DNA. gp5-S-S-trx has difficulty polymerizing nucleotides through sites of secondary structure on M13 DNA and stalls at these sites, resulting in lower processivity. However, gp5-S-S-trx has an identical processivity and rate of elongation when E. coli single-stranded DNA-binding protein (SSB protein) is used to remove secondary structure from M13 DNA. Upon completing synthesis on a DNA template lacking secondary structure, both complexes recycle intact, without dissociation of the processivity factor, to initiate synthesis on a new DNA template. However, a complex stalled at secondary structure becomes unstable, and both subunits dissociate from each other as the polymerase prematurely releases from M13 DNA.

Joosten, M., M. Blazquez-Domingo, et al. (2004). "Translational Control of Putative Protooncogene Nm23-M2 by Cytokines via Phosphoinositide 3-Kinase Signaling." *J. Biol. Chem.* **279**(37): 38169-38176.

<http://www.jbc.org/cgi/content/abstract/279/37/38169>

The expansion and differentiation of hematopoietic progenitors is regulated by cytokine and growth factor signaling. To examine how signal transduction controls the gene expression program required for progenitor expansion, we screened ATLAS filters with polysome-associated mRNA derived from erythroid progenitors stimulated with erythropoietin and/or stem cell factor. The putative proto-oncogene nucleoside diphosphate kinase B (ndpk-B or nm23-M2) was identified as an erythropoietin and stem cell factor target gene. Factor-induced expression of nm23-M2 was regulated specifically at the level of polysome association by a phosphoinositide 3-kinase-dependent mechanism. Identification of the transcription initiation site revealed that nm23-M2 mRNA starts with a terminal oligopyrimidine sequence, which is known to render mRNA translation dependent on mitogenic factors. Recently, the nm23-M2 locus was identified as a

common leukemia retrovirus integration site, suggesting that it plays a role in leukemia development. The expression of Nm23 from a retroviral vector in the absence of its 5'-untranslated region caused constitutive polysome association of nm23-M2. Polysome-association and protein expression of endogenous nm23-M2 declined during differentiation of erythroid progenitors, suggesting a role for Nm23-M2 in progenitor expansion. Taken together, nm23-m2 exemplifies that cytokine-dependent control of translation initiation is an important mechanism of gene expression regulation.

Jurado, L. A., S. Song, et al. (2002). "Conserved Amino Acids within CCAAT Enhancer-binding Proteins (C/EBPalpha and beta) Regulate Phosphoenolpyruvate Carboxykinase (PEPCK) Gene Expression." J. Biol. Chem. **277**(31): 27606-27612.

<http://www.jbc.org/cgi/content/abstract/277/31/27606>

Thyroid hormone and cAMP stimulate transcription of the gene for phosphoenolpyruvate carboxykinase (PEPCK). CCAAT enhancer-binding proteins (C/EBP[alpha] and [beta]) are involved in multiple aspects of the nutritional, developmental and hormonal regulation of PEPCK gene expression. Previously, we have identified a thyroid hormone response element in the PEPCK promoter and demonstrated that C/EBP proteins bound to the P3(I) site are participants in the induction of PEPCK gene expression by thyroid hormone and cAMP. Here, we identify several peptide regions within the transactivation domain of C/EBP[alpha] that enhance the ability of T3 to stimulate gene transcription. We also demonstrate that several conserved amino acids in the transactivation domain of C/EBP[alpha] and C/EBP[beta] are required for the stimulation of basal gene expression and identify amino acids within C/EBP[beta] that participate in the cAMP induction of the PEPCK gene. Finally, we show that the CREB-binding protein (CBP) enhanced the induction of PEPCK gene transcription by thyroid hormone and that CBP is associated with the PEPCK gene in vivo. Our results indicate that both C/EBP proteins and CBP participate in the regulation of PEPCK gene transcription by thyroid hormone.

Kaneto, H., T.-a. Matsuoka, et al. (2005). "A Crucial Role of MafA as a Novel Therapeutic Target for Diabetes." J. Biol. Chem. **280**(15): 15047-15052.

<http://www.jbc.org/cgi/content/abstract/280/15/15047>

MafA, a recently isolated pancreatic {beta}-cell-specific transcription factor, is a potent activator of insulin gene transcription. In this study, we show that MafA overexpression, together with PDX-1 (pancreatic and duodenal homeobox factor-1) and NeuroD, markedly increases insulin gene expression in the liver. Consequently, substantial amounts of insulin protein were induced by such combination. Furthermore, in streptozotocin-induced diabetic mice, MafA overexpression in the liver, together with PDX-1 and NeuroD, dramatically ameliorated glucose tolerance, while combination of PDX-1 and NeuroD was much less effective. These results suggest a crucial role of MafA as a novel therapeutic target for diabetes.

Kaneto, H., A. Sharma, et al. (2002). "Induction of c-Myc Expression Suppresses Insulin Gene Transcription by Inhibiting NeuroD/BETA2-mediated Transcriptional Activation." J. Biol. Chem. **277**(15): 12998-13006.

<http://www.jbc.org/cgi/content/abstract/277/15/12998>

Insulin biosynthesis and secretion are critical for pancreatic [beta]-cell function, but both are impaired under diabetic conditions. We have found that hyperglycemia induces the expression of the basic helix-loop-helix transcription factor c-Myc in islets in several different diabetic models. To examine the possible implication of c-Myc in [beta]-cell dysfunction, c-Myc was overexpressed in isolated rat islets using adenovirus. Adenovirus-mediated c-Myc overexpression suppressed both insulin gene transcription and glucose-stimulated insulin secretion. Insulin protein content, determined by immunostaining, was markedly decreased in c-Myc-overexpressing cells. In gel-shift assays c-Myc bound to the E-box in the insulin gene promoter region. Furthermore, in [beta]TC1, MIN6, and HIT-T15 cells and primary rat islets, wild type insulin gene promoter activity was dramatically decreased by c-Myc overexpression, whereas the activity of an E-box mutated insulin promoter was not affected. In HeLa and HepG2 cells c-Myc exerted a suppressive effect on the insulin promoter activity only in the presence of NeuroD/BETA2 but not PDX-1. Both c-Myc and NeuroD can bind the E-box element in the insulin promoter, but unlike NeuroD, the c-Myc transactivation domain lacked the ability to activate insulin gene expression. Additionally p300, a co-activator of NeuroD, did not function as a co-activator of c-Myc. In conclusion, increased expression of c-Myc in [beta]-cells suppresses the insulin gene transcription by inhibiting NeuroD-mediated transcriptional activation. This mechanism may explain some of the [beta]-cell dysfunction found in diabetes.

Kaneto, H., K. Suzuma, et al. (2002). "Involvement of Protein Kinase C beta 2 in c-myc Induction by High Glucose in Pancreatic beta -Cells." *J. Biol. Chem.* **277**(5): 3680-3685.

<http://www.jbc.org/cgi/content/abstract/277/5/3680>

The expression of the basic helix-loop-helix transcription factor c-Myc is induced in pancreatic islets of several different diabetic model animals and is possibly involved in suppression of the insulin gene transcription. In this study, we found that activity of protein kinase C is increased by high glucose, preceding the induction of c-myc expression and that PKC [beta]2 specifically regulates c-myc expression in pancreatic [beta]-cells. Since PKC [alpha], [beta]2, [delta], [epsilon], and [zeta] were expressed in rat pancreatic islets, we prepared each wild type (WT) and dominant negative type (DN) PKC isoform ([alpha], [beta]2, [delta], [epsilon], and [zeta])-expressing adenovirus to examine the effect of each PKC isoform on c-myc expression. In isolated rat pancreatic islets, adenovirus-mediated overexpression of WT PKC [beta]2, but not other PKC isoforms, markedly increased c-myc expression. Moreover, c-myc induction by high glucose was suppressed by adenovirus-mediated overexpression of DN PKC [beta]2 but not by other DN PKC isoforms. Finally, adenovirus-mediated overexpression of WT PKC [beta]2, but not of other PKC isoforms, leads to suppression of the insulin gene transcription in pancreatic islets. These results suggest that at least some of the reduction of insulin gene transcription found in the diabetic state is mediated by PKC [beta]2 regulation of c-myc expression.

Kaneto, H., G. Xu, et al. (2002). "Involvement of c-Jun N-terminal Kinase in Oxidative Stress-mediated Suppression of Insulin Gene Expression." *J. Biol. Chem.* **277**(33): 30010-30018.

<http://www.jbc.org/cgi/content/abstract/277/33/30010>

Oxidative stress, which is found in pancreatic [beta]-cells in the diabetic state, suppresses insulin gene transcription and secretion, but the signaling pathways involved in the [beta]-cell dysfunction induced by oxidative stress remain unknown. In this study, subjecting rat islets to oxidative stress activates JNK, p38 MAPK, and protein kinase C, preceding the decrease of insulin gene expression. Adenovirus-mediated overexpression of dominant-negative type (DN) JNK, but not the p38 MAPK inhibitor SB203580 nor the protein kinase C inhibitor GF109203X, protected insulin gene expression and secretion from oxidative stress. Moreover, wild type JNK

overexpression suppressed both insulin gene expression and secretion. These results were correlated with changes in the binding of the important transcription factor PDX-1 to the insulin promoter; adenoviral overexpression of DN-JNK preserved PDX-1 DNA binding activity in the face of oxidative stress, whereas wild type JNK overexpression decreased PDX-1 DNA binding activity. Furthermore, to examine whether suppression of the JNK pathway can protect [beta]-cells from the toxic effects of hyperglycemia, rat islets were infected with DN-JNK expressing adenovirus or control adenovirus and transplanted under renal capsules of streptozotocin-induced diabetic nude mice. In mice receiving DN-JNK overexpressing islets, insulin gene expression in islet grafts was preserved, and hyperglycemia was ameliorated compared with control mice. In conclusion, activation of JNK is involved in the reduction of insulin gene expression by oxidative stress, and suppression of the JNK pathway protects [beta]-cells from oxidative stress.

Kang, H.-Y., K.-E. Huang, et al. (2002). "Differential Modulation of Androgen Receptor-mediated Transactivation by Smad3 and Tumor Suppressor Smad4." *J. Biol. Chem.* **277**(46): 43749-43756.

<http://www.jbc.org/cgi/content/abstract/277/46/43749>

Smad proteins have been demonstrated to be key components in the transforming growth factor [beta] signaling cascade. Here we demonstrate that Smad4, together with Smad3, can interact with the androgen receptor (AR) in the DNA-binding and ligand-binding domains, which may result in the modulation of 5[alpha]-dihydrotestosterone-induced AR transactivation. Interestingly, in the prostate PC3 and LNCaP cells, addition of Smad3 can enhance AR transactivation, and co-transfection of Smad3 and Smad4 can then repress AR transactivation in various androgen response element-promoter reporter assays as well as Northern blot and reverse transcription-PCR quantitation assays with prostate-specific antigen mRNA expression. In contrast, in the SW480{middle dot}C7 cells, lacking endogenous functional Smad4, the influence of Smad3 on AR transactivation is dependent on the various androgen response element-promoters. The influence of Smad3/Smad4 on the AR transactivation may involve the acetylation since the treatment of trichostatin A or sodium butyrate can reverse Smad3/Smad4-repressed AR transactivation and Smad3/Smad4 complex can also decrease the acetylation level of AR. Together, these results suggest that the interactions between AR, Smad3, and Smad4 may result in the differential regulation of the AR transactivation, which further strengthens their roles in the prostate cancer progression.

Kang, Z., A. Pirskanen, et al. (2002). "Involvement of Proteasome in the Dynamic Assembly of the Androgen Receptor Transcription Complex." *J. Biol. Chem.* **277**(50): 48366-48371.

<http://www.jbc.org/cgi/content/abstract/277/50/48366>

We have used the chromatin immunoprecipitation technique to analyze the formation of the androgen receptor (AR) transcription complex onto prostate-specific antigen (PSA) and kallikrein 2 promoters in LNCaP cells. Our results show that loading of holo-AR and recruitment of RNA polymerase II to the promoters occur transiently. The cyclic nature of AR transcription complex assembly is also illustrated by transient association of coactivators GRIP1 and CREB-binding protein and acetylated histone H3 with the PSA promoter. Treatment of cells with the pure antiandrogen bicalutamide also elicits occupancy of the promoter by AR. In contrast to the agonist-liganded AR, bicalutamide-bound receptor is not capable of recruiting polymerase II, GRIP1, or CREB-binding protein, indicating that the conformation of AR bound to anti-androgen is not competent to assemble transcription complexes. Proteasome is involved in the regulation of AR-dependent transcription, as a proteasome inhibitor, MG-132, prevents the release of the receptor from the PSA promoter, and it also blocks the androgen-induced PSA mRNA

accumulation. Furthermore, occupancy of the PSA promoter by the 19 S proteasome subcomplex parallels that by AR. Collectively, formation of the AR transcription complex, encompassing AR, polymerase II, and coactivators, on a regulated promoter is a cyclic process involving proteasome function.

Karbarz, M. J., S. R. Kalb, et al. (2003). "Expression Cloning and Biochemical Characterization of a *Rhizobium leguminosarum* Lipid A 1-Phosphatase." *J. Biol. Chem.* **278**(41): 39269-39279.

<http://www.jbc.org/cgi/content/abstract/278/41/39269>

Lipid A of *Rhizobium leguminosarum*, a nitrogen-fixing plant endosymbiont, displays several significant structural differences when compared with *Escherichia coli*. An especially striking feature of *R. leguminosarum* lipid A is that it lacks both the 1- and 4'-phosphate groups. Distinct lipid A phosphatases that attack either the 1 or the 4' positions have previously been identified in extracts of *R. leguminosarum* and *Rhizobium etli* but not *Sinorhizobium meliloti* or *E. coli*. Here we describe the identification of a hybrid cosmid (pMJK-1) containing a 25-kb *R. leguminosarum* 3841 DNA insert that directs the overexpression of the lipid A 1-phosphatase. Transfer of pMJK-1 into *S. meliloti* 1021 results in heterologous expression of 1-phosphatase activity, which is normally absent in extracts of strain 1021, and confers resistance to polymyxin. Sequencing of a 7-kb DNA fragment derived from the insert of pMJK-1 revealed the presence of a lipid phosphatase ortholog (designated LpxE). Expression of lpxE in *E. coli* behind the T7lac promoter results in the appearance of robust 1-phosphatase activity, which is normally absent in *E. coli* membranes. Matrix-assisted laser-desorption/ionization time-of-flight and radiochemical analysis of the product generated in vitro from the model substrate lipid IVA confirms the selective removal of the 1-phosphate group. These findings show that lpxE is the structural gene for the 1-phosphatase. The availability of lpxE may facilitate the re-engineering of lipid A structures in diverse Gram-negative bacteria and allow assessment of the role of the 1-phosphatase in *R. leguminosarum* symbiosis with plants. Possible orthologs of LpxE are present in some intracellular human pathogens, including *Francisella tularensis*, *Brucella melitensis*, and *Legionella pneumophila*.

Karchner, S. I., D. G. Franks, et al. (2002). "Regulatory Interactions among Three Members of the Vertebrate Aryl Hydrocarbon Receptor Family: AHR Repressor, AHR1, and AHR2." *J. Biol. Chem.* **277**(9): 6949-6959.

<http://www.jbc.org/cgi/content/abstract/277/9/6949>

The effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds occur via the aryl hydrocarbon receptor (AHR), a member of the basic helix-loop-helix-Per-ARNT-Sim homology (bHLH-PAS) protein superfamily. A single AHR gene has been identified in mammals, whereas many fish species, including the Atlantic killifish (*Fundulus heteroclitus*) possess two distinct AHR genes (AHR1 and a novel form, AHR2). A mouse bHLH-PAS protein closely related to AHR and designated AHR repressor (AHRR) is induced by 3-methylcholanthrene and represses the transcriptional activity of the AHR. To determine whether AHRR is the mammalian ortholog of fish AHR2 and to investigate the mechanisms by which AHRR regulates AHR function, we cloned an AHRR ortholog in *F. heteroclitus* with high sequence identity to the mouse and human AHRRs. Killifish AHRR encodes a 680-residue protein with a predicted molecular mass of 75.2 kDa. We show that in vitro expressed AHRR proteins from human, mouse, and killifish all fail to bind [³H]TCDD or [³H][β]-naphthoflavone. In transient transfection experiments using a luciferase reporter gene under control of AHR response elements, killifish AHRR inhibited the TCDD-dependent transactivation function of both AHR1 and AHR2. AHRR mRNA is widely expressed in killifish tissues and is inducible by TCDD or polychlorinated biphenyls, but its expression is not altered in a population of fish exhibiting genetic resistance to

these compounds. The *F. heteroclitus* AHRR promoter contains three putative AHR response elements. Both AHR1 and AHR2 activated transcription of luciferase driven by the AHRR promoter, and AHRR could repress its own promoter. Thus, AHRR is an evolutionarily conserved, TCDD-inducible repressor of AHR1 and AHR2 function. Phylogenetic analysis shows that AHRR, AHR1, and AHR2 are distinct genes, members of an AHR gene family; these three vertebrate AHR-like genes descended from a single invertebrate AHR.

Kawaguchi, T., K. Osatomi, et al. (2002). "Mechanism for Fatty Acid "Sparing" Effect on Glucose-induced Transcription. REGULATION OF CARBOHYDRATE-RESPONSIVE ELEMENT-BINDING PROTEIN BY AMP-ACTIVATED PROTEIN KINASE." *J. Biol. Chem.* **277**(6): 3829-3835.

<http://www.jbc.org/cgi/content/abstract/277/6/3829>

Carbohydrate-responsive element-binding protein (ChREBP) is a new transcription factor that binds to the carbohydrate-responsive element of the L-type pyruvate kinase gene (L-PK). The aim of this study was to investigate the mechanism by which feeding high fat diets results in decreased activity of ChREBP in the liver (Yamashita, H., Takenoshita, M., Sakurai, M., Bruick, R. K., Henzel, W. J., Shillinglaw, W., Arnot, D., and Uyeda, K. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98**, 9116-9121). We cloned the rat liver ChREBP gene for use throughout this study. Acetate, octanoate, and palmitate inhibited the glucose-induced activation of L-PK transcription in ChREBP-overexpressed hepatocytes. In these hepatocytes, the cytosolic AMP concentration increased 30-fold and AMP-activated protein kinase activity was activated 2-fold. Similarly to the fatty acids, 5-amino-4-imidazolecarboxamide ribotide, a specific activator of AMP-activated protein kinase (AMPK) also inhibited the L-PK transcription activity in ChREBP-overexpressed hepatocytes. Using as a substrate a truncated ChREBP consisting of the C-terminal region, we demonstrated that phosphorylation by AMPK resulted in inactivation of the DNA binding activity. AMPK specifically phosphorylated Ser568 of ChREBP. A S568A mutant of the ChREBP gene showed tight DNA binding and lost its fatty acid sensitivity, whereas a S568D mutant showed weak DNA binding and inhibited L-PK transcription activity even in the absence of fatty acid. These results strongly suggested that the fatty acid inhibition of glucose-induced L-PK transcription resulted from AMPK phosphorylation of ChREBP at Ser568, which inactivated the DNA binding activity. AMPK was activated by the increased AMP that was generated by the fatty acid activation.

Kessler, N., H. Schuhmann, et al. (2004). "The Linear Pentadecapeptide Gramicidin Is Assembled by Four Multimodular Nonribosomal Peptide Synthetases That Comprise 16 Modules with 56 Catalytic Domains." *J. Biol. Chem.* **279**(9): 7413-7419.

<http://www.jbc.org/cgi/content/abstract/279/9/7413>

Linear gramicidin is a membrane channel forming pentadecapeptide that is produced via the nonribosomal pathway. It consists of 15 hydrophobic amino acids with alternating L- and D-configuration forming a {beta}-helix-like structure. It has an N-formylated valine and a C-terminal ethanolamine. Here we report cloning and sequencing of the entire biosynthetic gene cluster as well as initial biochemical analysis of a new reductase domain. The biosynthetic gene cluster was identified on two nonoverlapping fosmids and a 13-kilobase pair (kbp) interbridge fragment covering a region of 74 kbp. Four very large open reading frames, *lgrA*, *lgrB*, *lgrC*, and *lgrD* with 6.8, 15.5, 23.3, and 15.3 kbp, were identified and shown to encode nonribosomal peptide synthetases with two, four, six, and four modules, respectively. Within the 16 modules identified, seven epimerization domains in alternating positions were detected as well as a putative formylation domain fused to the first module *LgrA* and a putative reductase domain attached to the C-terminal module of *LgrD*. Analysis of the substrate specificity by phylogenetic studies using

the residues of the substrate-binding pockets of all 16 adenylation domains revealed a good agreement of the substrate amino acids predicted with the sequence of linear gramicidin. Additional biochemical analysis of the three adenylation domains of modules 1, 2, and 3 confirmed the colinearity of this nonribosomal peptide synthetase assembly line. Module 16 was predicted to activate glycine, which would then, being the C-terminal residue of the peptide chain, be reduced by the adjacent reductase domain to give ethanolamine, thereby releasing the final product N-formyl-pentadecapeptide-ethanolamine. However, initial biochemical analysis of this reductase showed only a one-step reduction yielding the corresponding aldehyde in vitro.

Kiefer, H. L. B., T. M. Hanley, et al. (2004). "Retinoic Acid Inhibition of Chromatin Remodeling at the Human Immunodeficiency Virus Type 1 Promoter: UNCOUPLING OF HISTONE ACETYLATION AND CHROMATIN REMODELING." *J. Biol. Chem.* **279**(42): 43604-43613.

<http://www.jbc.org/cgi/content/abstract/279/42/43604>

All-trans retinoic acid (RA) represses HIV-1 transcription and replication in cultured monocytic cells and in primary monocyte-derived macrophages. Here we examine the role of histone acetylation and chromatin remodeling in RA-mediated repression. RA pretreatment of latently infected U1 promonocytes inhibits HIV-1 expression in response to the histone deacetylase (HDAC) inhibitor, trichostatin A (TSA). TSA is thought to activate HIV-1 transcription by inducing histone hyperacetylation within a regulatory nucleosome, nuc-1, positioned immediately downstream from the transcription start site. Acetylation of nuc-1 is thought to be a critical step in activation that precedes nuc-1 remodeling and, subsequently, transcriptional initiation. Here we demonstrate that TSA treatment induces H3 and H4 hyperacetylation and nuc-1 remodeling. Although RA pretreatment inhibits nuc-1 remodeling and HIV-1 transcription, it has no effect on histone acetylation. This suggests that acetylation and remodeling are not obligatorily coupled. We also show that growth of U1 cells in retinoid-deficient medium induces nuc-1 remodeling and HIV-1 expression but does not induce histone hyperacetylation. These findings suggest that remodeling, not histone hyperacetylation, is the limiting step in transcriptional activation in these cells. Together, these data suggest that RA signaling maintains the chromatin structure of the HIV-1 promoter in a transcriptionally non-permissive state that may contribute to the establishment of latency in monocyte/macrophages.

Kim, S., C. Domon-Dell, et al. (2004). "Down-regulation of the Tumor Suppressor PTEN by the Tumor Necrosis Factor- α /Nuclear Factor- κ B (NF- κ B)-inducing Kinase/NF- κ B Pathway Is Linked to a Default I κ B- α Autoregulatory Loop." *J. Biol. Chem.* **279**(6): 4285-4291.

<http://www.jbc.org/cgi/content/abstract/279/6/4285>

The PTEN (phosphatase and tensin homolog deleted on chromosome ten) tumor suppressor gene affects multiple cellular processes including cell growth, proliferation, and cell migration by antagonizing phosphatidylinositol 3-kinase (PI3K). However, mechanisms by which PTEN expression is regulated have not been studied extensively. Similar to PTEN, tumor necrosis factor- α (TNF- α) affects a wide spectrum of diseases including inflammatory processes and cancer by acting as a mediator of apoptosis, inflammation, and immunity. In this study, we show that treatment of cancer cell lines with TNF- α decreases PTEN expression. In addition, overexpression of TNF- α downstream signaling targets, nuclear factor- κ B (NF- κ B)-inducing kinase (NIK) and p65 nuclear factor NF- κ B, lowers PTEN expression, suggesting that TNF- α -induced down-regulation of PTEN is mediated through a TNF- α /NIK/NF- κ B pathway. Down-regulation of PTEN by NIK/NF- κ B results in activation of the PI3K/Akt pathway and augmentation of TNF- α -induced PI3K/Akt

stimulation. Importantly, we demonstrate that this effect is associated with a lack of an inhibitor of κ B (κ B)- α autoregulatory loop. Moreover, these findings suggest the interaction between PI3K/Akt and NF- κ B via transcriptional regulation of PTEN and offer one possible explanation for increased tumorigenesis in systems in which NF- κ B is chronically activated. In such a tumor system, these findings suggest a positive feedback loop whereby Akt activation of NF- κ B further stimulates Akt via down-regulation of the PI3K inhibitor PTEN.

Ko, Y.-G., K. Nishino, et al. (2005). "Stage-by-Stage Change in DNA Methylation Status of Dnmt1 Locus during Mouse Early Development." *J. Biol. Chem.* **280**(10): 9627-9634.

<http://www.jbc.org/cgi/content/abstract/280/10/9627>

Methylation of DNA is involved in tissue-specific gene control, and establishment of DNA methylation pattern in the genome is thought to be essential for embryonic development. Three isoforms of Dnmt1 (DNA methyltransferase 1) transcripts, Dnmt1s, Dnmt1o, and Dnmt1p, are produced by alternative usage of multiple first exons. Dnmt1s is expressed in somatic cells. Dnmt1p is found only in pachytene spermatocytes, whereas Dnmt1o is specific to oocytes and preimplantation embryos. Here we determined that there is a tissue-dependent differentially methylated region (T-DMR) in the 5' region of Dnmt1o but not in that of the Dnmt1s/1p. The methylation status of the Dnmt1o T-DMR was distinctively different in the oocyte from that in the sperm and adult somatic tissues and changed at each stage from fertilization to blastocyst stage, suggesting that active methylation and demethylation occur during preimplantation development. The T-DMR was highly methylated in somatic cells and embryonic stem cells. Analysis using Dnmt-deficient embryonic stem cell lines revealed that Dnmt1, Dnmt3a, and Dnmt3b are each partially responsible for maintenance of methylation of Dnmt1o T-DMR. In particular, there are compensatory and cooperative roles between Dnmt3a and Dnmt3b. Thus, the regulatory region of Dnmt1o, but not of Dnmt1s/1p, appeared to be a target of DNA methylation. The present study also suggested that the DNA methylation status of the gene region dynamically changes during embryogenesis independently of the change in the bulk DNA methylation status.

Kominato, Y., Y. Hata, et al. (2002). "Alternative Promoter Identified between a Hypermethylated Upstream Region of Repetitive Elements and a CpG Island in Human ABO Histo-blood Group Genes." *J. Biol. Chem.* **277**(40): 37936-37948.

<http://www.jbc.org/cgi/content/abstract/277/40/37936>

We have studied the expression of human histo-blood group ABO genes during erythroid differentiation, using an ex vivo culture of AC133[-]CD34+ cells obtained from peripheral blood. 5'-Rapid amplification of cDNA ends analysis of RNA from those cells revealed a novel transcription start site, which appeared to mark an alternative starting exon (1a) comprising 27 bp at the 5'-end of a CpG island in ABO genes. Results from reverse transcription-PCR specific to exon 1a indicated that the cells of both erythroid and epithelial lineages utilize this exon as the transcription starting exon. Transient transfection experiments showed that the region just upstream from the transcription start site possesses promoter activity in a cell type-specific manner when placed 5' adjacent to the reporter luciferase gene. Results from bisulfite genomic sequencing and reverse transcription-PCR analysis indicated that hypermethylation of the distal promoter region correlated with the absence of transcripts containing exon 1a, whereas hypermethylation in the interspersed repeats 5' adjacent to the distal promoter was commonly observed in all of the cell lines examined. These results suggest that a functional alternative promoter is located between the hypermethylated region of repetitive elements and the CpG island in the ABO genes.

Kondapalli, J., A. S. Flozak, et al. (2004). "Laminar Shear Stress Differentially Modulates Gene Expression of p120 Catenin, Kaiso Transcription Factor, and Vascular Endothelial Cadherin in Human Coronary Artery Endothelial Cells." *J. Biol. Chem.* **279**(12): 11417-11424.

<http://www.jbc.org/cgi/content/abstract/279/12/11417>

We demonstrated previously that laminar shear stress (LSS) enhances human coronary artery endothelial cell (HCAEC) wound closure via a vascular endothelial cadherin (VE-cadherin)-dependent mechanism. VE-cadherin can interact with p120 catenin (p120ctn) to mediate cell locomotion and proliferation. In this study, we hypothesized that p120ctn and an interacting protein, Kaiso, a transcriptional factor with which p120ctn may interact, would be expressed differentially at the wound border and away from the wound border in HCAEC exposed to LSS. One of the major goals in this study was to assess the differential gene expression of p120ctn, Kaiso, and VE-cadherin in HCAEC at specific locations along the wound border to further our understanding of the molecular mechanisms involved in wound closure. We combined the technique of laser capture microdissection with quantitative real time PCR to compare p120ctn, Kaiso, and VE-cadherin mRNA expression in HCAEC at and away from the wound border under LSS. Total RNA was isolated from 200-1,000 laser-captured HCAEC and reverse transcribed into cDNA. Detection of p120ctn, Kaiso, and VE-cadherin mRNA was carried out using quantitative real time PCR. Normalization of cDNA templates was achieved by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) quantification. Quantitative real time PCR analysis revealed p120ctn:GAPDH ratios, Kaiso: GAPDH ratios, and VE-cadherin:GAPDH ratios, relative to static control for each set, of 0.99-4.18 (mean $\pm$ S.E., 1.94 $\pm$ 0.404), 1.0-5.24 (2.11 $\pm$ 0.51), and 0.99-1.42 (1.09 $\pm$ 0.09) after 3 h of LSS, respectively. With these techniques, we found that p120ctn and Kaiso transcripts were increased in laser-captured HCAEC at the wound border compared with HCAEC away from the wound border. In addition, differential expression of p120ctn and Kaiso mRNA was observed in HCAEC depending on how LSS was applied in relation to the wounding process. These techniques may have wide applicability for studying wound healing because gene expression of key adhesion molecules in HCAEC may now be determined from select regions of the endothelial wound border.

Koo, I. C. and R. S. Stephens (2003). "A Developmentally Regulated Two-component Signal Transduction System in Chlamydia." *J. Biol. Chem.* **278**(19): 17314-17319.

<http://www.jbc.org/cgi/content/abstract/278/19/17314>

Two-component systems allow bacteria to adapt to changing environmental conditions and may induce developmental changes necessary for survival. Chlamydia trachomatis alternates between two distinct developmental forms, each optimized for survival in a separate niche. Transcriptional regulation of development is not understood. The C. trachomatis genome sequence revealed a single pair of genes (ctcB-ctcC) predicted to encode proteins with sequence conservation to bacterial two-component systems. Sequence analysis revealed that the sensor kinase, CtcB, possessed an energy-sensing PAS domain and phosphorylation site. The response regulator, CtcC, had homology to σ^{54} activators, possessing conserved receiver and ATPase domains and phosphorylation site, but lacked the C-terminal DNA-binding domain. ctcB and ctcC were expressed late in the developmental cycle, and both proteins were detected in EB lysates. Recombinant CtcB and CtcC were purified from denatured Escherichia coli inclusion bodies and refolded. CtcC was found to aggregate as dimers and tetramers in solution. In vitro phosphorylation assays showed that CtcB autophosphorylated in the presence of Mg^{2+} , Mn^{2+} , and Fe^{2+} and transferred the phosphoryl group in the presence of CtcC. Collectively, these results show that CtcB and CtcC function as a two-component system and are likely responsible for transcriptional regulation by σ^{54} holoenzyme during late-stage chlamydial development.

Kruger, R. P., H. C. Winter, et al. (2002). "Cloning, Expression, and Characterization of the Gal α 1,3Gal High Affinity Lectin from the Mushroom *Marasmius oreades*." *J. Biol. Chem.* **277**(17): 15002-15005.

<http://www.jbc.org/cgi/content/abstract/277/17/15002>

The purification and unique carbohydrate binding properties, including blood group B-specific agglutination and preferential binding to Gal α 1,3Gal-containing sugar epitopes, of the *Marasmius oreades* agglutinin (MOA) are reported in an accompanying paper (Winter, H. C., Mostafapour, K., and Goldstein, I. J. (2002) *J. Biol. Chem.* **277**, 14996-15001). Here we describe the cloning, characterization, and expression of MOA. MOA was digested with trypsin and endoproteinase Asp-N, and the peptide fragments were purified by high performance liquid chromatography. Amino acid sequence data were obtained for eight peptides. Using oligonucleotides deduced from the peptide sequences for a reverse transcriptase-PCR, a 41-base pair cDNA was obtained. The 41-base pair fragment allowed the generation a full-length cDNA using 5' and 3' rapid amplification of cDNA ends. MOA cDNA encodes a protein of 293 amino acids that contains a ricin domain. These carbohydrate binding domains were first described in subunits of bacterial toxins and are also commonly found in polysaccharide-degrading enzymes. Whereas these proteins are known to display a variety of sugar binding specificities, none to date are known to share MOA's high affinity for Gal α 1,3Gal and Gal α 1,3Gal β 1,4GlcNAc. Recombinantly expressed and purified MOA retains the specificity and affinity observed with the native protein. This study provides the basis for analyzing the underlying cause for the unusual binding specificity of MOA.

Kubota, H., R. W. Storms, et al. (2002). "Variant Forms of α -Fetoprotein Transcripts Expressed in Human Hematopoietic Progenitors. IMPLICATIONS FOR THEIR DEVELOPMENTAL POTENTIAL TOWARDS ENDODERM." *J. Biol. Chem.* **277**(31): 27629-27635.

<http://www.jbc.org/cgi/content/abstract/277/31/27629>

Hematopoietic stem cells have been identified as multipotent cells that give rise to all adult hematopoietic lineages. Although the hematopoietic lineage is derived from the mesodermal germ layer in the embryo, recent data suggest that bone marrow cells with an antigenic profile consistent with that of hematopoietic stem cells can also differentiate to cell types of the endodermal lineages, such as hepatocytes. However, the molecular mechanisms associated with these events are entirely unknown. For decades, α -fetoprotein (AFP) has been used as a differentiation marker for endodermal cells, because it was thought that the transcription of AFP mRNA is tightly regulated in a developmental and tissue-specific process. In this report we describe two new variant forms of AFP transcripts in human hematopoietic progenitors that are not expressed in mature cells. The variant AFP (vAFP) cDNA sequences isolated from a multipotent hematopoietic cell line, K562, revealed that the vAFP differed from the authentic transcript, consisting of 15 exons, by replacing exon 1 of AFP with one or two exons located in the 5'-untranslated region of the AFP gene. In addition to the K562 cell line, vAFP transcripts were detected in normal bone marrow, thymus, and brain but were not detected in normal spleen, intestine, liver, or the hepatocellular carcinoma cell line, HepG2. This suggests expression in normal hematopoietic progenitors. This hypothesis was confirmed by the finding that CD34⁺Lin⁻ hematopoietic progenitor cells purified from cord blood by flow cytometric sorting also expressed the variant transcripts. These results suggest that some hematopoietic progenitors are in a state that permits them to express certain types of transcripts that have been considered unique to endoderm.

Kumar, S., L. Gupta, et al. (2004). "Inducible Peroxidases Mediate Nitration of Anopheles Midgut Cells Undergoing Apoptosis in Response to Plasmodium Invasion." *J. Biol. Chem.* **279**(51): 53475-53482.

<http://www.jbc.org/cgi/content/abstract/279/51/53475>

Plasmodium berghei invasion of Anopheles stephensi midgut cells causes severe damage, induces expression of nitric-oxide synthase, and leads to apoptosis. The present study indicates that invasion results in tyrosine nitration, catalyzed as a two-step reaction in which nitric-oxide synthase induction is followed by increased peroxidase activity. Ookinete invasion induced localized expression of peroxidase enzymes, which catalyzed protein nitration in vitro in the presence of nitrite and H₂O₂. Histochemical stainings revealed that when a parasite migrates laterally and invades more than one cell, the pattern of induced peroxidase activity is similar to that observed for tyrosine nitration. In Anopheles gambiae, ookinete invasion elicited similar responses; it induced expression of 5 of the 16 peroxidase genes predicted by the genome sequence and decreased mRNA levels of one of them. One of these inducible peroxidases has a C-terminal oxidase domain homologous to the catalytic moiety of phagocyte NADPH oxidase and could provide high local levels of superoxide anion ([IMG]f1.gif" BORDER="0">), that when dismutated would generate the local increase in H₂O₂ required for nitration. Chemically induced apoptosis of midgut cells also activated expression of four ookinete-induced peroxidase genes, suggesting their involvement in general apoptotic responses. The two-step nitration reaction provides a mechanism to precisely localize and circumscribe the toxic products generated by defense reactions involving nitration. The present study furthers our understanding of the biochemistry of midgut defense reactions to parasite invasion and how these may influence the efficiency of malaria transmission by anopheline mosquitoes.

Lau, P., S. J. Nixon, et al. (2004). "ROR{alpha} Regulates the Expression of Genes Involved in Lipid Homeostasis in Skeletal Muscle Cells: CAVEOLIN-3 AND CPT-1 ARE DIRECT TARGETS OF ROR." *J. Biol. Chem.* **279**(35): 36828-36840.

<http://www.jbc.org/cgi/content/abstract/279/35/36828>

The staggerer mice carry a deletion in the ROR{alpha} gene and have a prolonged humoral response, overproduce inflammatory cytokines, and are immunodeficient. Furthermore, the staggerer mice display lowered plasma apoA-I/-II, decreased plasma high density lipoprotein cholesterol and triglycerides, and develop hypo-{alpha}-lipoproteinemia and atherosclerosis. However, relatively little is known about ROR{alpha} in the context of target tissues, target genes, and lipid homeostasis. For example, ROR{alpha} is abundantly expressed in skeletal muscle, a major mass peripheral tissue that accounts for [~]40% of total body weight and 50% of energy expenditure. This lean tissue is a primary site of glucose disposal and fatty acid oxidation. Consequently, muscle has a significant role in insulin sensitivity, obesity, and the blood-lipid profile. In particular, the role of ROR{alpha} in skeletal muscle metabolism has not been investigated, and the contribution of skeletal muscle to the ROR-/- phenotype has not been resolved. We utilize ectopic dominant negative ROR{alpha} expression in skeletal muscle cells to understand the regulatory role of RORs in this major mass peripheral tissue. Exogenous dominant negative ROR{alpha} expression in skeletal muscle cells represses the endogenous levels of ROR{alpha} and -{gamma} mRNAs and ROR-dependent gene expression. Moreover, we observed attenuated expression of many genes involved in lipid homeostasis. Furthermore, we show that the muscle carnitine palmitoyltransferase-1 and caveolin-3 promoters are directly regulated by ROR and coactivated by p300 and PGC-1. This study implicates RORs in the control of lipid homeostasis in skeletal muscle. In conclusion, we speculate that ROR agonists would increase fatty acid catabolism in muscle and suggest selective activators of ROR may have therapeutic utility in the treatment of obesity and atherosclerosis.

Laybutt, D. R., M. Glandt, et al. (2003). "Critical Reduction in beta -Cell Mass Results in Two Distinct Outcomes over Time. ADAPTATION WITH IMPAIRED GLUCOSE TOLERANCE OR DECOMPENSATED DIABETES." *J. Biol. Chem.* **278**(5): 2997-3005.

<http://www.jbc.org/cgi/content/abstract/278/5/2997>

We have proposed that hyperglycemia-induced dedifferentiation of [beta]-cells is a critical factor for the loss of insulin secretory function in diabetes. Here we examined the effects of the duration of hyperglycemia on gene expression in islets of partially pancreatectomized (Px) rats. Islets were isolated, and mRNA was extracted from rats 4 and 14 weeks after Px or sham Px surgery. Px rats developed different degrees of hyperglycemia; low hyperglycemia was assigned to Px rats with fed blood glucose levels less than 150 mg/dl, and high hyperglycemia was assigned above 150 mg/dl. [beta]-Cell hypertrophy was present at both 4 and 14 weeks. At the same time points, high hyperglycemia rats showed a global alteration in gene expression with decreased mRNA for insulin, IAPP, islet-associated transcription factors (pancreatic and duodenal homeobox-1, BETA2/NeuroD, Nkx6.1, and hepatocyte nuclear factor 1[alpha]), [beta]-cell metabolic enzymes (glucose transporter 2, glucokinase, mitochondrial glycerol phosphate dehydrogenase, and pyruvate carboxylase), and ion channels/pumps (Kir6.2, VDCC[beta], and sarcoplasmic reticulum Ca²⁺-ATPase 3). Conversely, genes normally suppressed in [beta]-cells, such as lactate dehydrogenase-A, hexokinase I, glucose-6-phosphatase, stress genes (heme oxygenase-1, A20, and Fas), and the transcription factor c-Myc, were markedly increased. In contrast, gene expression in low hyperglycemia rats was only minimally changed at 4 weeks but significantly changed at 14 weeks, indicating that even low levels of hyperglycemia induce [beta]-cell dedifferentiation over time. In addition, whereas 2 weeks of correction of hyperglycemia completely reverses the changes in gene expression of Px rats at 4 weeks, the changes at 14 weeks were only partially reversed, indicating that the phenotype becomes resistant to reversal in the long term. In conclusion, chronic hyperglycemia induces a progressive loss of [beta]-cell phenotype with decreased expression of [beta]-cell-associated genes and increased expression of normally suppressed genes, these changes being present with even minimal levels of hyperglycemia. Thus, both the severity and duration of hyperglycemia appear to contribute to the deterioration of the [beta]-cell phenotype found in diabetes.

Laybutt, D. R., A. Sharma, et al. (2002). "Genetic Regulation of Metabolic Pathways in beta -Cells Disrupted by Hyperglycemia." *J. Biol. Chem.* **277**(13): 10912-10921.

<http://www.jbc.org/cgi/content/abstract/277/13/10912>

In models of type 2 diabetes the expression of [beta]-cell genes is altered, but these changes have not fully explained the impairment in [beta]-cell function. We hypothesized that changes in [beta]-cell phenotype and global alterations in both carbohydrate and lipid pathways are likely to contribute to secretory abnormalities. Therefore, expression of genes involved in carbohydrate and lipid metabolism were analyzed in islets 4 weeks after 85-95% partial pancreatectomy (Px) when [beta]-cells have impaired glucose-induced insulin secretion and ATP synthesis. Px rats after 1 week developed mild to severe hyperglycemia that was stable for the next 3 weeks, whereas neither plasma triglyceride, non-esterified fatty acid, or islet triglyceride levels were altered. Expression of peroxisome proliferator-activated receptors (PPARs), with several target genes, were reciprocally regulated; PPAR[alpha] was markedly reduced even at low level hyperglycemia, whereas PPAR[gamma] was progressively increased with increasing hyperglycemia. Uncoupling protein 2 (UCP-2) was increased as were other genes barely expressed in sham islets including lactate dehydrogenase-A (LDH-A), lactate (monocarboxylate) transporters, glucose-6-phosphatase, fructose-1,6-bisphosphatase, 12-lipoxygenase, and cyclooxygenase 2. On the other hand, the expression of [beta]-cell-associated genes, insulin, and

GLUT2 were decreased. Treating Px rats with phlorizin normalized hyperglycemia without effecting plasma fatty acids and reversed the changes in gene expression implicating the importance of hyperglycemia per se in the loss of [beta]-cell phenotype. In addition, parallel changes were observed in [beta]-cell-enriched tissue dissected by laser capture microdissection from the central core of islets. In conclusion, chronic hyperglycemia leads to a critical loss of [beta]-cell differentiation with altered expression of genes involved in multiple metabolic pathways diversionary to normal [beta]-cell glucose metabolism. This global maladaptation in gene expression at the time of increased secretory demand may contribute to the [beta]-cell dysfunction found in diabetes.

Leavey, P. J., C. Gonzalez-Aller, et al. (2002). "A 29-kDa Protein Associated with p67phox Expresses Both Peroxiredoxin and Phospholipase A2 Activity and Enhances Superoxide Anion Production by a Cell-free System of NADPH Oxidase Activity." *J. Biol. Chem.* **277**(47): 45181-45187.

<http://www.jbc.org/cgi/content/abstract/277/47/45181>

Production of toxic oxygen metabolites provides a mechanism for microbicidal activity of the neutrophil. The NADPH oxidase enzyme system initiates the production of oxygen metabolites by reducing oxygen to form superoxide anion (O₂⁻). With stimulation of the respiratory burst, cytosolic oxidase components, p47phox, p67phox, and Rac, translocate to the phagolysosomal and plasma membranes where they form a complex with cytochrome b558 and express enzyme activity. A 29-kDa neutrophil protein (p29) was identified by co-immunoprecipitation with p67phox. N-terminal sequence analysis of p29 revealed homology to an open reading frame gene described in a myeloid leukemia cell line. A cDNA for p29 identical to the open reading frame protein was amplified from RNA of neutrophils. Significant interaction between p29 and p67phox was demonstrated using a yeast two-hybrid system. A recombinant (rh) p29 was expressed in Sf9 cells resulting in a protein with an apparent molecular weight of 34,000. The rh-p29 showed immunoreactivity with the original rabbit antiserum that detected p47phox and p67phox. In addition, rh-p29 exhibited PLA2 activity, which was Ca²⁺ independent, optimal at low pH, and preferential for phosphatidylcholine substrates. The recombinant protein protected glutathione synthetase and directly inactivated H₂O₂. By activity and sequence homology, rh-p29 can be classified as a peroxiredoxin. Finally, O₂⁻ production by plasma membrane and recombinant cytosolic oxidase components in the SDS-activated, cell-free NADPH oxidase system were enhanced by rh-p29. This effect was not inhibited by PLA2 inhibitors. Thus, p29 is a novel protein that associates with p67 and has peroxiredoxin activity. This protein has a potential role in protecting the NADPH oxidase by inactivating H₂O₂ or altering signaling pathways affected by H₂O₂.

Lee, C.-G., T. W. Reichman, et al. (2004). "MLE Functions as a Transcriptional Regulator of the roX2 Gene." *J. Biol. Chem.* **279**(46): 47740-47745.

<http://www.jbc.org/cgi/content/abstract/279/46/47740>

Dosage compensation is a process that equalizes transcription activity between the sexes. In *Drosophila*, two non-coding RNA, roX1 and roX2, and at least six protein regulators, MSL-1, MSL-2, MSL-3, MLE, MOF, and JIL-1, have been identified as essential for dosage compensation. Although there is accumulating evidence of the intricate functional and physical interactions between protein and RNA regulators, little is known about how roX RNA expression and function are modulated in coordination with protein regulators. In this report, we have found that a relatively short (about 350 bp) upstream genomic region of the roX2 gene, Prox2, harbors

an activity that drives transcription of the downstream gene. Our study has shown that MLE can stimulate the transcription activity of Prox2 and that MLE associates with Prox2 through direct interaction with a newly identified 54-bp repeat, Prox. Our observations suggest a novel mechanism by which roX2 RNA is regulated at the transcriptional level.

Lee, H.-W., D.-H. Ahn, et al. (2002). "Phorbol 12-Myristate 13-Acetate Up-regulates the Transcription of MUC2 Intestinal Mucin via Ras, ERK, and NF-kappa B." J. Biol. Chem. **277**(36): 32624-32631.

<http://www.jbc.org/cgi/content/abstract/277/36/32624>

MUC2 is a secretory mucin normally expressed by goblet cells of the intestinal epithelium. It is overexpressed in mucinous type colorectal cancers but down-regulated in colorectal adenocarcinoma. Phorbol 12-myristate 13-acetate (PMA) treatment of colon cancer cell lines increases MUC2 expression, so we have undertaken a detailed analysis of the effects of PMA on the promoter activity of the 5'-flanking region of the MUC2 gene using stably and transiently transfected promoter reporter vectors. Protein kinase C inhibitors (bisindolylmaleimide, calphostin C) and inhibitors of mitogen-activated protein/extracellular signal regulated kinase kinase (MEK) (PD98059 and U0126) suppressed up-regulation of MUC2. Src tyrosine kinase inhibitor PP2, a protein kinase A inhibitor (KT5720), and a p38 inhibitor (SB 203580) did not affect transcription. Western blotting and reverse transcription-PCR analysis confirmed these results. In addition, co-transfections with mutants of Ras, Raf, and MEK showed that the induction of MUC2 promoter activity by PMA required these three signaling proteins. Our results demonstrate that PMA activates protein kinase C, stimulating MAP kinase through a Ras- and Raf-dependent mechanism. An important role for nuclear factor [kappa]B (NF-[kappa]B) was also demonstrated using the inhibitor caffeic acid phenethyl ester and electrophoretic mobility shift assays. Such identification of pathways involved in MUC2 up-regulation by PMA in the HM3 colon cancer cell line may serve as a model for the effects of cytokines and growth factors, which regulate MUC2 expression during the progression of colorectal cancer.

Leite, M. F., K. Hirata, et al. (2002). "Molecular Basis for Pacemaker Cells in Epithelia." J. Biol. Chem. **277**(18): 16313-16323.

<http://www.jbc.org/cgi/content/abstract/277/18/16313>

Intercellular signaling is highly coordinated in excitable tissues such as heart, but the organization of intercellular signaling in epithelia is less clear. We examined Ca²⁺ signaling in hepatoma cells expressing the hepatocyte gap junction protein connexin32 (cx32) or the cardiac gap junction protein cx43, plus a fluorescently tagged V1a vasopressin receptor (V1aR). Release of inositol 1,4,5-trisphosphate (InsP3) in wild type cells increased Ca²⁺ in the injected cell but not in neighboring cells, while the Ca²⁺ signal spread to neighbors when gap junctions were expressed. Photorelease of caged Ca²⁺ rather than InsP3 resulted in a small increase in Ca²⁺ that did not spread to neighbors with or without gap junctions. However, photorelease of Ca²⁺ in cells stimulated with low concentrations of vasopressin resulted in a much larger increase in Ca²⁺, which spread to neighbors via gap junctions. Cells expressing tagged V1aR similarly had increased sensitivity to vasopressin, and could signal to neighbors via gap junctions. Higher concentrations of vasopressin elicited Ca²⁺ signals in all cells. In cx32 or cx43 but not in wild type cells, this signaling was synchronized and began in cells expressing the tagged V1aR. Thus, intercellular Ca²⁺ signals in epithelia are organized by three factors: 1) InsP3 must be generated in each cell to support a Ca²⁺ signal in that cell; 2) gap junctions are necessary to synchronize Ca²⁺ signals among cells; and 3) cells with relatively increased expression of hormone receptor will initiate Ca²⁺ signals and thus serve as pacemakers for their neighbors. Together, these factors may allow epithelia to act in an integrated, organ-level fashion rather than as a collection

of isolated cells.

Lemercier, G., B. Espiau, et al. (2004). "A Pyrophosphatase Regulating Polyphosphate Metabolism in Acidocalcisomes Is Essential for *Trypanosoma brucei* Virulence in Mice." *J. Biol. Chem.* **279**(5): 3420-3425.

<http://www.jbc.org/cgi/content/abstract/279/5/3420>

We report the functional characterization of a soluble pyrophosphatase (TbVSP1), which localizes to acidocalcisomes, a vesicular acidic compartment of *Trypanosoma brucei*. Depending on the pH and the cofactors Mg²⁺ or Zn²⁺, both present in the compartment, the enzyme hydrolyzes either inorganic pyrophosphate (PPi) (k_{cat} = 385 s⁻¹) or tripolyP (polyP₃) and polyphosphate (polyP) of 28 residues (polyP₂₈) with k_{cat} values of 52 and 3.5 s⁻¹, respectively. An unusual N-terminal domain of 160 amino acids, containing a putative calcium EF-hand-binding domain, is involved in protein oligomerization. Using double-stranded RNA interference methodology, we produced an inducible bloodstream form (BF) deficient in the TbVSP1 protein (BFiVSP1). The long-chain polyP levels of these mutants were reduced by 60%. Their phenotypes revealed a deficient polyP metabolism, as indicated by their defective response to phosphate starvation and hypotonic stress. BFiVSP1 did not cause acute virulent infection in mice, demonstrating that TbVSP1 is essential for growth of bloodstream forms in the mammalian host.

Levitin, F., A. Baruch, et al. (2005). "A Novel Protein Derived from the MUC1 Gene by Alternative Splicing and Frameshifting." *J. Biol. Chem.* **280**(11): 10655-10663.

<http://www.jbc.org/cgi/content/abstract/280/11/10655>

Genes that have been designated the name "MUC" code for proteins comprising mucin domains. These proteins may be involved in barrier and protective functions. The first such gene to be characterized and sequenced is the MUC1 gene. Here we report a novel small protein derived from the MUC1 gene by alternative splicing that does not contain the hallmark of mucin proteins, the mucin domain. This protein termed MUC1/ZD retains the same N-terminal MUC1 sequences as all of the other known MUC1 protein isoforms. The common N-terminal sequences comprise the signal peptide and a subsequent stretch of 30 amino acids. In contrast, the MUC1/ZD C-terminal 43 amino acids are novel and result from a reading frameshift engendered by a splicing event that forms MUC1/ZD. The expression of MUC1/ZD at the protein level in human tissues is demonstrated by Western blotting, immunohistochemistry, immunoprecipitation, and an ELISA. Utilization was made of affinity-purified MUC1/ZD-specific polyclonal antibodies as well as two different monoclonal antibodies that are monospecific for the MUC1/ZD protein. The MUC1/ZD protein is expressed in tissues as an oligomeric complex composed of monomers linked by disulfide bonds contributed by MUC1/ZD cysteine residues. MUC1/ZD protein expression did not parallel that of the tandem-repeat array-containing MUC1 protein. Results presented here demonstrate for the first time the expression of a novel MUC1 protein isoform MUC1/ZD, which is generated by an alternative splicing event that both deletes the tandem-repeat array and leads to a C-terminal reading frameshift.

Li, B., N. M. Nowak, et al. (2005). "Random Mutagenesis of the M3 Muscarinic Acetylcholine Receptor Expressed in Yeast: IDENTIFICATION OF SECOND-SITE MUTATIONS THAT RESTORE FUNCTION TO A COUPLING-DEFICIENT MUTANT M3 RECEPTOR." *J. Biol. Chem.* **280**(7): 5664-5675.

<http://www.jbc.org/cgi/content/abstract/280/7/5664>

The M3 muscarinic receptor is a prototypical member of the class A family of G protein-coupled receptors (GPCRs). To gain insight into the structural mechanisms governing agonist-mediated M3 receptor activation, we recently developed a genetically modified yeast strain (*Saccharomyces cerevisiae*) which allows the efficient screening of large libraries of mutant M3 receptors to identify mutant receptors with altered/novel functional properties. Class A GPCRs contain a highly conserved Asp residue located in transmembrane domain II (TM II; corresponding to Asp-113 in the rat M3 muscarinic receptor) which is of fundamental importance for receptor activation. As observed previously with other GPCRs analyzed in mammalian expression systems, the D113N point mutation abolished agonist-induced receptor/G protein coupling in yeast. We then subjected the D113N mutant M3 receptor to PCR-based random mutagenesis followed by a yeast genetic screen to recover point mutations that can restore G protein coupling to the D113N mutant receptor. A large scale screening effort led to the identification of three such second-site suppressor mutations, R165W, R165M, and Y250D. When expressed in the wild-type receptor background, these three point mutations did not lead to an increase in basal activity and reduced the efficiency of receptor/G protein coupling. Similar results were obtained when the various mutant receptors were expressed and analyzed in transfected mammalian cells (COS-7 cells). Interestingly, like Asp-113, Arg-165 and Tyr-250, which are located at the cytoplasmic ends of TM III and TM V, respectively, are also highly conserved among class A GPCRs. Our data suggest a conformational link between the highly conserved Asp-113, Arg-165, and Tyr-250 residues which is critical for receptor activation.

Li, Q., A. K.-K. Ching, et al. (2004). "A Death Receptor-associated Anti-apoptotic Protein, BRE, Inhibits Mitochondrial Apoptotic Pathway." *J. Biol. Chem.* **279**(50): 52106-52116.

<http://www.jbc.org/cgi/content/abstract/279/50/52106>

BRE, brain and reproductive organ-expressed protein, was found previously to bind the intracellular juxtamembrane domain of a ubiquitous death receptor, tumor necrosis factor receptor 1 (TNF-R1), and to down-regulate TNF- α -induced activation of NF- κ B. Here we show that BRE also binds to another death receptor, Fas, and upon overexpression conferred resistance to apoptosis induced by TNF- α , anti-Fas agonist antibody, cycloheximide, and a variety of stress-related stimuli. However, down-regulation of the endogenous BRE by small interfering RNA increased apoptosis to TNF- α , but not to etoposide, indicating that the physiological antiapoptotic role of this protein is specific to death receptor-mediated apoptosis. We further demonstrate that BRE mediates antiapoptosis by inhibiting the mitochondrial apoptotic machinery but without translocation to the mitochondria or nucleus or down-regulation of the cellular level of truncated Bid. Dissociation of BRE rapidly from TNF-R1, but not from Fas, upon receptor ligation suggests that this protein interacts with the death inducing signaling complex during apoptotic induction. Increased association of BRE with phosphorylated, sumoylated, and ubiquitinated proteins after death receptor stimulation was also detected. We conclude that in contrast to the truncated Bid that integrates mitochondrial apoptosis to death receptor-triggered apoptotic cascade, BRE inhibits the integration. We propose that BRE inhibits, by ubiquitination-like activity, components in or proximal to the death-inducing signaling complexes that are necessary for activation of the mitochondria.

Lim, I. A., D. D. Hall, et al. (2002). "Selectivity and Promiscuity of the First and Second PDZ Domains of PSD-95 and Synapse-associated Protein 102." *J. Biol. Chem.* **277**(24): 21697-21711.

<http://www.jbc.org/cgi/content/abstract/277/24/21697>

PDZ domains typically interact with the very carboxyl terminus of their binding partners. Type 1 PDZ domains usually require valine, leucine, or isoleucine at the very COOH-terminal (P0) position, and serine or threonine 2 residues upstream at P[-]2. We quantitatively defined the contributions of carboxyl-terminal residues to binding selectivity of the prototypic interactions of the PDZ domains of postsynaptic density protein 95 (PSD-95) and its homolog synapse-associated protein 90 (SAP102) with the NR2b subunit of the N-methyl-D-aspartate-type glutamate receptor. Our studies indicate that all of the last five residues of NR2b contribute to the binding selectivity. Prominent were a requirement for glutamate or glutamine at P[-]3 and for valine at P0 for high affinity binding and a preference for threonine over serine at P[-]2, in the context of the last 11 residues of the NR2b COOH terminus. This analysis predicts a COOH-terminal (E/Q)(S/T)XV consensus sequence for the strongest binding to the first two PDZ domains of PSD-95 and SAP102. A search of the human genome sequences for proteins with a COOH-terminal (E/Q)(S/T)XV motif yielded 50 proteins, many of which have not been previously identified as PSD-95 or SAP102 binding partners. Two of these proteins, brain-specific angiogenesis inhibitor 1 and protein kinase C[alpha], co-immunoprecipitated with PSD-95 and SAP102 from rat brain extracts.

Lin, S., Q. Shi, et al. (2002). "A Novel S-Adenosyl-L-methionine:Arсенic(III) Methyltransferase from Rat Liver Cytosol." *J. Biol. Chem.* **277**(13): 10795-10803.

<http://www.jbc.org/cgi/content/abstract/277/13/10795>

S-Adenosyl-L-methionine (AdoMet):arsenic(III) methyltransferase, purified from liver cytosol of adult male Fischer 344 rats, catalyzes transfer of a methyl group from AdoMet to trivalent arsenicals producing methylated and dimethylated arsenicals. The kinetics of production of methylated arsenicals in reaction mixtures containing enzyme, AdoMet, dithiothreitol, glutathione (GSH), and arsenite are consistent with a scheme in which monomethylated arsenical produced from arsenite is the substrate for a second methylation reaction that yields dimethylated arsenical. The mRNA for this protein predicts a 369-amino acid residue protein (molecular mass 41056) that contains common methyltransferase sequence motifs. Its sequence is similar to Cyt19, a putative methyltransferase, expressed in human and mouse tissues. Reverse transcription-polymerase chain reaction detects S-adenosyl-L-methionine:arsenic(III) methyltransferase mRNA in rat tissues and in HepG2 cells, a human cell line that methylates arsenite and methylarsonous acid. S-Adenosyl-L-methionine:arsenic(III) methyltransferase mRNA is not detected in UROtsa cells, an immortalized human urothelial cell line that does not methylate arsenite. Because methylation of arsenic is a critical feature of its metabolism, characterization of this enzyme will improve our understanding of this metalloid's metabolism and its actions as a toxin and a carcinogen.

Lindvall, H., P. Nevsten, et al. (2004). "A Novel Hormone-sensitive Lipase Isoform Expressed in Pancreatic {beta}-Cells." *J. Biol. Chem.* **279**(5): 3828-3836.

<http://www.jbc.org/cgi/content/abstract/279/5/3828>

Hormone-sensitive lipase (HSL) is a key enzyme in fatty acid mobilization in many cell types. Two isoforms of HSL are known to date, namely HSLadi (84 kDa in rat) and HSLtes (130 kDa in rat). These are encoded by the same gene, with exons 1-9 encoding the parts that are common to both and an additional 5'-exon encoding the additional amino acids in HSLtes. HSL of various tissues, among these the islet of Langerhans, is larger than HSLadi, but not as large as HSLtes, indicating that there may be other 5'-coding exons. Here we describe the molecular basis for a novel 89-kDa HSL isoform that is expressed in {beta}-cells, adipocytes, adrenal glands, and ovaries in the rat and that is encoded by exons 1-9 and exon A, which is spliced to exon 1 and thereby introducing an upstream start codon. The additional 5'-base pairs encode a 43-amino

acid peptide, which is highly positively charged. Conglomerates of HSL molecules are in close association with the secretory granules of the β -cell, as determined by immunoelectron microscopy with antibodies targeting two separate regions of HSL. We have also determined that the human genomic sequence upstream of exon A has promoter activity in INS-1 cells as well as glucose sensing capability, mediating an increase in expression at high glucose concentration. The minimal promoter is present within 170 bp from the transcriptional start site and maximal glucose responsiveness is conferred by sequence within 850 bp from the transcriptional start site.

Lodhi, K. M., M. H. Ozdener, et al. (2003). "The Upstream Open Reading Frame Mediates Constitutive Effects on Translation of Cytochrome P-450c27 from the Seventh In-frame AUG Codon in Rat Liver." *J. Biol. Chem.* **278**(42): 40647-40657.

<http://www.jbc.org/cgi/content/abstract/278/42/40647>

The 2.3-kb mRNA that codes for cytochrome P-450c27 (CYP27) has an unexpectedly long 5'-untranslated region (UTR) that holds six AUGs, leading to several upstream open reading frames (uORFs). The initiation of translation from the seventh AUG forms a putative 55-kDa precursor, which is processed in mitochondria to form a 52-kDa mature protein. The first three AUGs form fully overlapping uORF1, uORF2, and uORF3 that are in-frame with the seventh AUG and next two form fully overlapping uORF4 and uORF5 that are out-of-frame with the seventh AUG. Although not recognized by the scanning ribosomes under normal conditions, the sixth in-frame AUG forms a putative 57-kDa extension of the main open reading frame. The purpose of this study was to identify the elements in the 5'-UTR that direct CYP27 mRNA translation exclusively from the seventh AUG. Expression of 5' deletion mutants in COS cells reveal that the intact 5'-UTR not only directs the initiation of translation from the seventh AUG but also acts as a negative regulator. A 2-kb deletion mutant that lacks uORF1 initiates translation equally from the sixth and the seventh AUGs, forming both 57- and 55-kDa precursor proteins with a 2-fold increase in rate of translation. However, induction in translation does not affect the levels of the mature 52-kDa form in mitochondria but causes accumulation of the precursor form in cytosol not seen in COS cells transfected with wild-type cDNA. Mutation of the stop codon that terminates uORF1 completely shifts the initiation of translation from the seventh to the first AUG, forming a 67-kDa precursor that is processed into a 52-kDa mature protein in mitochondria. Confirmation of the bicistronic nature of CYP27 mRNA by epitope mapping of uORF1 suggests that translation of CYP27 mRNA from the seventh AUG is directed and regulated by uORF1 expression.

Longo, K. A., J. A. Kennell, et al. (2002). "Wnt Signaling Protects 3T3-L1 Preadipocytes from Apoptosis through Induction of Insulin-like Growth Factors." *J. Biol. Chem.* **277**(41): 38239-38244.

<http://www.jbc.org/cgi/content/abstract/277/41/38239>

Ectopic expression of Wnt-1 in 3T3-L1 preadipocytes stabilizes β -catenin, activates TCF-dependent gene transcription, and blocks adipogenesis. Here we report that upon serum withdrawal, Wnt-1 causes 3T3-L1 cells to resist apoptosis through a mechanism that is partially dependent on phosphatidylinositol 3-kinase. Although activation of Wnt signaling by inhibition of GSK-3 activity or ectopic expression of dominant stable β -catenin blocks apoptosis, inhibition of Wnt signaling through expression of dominant negative TCF-4 increases apoptosis. Wnt-1 stimulates 3T3-L1 preadipocytes to secrete factors that increase PKB/Akt phosphorylation at levels comparable with treatment with 10% serum. With DNA microarrays, we identified several secreted antiapoptotic genes that are induced by Wnt-1, notably insulin-like growth factor I (IGF-I) and IGF-II. Consistent with IGFs mediating the antiapoptotic effects of Wnt-1 in preadipocytes, conditioned medium from Wnt-1 expressing 3T3-L1 cells was unable to promote protein kinase B phosphorylation after the addition of recombinant IGFBP-4. Thus, we demonstrated that Wnt-1

induces expression of antiapoptotic genes in 3T3-L1 preadipocytes such as IGF-I and IGF-II, which allows these cells to resist apoptosis in response to serum deprivation.

Lu, K. P. and K. S. Ramos (2003). "Redox Regulation of a Novel L1Md-A2 Retrotransposon in Vascular Smooth Muscle Cells." J. Biol. Chem. **278**(30): 28201-28209.

<http://www.jbc.org/cgi/content/abstract/278/30/28201>

Activation and reintegration of retrotransposons into the genome is linked to several diseases in human and rodents, but mechanisms of gene activation remain largely unknown. Here we identify a novel gene of L1Md-A2 lineage in vascular smooth muscle cells and show that environmental hydrocarbons enhance gene expression and activate monomer-driven transcription via a redox-sensitive mechanism. Site-directed mutagenesis and progressive deletion analyses identified two antioxidant/electrophile response-like elements (5'-GTGACTCGAGC-3') within the A2/3 and A3 region. These elements mediated activation, with the A3 monomer playing an essential role in transactivation. This signaling pathway may contribute to gene instability during the course of atherogenesis.

Lu, S., Y. Yao, et al. (2002). "Overexpression of Apolipoprotein A-IV Enhances Lipid Transport in Newborn Swine Intestinal Epithelial Cells." J. Biol. Chem. **277**(35): 31929-31937.

<http://www.jbc.org/cgi/content/abstract/277/35/31929>

Apolipoprotein A-IV (apoA-IV) has myriad functions, including roles as a post-prandial satiety factor and lipid antioxidant. ApoA-IV is expressed in mammalian small intestine and is up-regulated in response to lipid absorption. In newborn swine jejunum, a high fat diet acutely induces a 7-fold increase in apoA-IV expression. To determine whether apoA-IV plays a role in the transport of absorbed lipid, swine apoA-IV was overexpressed in a newborn swine enterocyte cell line, IPEC-1, followed by analysis of the expression of genes related to lipoprotein assembly and lipid transport, as well as quantitation of lipid synthesis and secretion. A full-length swine apoA-IV cDNA was cloned, sequenced, and inserted into a Vp and Rep gene-deficient adeno-associated viral vector, containing the cytomegalovirus immediate early promoter/enhancer and neomycin resistance gene, and was used to transfect IPEC-1 cells. Control cells were transfected with the same vector minus the apoA-IV insert. Using neomycin selection, apoA-IV-overexpressing (+AIV) and control ([-]AIV) clones were isolated for further study. Both undifferentiated ([-]D) and differentiated (+D) +AIV cells expressed 40- to 50-fold higher levels of apoA-IV mRNA and both intracellular and secreted apoA-IV protein compared with [-]AIV cells. Expression of other genes was not affected by apoA-IV overexpression in a manner that would contribute to enhanced lipid secretion. +D +AIV cells secreted 4.9-fold more labeled triacylglycerol (TG), 4.6-fold more labeled cholesteryl ester (CE), and 2-fold more labeled phospholipid (PL) as lipoproteins, mostly in the chylomicron/very low density lipoprotein (VLDL) density range. ApoA-IV overexpression in IPEC-1 cells enhances basolateral TG, CE, and PL secretion in chylomicron/VLDL particles. This enhancement is not associated with up-regulation of other genes involved in lipid transport. ApoA-IV may play a role in facilitating enterocyte lipid transport, particularly in the neonate receiving a diet of high fat breast milk.

Ma, X., J. Hu, et al. (2002). "Mutational Analysis of Human Thioredoxin Reductase 1. EFFECTS ON p53-MEDIATED GENE EXPRESSION AND INTERFERON AND RETINOIC ACID-INDUCED CELL DEATH." J. Biol. Chem. **277**(25): 22460-22468.

<http://www.jbc.org/cgi/content/abstract/277/25/22460>

The interferon (IFN)-[beta] and all-trans-retinoic acid combination suppresses tumor growth by inducing apoptosis in several tumor cell lines. A genetic technique permitted the isolation of human thioredoxin reductase (TR) as a critical regulator of IFN/all-trans-retinoic acid-induced cell death. Our recent studies have shown that TR1:thioredoxin 1-regulated cell death is effected in part through the activation of p53-dependent responses. To understand its death regulatory function, we have performed a mutational analysis of TR. Human TR1 has three major structural domains, the FAD binding domain, the NADPH binding domain, and an interface domain (ID). Here, we show that the deletion of the C-terminal interface domain results in a constitutive activation of TR-dependent death responses and promotes p53-dependent gene expression. TR mutant without the ID still retains its dependence on thioredoxin for promoting these responses. Thus, our data suggest that TR-ID acts as a regulatory domain.

Malhotra, J. D., M. C. Koopmann, et al. (2002). "Structural Requirements for Interaction of Sodium Channel beta 1 Subunits with Ankyrin." *J. Biol. Chem.* **277**(29): 26681-26688.

<http://www.jbc.org/cgi/content/abstract/277/29/26681>

Sodium channel [beta] subunits modulate channel kinetic properties and cell surface expression levels and function as cell adhesion molecules. [beta]1 and [beta]2 participate in homophilic cell adhesion resulting in ankyrin recruitment to cell contact sites. We hypothesized that a tyrosine residue in the cytoplasmic domain of [beta]1 may be important for ankyrin recruitment and tested our hypothesis using [beta]1 mutants replacing Tyr181 with alanine ([beta]1Y181A), phenylalanine ([beta]1Y181F), or glutamate ([beta]1Y181E), or a truncated construct deleting all residues beyond Tyr181 ([beta]1L182STOP). Ankyrin recruitment was observed in [beta]1L182STOP, showing that residues Ile166-Tyr181 contain the major ankyrin recruiting activity of [beta]1. Ankyrin recruitment was abolished in [beta]1Y181E, suggesting that tyrosine phosphorylation of [beta]1 may inhibit [beta]1-ankyrin interactions. AnkyrinG and [beta]1 associate in rat brain membranes and in transfected cells expressing [beta]1 and ankyrinG in the absence of sodium channel [alpha] subunits. [beta]1 subunits are recognized by anti-phosphotyrosine antibodies following treatment of these cell lines with fibroblast growth factor. [beta]1 and ankyrinG association is not detectable in cells following treatment with fibroblast growth factor. AnkyrinG and [beta]1Y181E do not associate even in the absence of fibroblast growth factor treatment. [beta]1 subunit-mediated cell adhesion and ankyrin recruitment may contribute to sodium channel placement at nodes of Ranvier. The phosphorylation state of [beta]1Y181 may be a critical regulatory step in these developmental processes.

Mansouri, A., L. D. Ridgway, et al. (2003). "Sustained Activation of JNK/p38 MAPK Pathways in Response to Cisplatin Leads to Fas Ligand Induction and Cell Death in Ovarian Carcinoma Cells." *J. Biol. Chem.* **278**(21): 19245-19256.

<http://www.jbc.org/cgi/content/abstract/278/21/19245>

The efficacy of cisplatin in cancer chemotherapy is limited by the development of resistance. Although the molecular mechanisms involved in chemoresistance are poorly understood, cellular response to cisplatin is known to involve activation of MAPK and other signal transduction pathways. An understanding of early signal transduction events in the response to cisplatin could be valuable for improving the efficacy of cancer therapy. We compared cisplatin-induced activation of three MAPKs, JNK, p38, and ERK, in a cisplatin-sensitive human ovarian carcinoma cell line (2008) and its resistant subclone (2008C13). The JNK and p38 pathways were activated

differentially in response to cisplatin, with the cisplatin-sensitive cells showing prolonged activation (8-12 h) and the cisplatin-resistant cells showing only transient activation (1-3 h) of JNK and p38. In the sensitive cells, inhibition of cisplatin-induced JNK and p38 activation blocked cisplatin-induced apoptosis; persistent activation of JNK resulted in hyperphosphorylation of the c-Jun transcription factor, which in turn stimulated the transcription of an immediate downstream target, the death inducer Fas ligand (FasL). Sequestration of FasL by incubation with a neutralizing anti-FasL antibody inhibited cisplatin-induced apoptosis. In contrast, chemoresistance in 2008C13 cells was associated with failure to up-regulate FasL. Moreover, in these cells, selective stimulation of the JNK/p38 MAPK pathways by adenovirus-mediated delivery of recombinant MKK7 or MKK3 led to sensitization to apoptosis through reactivating FasL expression. Thus, the JNK > c-Jun > FasL > Fas pathway plays an important role in mediating cisplatin-induced apoptosis in ovarian cancer cells, and the duration of JNK activation is critical in determining whether cells survive or undergo apoptosis.

Marchesini, N., W. Osta, et al. (2004). "Role for Mammalian Neutral Sphingomyelinase 2 in Confluence-induced Growth Arrest of MCF7 Cells." *J. Biol. Chem.* **279**(24): 25101-25111.

<http://www.jbc.org/cgi/content/abstract/279/24/25101>

Recently, we reported that neutral sphingomyelinase 2 (nSMase2) functions as a bona fide neutral sphingomyelinase and that overexpression of nSMase2 in MCF7 breast cancer cells caused a decrease in cell growth (Marchesini, N., Luberto, C., and Hannun, Y. A. (2003) *J. Biol. Chem.* **278**, 13775-13783). In this study, the role of endogenous nSMase2 in regulating growth arrest was investigated. The results show that endogenous nSMase2 mRNA was up-regulated [~]5-fold when MCF7 cells became growth-arrested at confluence, and total neutral SMase activity was increased by 119 {+/-} 41% with respect to control. Cell cycle analysis showed that up-regulation of endogenous nSMase2 correlated with G0/G1 cell cycle arrest and an increase in total ceramide levels (2.4-fold). Analysis of ceramide species showed that confluence caused selective increases in very long chain ceramide C24:1 (370 {+/-} 54%) and C24:0 (266 {+/-} 81%) during arrest. The role of endogenous nSMase2 in growth regulation and ceramide metabolism was investigated using short interfering RNA (siRNA)-mediated loss-of-function analysis. Down-regulation of nSMase2 with specific siRNA increased the cell population of cells in S phase of the cell cycle by 59 {+/-} 14% and selectively reverted the effects of growth arrest on the increase in levels of very long chain ceramides. Mechanistically, confluence arrest also induced hypophosphorylation of the retinoblastoma protein (6-fold) and induction of p21WAF1 (3-fold). Down-regulation of nSMase2 with siRNA largely prevented the dephosphorylation of the retinoblastoma protein and the induction of p21WAF1, providing a link between the action of nSMase2 and key regulators of cell cycle progression. Moreover, studies on nSMase2 localization in MCF7 cells showed that nSMase2 distributed throughout the cells in subconfluent, proliferating cultures. In contrast, nSMase2 became nearly exclusively located at the plasma membrane in confluent, contact-inhibited cells. Hence, we demonstrate for the first time that nSMase2 functions as a growth suppressor in MCF7 cells, linking confluence to the G0/G1 cell cycle check point.

Martin, V., R. Bredoux, et al. (2002). "Three Novel Sarco/endoplasmic Reticulum Ca²⁺-ATPase (SERCA) 3 Isoforms. EXPRESSION, REGULATION, AND FUNCTION OF THE MEMBERS OF THE SERCA3 FAMILY." *J. Biol. Chem.* **277**(27): 24442-24452.

<http://www.jbc.org/cgi/content/abstract/277/27/24442>

Sarco/endoplasmic reticulum Ca²⁺-ATPases (SERCAs) pump Ca²⁺ into the endoplasmic reticulum. Recently, three human SERCA3 (h3a-c) proteins and a previously unknown rat

SERCA3 (r3b/c) mRNA have been described. Here, we (i) document two novel human SERCA3 splice variants h3d and h3e, (ii) provide data for the expression and mechanisms regulating the expression of all known SERCA3 variants (r3a, r3b/c, and h3a-e), and (iii) show functional characteristics of the SERCA3 isoforms. h3d and h3e are issued from the insertion of an additional penultimate exon 22 resulting in different carboxyl termini for these variants. Distinct distribution patterns of the SERCA3 gene products were observed in a series of cell lines of hematopoietic, epithelial, embryonic origin, and several cancerous types, as well as in panels of rat and human tissues. Hypertension and protein kinase C, calcineurin, or retinoic acid receptor signaling pathways were found to differently control rat and human splice variant expression, respectively. Stable overexpression of each variant was performed in human embryonic kidney 293 cells, and the SERCA3 isoforms were fully characterized. All SERCA3 isoforms were found to pump Ca^{2+} with similar affinities. However, they modulated the cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_c$) and the endoplasmic reticulum Ca^{2+} content ($[\text{Ca}^{2+}]_{er}$) in different manners. A newly generated polyclonal antibody and a pan-SERCA3 antibody proved the endogenous expression of the three novel SERCA3 proteins, h3d, h3e, and r3b/c. All these data suggest that the SERCA3 gene products have a more widespread role in cellular Ca^{2+} signaling than previously appreciated.

Massimi, I., E. Park, et al. (2002). "Identification of a Novel Maturation Mechanism and Restricted Substrate Specificity for the SspB Cysteine Protease of *Staphylococcus aureus*." J. Biol. Chem. **277**(44): 41770-41777.

<http://www.jbc.org/cgi/content/abstract/277/44/41770>

The SspB cysteine protease of *Staphylococcus aureus* is expressed in an operon, flanked by the sspA serine protease, and sspC, encoding a 12.9-kDa protein of unknown function. SspB was expressed as a 40-kDa prepropeptide pSspB, which did not undergo autocatalytic maturation. Activity of pSspB was reduced compared with 22-kDa mature SspB, but it was equivalent to mature SspB after incubation with SspA, which specifically removed the pSspB N-terminal propeptide. SspC abrogated the activity of pSspB when incubated in a 1:1 complex but had no effect on SspA or papain. Activity of the pSspB{middle dot}SspC complex was restored when incubated with SspA, and SspC was cleaved by SspA but not pSspB. Thus, SspC maintains pSspB as an inert zymogen, and SspA is required for removal of the propeptide and inactivation of SspC. Like the papain protease family, SspB cleaved substrates with a hydrophobic amino acid at P2 but had a strong preference for arginine at P1. It did not cleave casein, serum albumin, IgG, or IgA, but it promoted detachment of cultured keratinocytes and cleaved fibronectin and fibrinogen at sites recognized by urokinase plasminogen activator and plasmin, respectively. It also processed high molecular weight kininogen in a manner resembling plasma kallikrein. Thus, SspB exhibits a novel maturation mechanism and mimics the specificity of plasma serine proteases.

Mateo, J., S. Kreda, et al. (2003). "Requirement of Cys399 for Processing of the Human Ecto-ATPase (NTPDase2) and Its Implications for Determination of the Activities of Splice Variants of the Enzyme." J. Biol. Chem. **278**(41): 39960-39968.

<http://www.jbc.org/cgi/content/abstract/278/41/39960>

Ecto-ATPase (CD39L1) corresponds to the type 2 enzyme of the ecto-nucleoside triphosphate diphosphohydrolase family (E-NTPDase). We have isolated from human ECV304 cells three cDNAs with high homology with members of the E-NTPDase family that encode predicted proteins of 495, 472, and 450 amino acids. Sequencing of a genomic DNA clone confirmed that these three sequences correspond to splice variants of the human ecto-ATPase

(NTPDase2{alpha}, -2{beta}, and -2{gamma}). Although all three enzyme forms were expressed heterologously to similar levels in Chinese hamster ovary cells clone K-1 (CHO-K1) cells, only the 495-amino acid protein (NTPDase2{alpha}) exhibited ecto-ATPase activity. Immunolocalization studies demonstrated that NTPDase2{alpha} is fully processed and trafficked to the plasma membrane, whereas the NTPDase2{beta} and -2{gamma} splice variants were retained in not fully glycosylated forms in the endoplasmic reticulum. The potential roles of two highly conserved residues, Cys399 and Asn443, in the activity and cellular trafficking of the ecto-ATPase were examined. Mutation of Cys399, which is absent in NTPDase2{beta} and -2{gamma}, produced a protein completely devoid of nucleotidase activity, while mutation of Asn443 to Asp resulted in substantial loss of activity. Neither the Cys399 nor Asn443 mutants were fully glycosylated, and both were retained in the endoplasmic reticulum. These results indicate that the lack of ecto-nucleotidase activity exhibited by NTPDase2{beta} and -2{gamma} and the C399S mutant, as well as the large reduction of activity in the N443D mutant are due to alterations in the folding/maturation of these proteins.

Maxwell, M. A., M. E. Cleasby, et al. (2005). "Nur77 Regulates Lipolysis in Skeletal Muscle Cells: EVIDENCE FOR CROSS-TALK BETWEEN THE {beta}-ADRENERGIC AND AN ORPHAN NUCLEAR HORMONE RECEPTOR PATHWAY." *J. Biol. Chem.* **280**(13): 12573-12584.

<http://www.jbc.org/cgi/content/abstract/280/13/12573>

Skeletal muscle is a major mass peripheral tissue that accounts for [~]40% of total body weight and 50% of energy expenditure and is a primary site of glucose disposal and fatty acid oxidation. Consequently, muscle has a significant role in insulin sensitivity, obesity, and the blood-lipid profile. Excessive caloric intake is sensed by the brain and induces {beta}-adrenergic receptor ({beta}-AR)-mediated adaptive thermogenesis. {beta}-AR null mice develop severe obesity on a high fat diet. However, the target gene(s), target tissues(s), and molecular mechanism involved remain obscure. We observed that 30-60 min of {beta}-AR agonist (isoprenaline) treatment of C2C12 skeletal muscle cells strikingly activated (>100-fold) the expression of the mRNA encoding the nuclear hormone receptor, Nur77. In contrast, the expression of other nuclear receptors that regulate lipid and carbohydrate metabolism was not induced. Stable transfection of Nur77-specific small interfering RNAs (siNur77) into skeletal muscle cells repressed endogenous Nur77 mRNA expression. Moreover, we observed attenuation of gene and protein expression associated with the regulation of energy expenditure and lipid homeostasis, for example AMP-activated protein kinase {gamma}3, UCP3, CD36, adiponectin receptor 2, GLUT4, and caveolin-3. Attenuation of Nur77 expression resulted in decreased lipolysis. Finally, in concordance with the cell culture model, injection and electrotransfer of siNur77 into mouse tibialis cranialis muscle resulted in the repression of UCP3 mRNA expression. This study demonstrates regulatory cross-talk between the nuclear hormone receptor and {beta}-AR signaling pathways. Moreover, it suggests Nur77 modulates the expression of genes that are key regulators of skeletal muscle lipid and energy homeostasis. In conclusion, we speculate that Nur77 agonists would stimulate lipolysis and increase energy expenditure in skeletal muscle and suggest selective activators of Nur77 may have therapeutic utility in the treatment of obesity.

McDermott, N. B., D. F. Gordon, et al. (2002). "Isolation and Functional Analysis of the Mouse RXRgamma 1 Gene Promoter in Anterior Pituitary Cells." *J. Biol. Chem.* **277**(39): 36839-36844.

<http://www.jbc.org/cgi/content/abstract/277/39/36839>

The retinoid X receptor (RXR) isoform RXR[gamma] has limited tissue expression, including brain, skeletal muscle, and anterior pituitary gland. Within the anterior pituitary gland, RXR[gamma] expression is limited primarily to the thyrotropes. In this report, we have isolated ~3

kb of 5'-flanking DNA of the mouse RXR[gamma]1 gene. We have identified the major transcription start site in the thyrotrope-derived TtT-97 cells. Transient transfection studies show that a 1.4-kb promoter fragment has full promoter activity in TtT-97 cells. This promoter has much less activity in thyrotrope-derived [alpha]TSH cells, pituitary-derived GH3 somatomammotropes, and non-pituitary CV-1 cells. None of these cell lines has detectable RXR[gamma]1 mRNA. A previous report has identified a non-consensus direct repeat (DR-1) element in the RXR[gamma]2 gene promoter region that mediates stimulation of promoter activity by 9-cis-retinoic acid (9-cis-RA). Inspection of the RXR[gamma]1 promoter region revealed a non-consensus DR-1 element at [-]232 bp from the transcription start site. Interestingly, RXR[gamma]1 promoter activity was suppressed 50% by 9-cis-RA in the TtT-97 thyrotropes. Further experiments in non-pituitary cells showed that suppression of RXR[gamma]1 promoter activity was RXR-dependent. Mutagenesis of the DR-1 element abrogated suppression of promoter activity by 9-cis-RA, suggesting that this negative regulation requires both RXR and this specific DR-1 element. In summary, we have isolated the mouse RXR[gamma]1 gene promoter region and identified the major start site in thyrotropes. Promoter activity is uniquely suppressed by 9-cis-RA through a DR-1 element. Isolation and characterization of the mouse RXR[gamma]1 promoter region provides a tool for further investigation focusing on thyrotrope-specific gene expression as well as negative regulation of genes by retinoic acid.

Merckx, A., K. Le Roch, et al. (2003). "Identification and Initial Characterization of Three Novel Cyclin-related Proteins of the Human Malaria Parasite *Plasmodium falciparum*." *J. Biol. Chem.* **278**(41): 39839-39850.

<http://www.jbc.org/cgi/content/abstract/278/41/39839>

The molecular mechanisms regulating cell proliferation and development during the life cycle of malaria parasites remain to be elucidated. The peculiarities of the cell cycle organization during *Plasmodium falciparum* schizogony suggest that the modalities of cell cycle control in this organism may differ from those in other eukaryotes. Indeed, existing data concerning *Plasmodium* cell cycle regulators such as cyclin-dependent kinases reveal structural and functional properties that are divergent from those of their homologues in other systems. The work presented here lies in the context of the exploitation of the recently available *P. falciparum* genome sequence toward the characterization of putative cell cycle regulators. We describe the *in silico* identification of three open reading frames encoding proteins with maximal homology to various members of the cyclin family and demonstrate that the corresponding polypeptides are expressed in the erythrocytic stages of the infection. We present evidence that these proteins possess cyclin activity by demonstrating either their association with histone H1 kinase activity in parasite extracts or their ability to activate PfPK5, a *P. falciparum* cyclin-dependent kinase homologue, *in vitro*. Furthermore, we show that RINGO, a protein with no sequence homology to cyclins but that is nevertheless a strong activator of mammalian CDK1/2, is also a strong activator of PfPK5 *in vitro*. This raises the possibility that "cryptic" cell cycle regulators may be found among the 50% of the open reading frames in the *P. falciparum* genome that display no homology to any known proteins.

Mesquita, P., N. Jonckheere, et al. (2003). "Human MUC2 Mucin Gene Is Transcriptionally Regulated by Cdx Homeodomain Proteins in Gastrointestinal Carcinoma Cell Lines." *J. Biol. Chem.* **278**(51): 51549-51556.

<http://www.jbc.org/cgi/content/abstract/278/51/51549>

In intestinal metaplasia and 30% of gastric carcinomas, MUC2 intestinal mucin and the intestine-specific transcription factors Cdx-1 and Cdx-2 are aberrantly expressed. The involvement of Cdx-

1 and Cdx-2 in the intestinal development and their role in transcription of several intestinal genes support the hypothesis that Cdx-1 and/or Cdx-2 play important roles in the aberrant intestinal differentiation program of intestinal metaplasia and gastric carcinoma. To clarify the mechanisms of transcriptional regulation of the MUC2 mucin gene in gastric cells, pGL3 deletion constructs covering 2.6 kb of the human MUC2 promoter were used in transient transfection assays, enabling us to identify a relevant region for MUC2 transcription in all gastric cell lines. To evaluate the role of Cdx-1 and Cdx-2 in MUC2 transcription we performed co-transfection experiments with expression vectors encoding Cdx-1 and Cdx-2. In two of the four gastric carcinoma cell lines and in all colon carcinoma cell lines we observed transactivation of the MUC2 promoter by Cdx-2. Using gel shift assays we identified two Cdx-2 binding sites at -177/-171 and -191/-187. Only simultaneous mutation of the two sites resulted in inhibition of Cdx-2-mediated transactivation of MUC2 promoter, implying that both Cdx-2 sites are active. Finally, stable expression of Cdx-2 in a gastric cell line initially not expressing Cdx-2, led to induction of MUC2 expression. In conclusion, this work demonstrates that Cdx-2 activates the expression of MUC2 mucin gene in gastric cells, inducing an intestinal transdifferentiation phenotype that parallels what is observed both in intestinal metaplasia and some gastric carcinomas.

Mills, J. A., K. Motichka, et al. (2004). "Inactivation of the Mycobacterial Rhamnosyltransferase, Which Is Needed for the Formation of the Arabinogalactan-Peptidoglycan Linker, Leads to Irreversible Loss of Viability." *J. Biol. Chem.* **279**(42): 43540-43546.

<http://www.jbc.org/cgi/content/abstract/279/42/43540>

Temperature-sensitive mutant 2-20/32 of *Mycobacterium smegmatis* mc2155 was isolated and genetically complemented with a *Mycobacterium tuberculosis* H37Rv DNA fragment that contained a single open reading frame. This open reading frame is designated Rv3265c in the *M. tuberculosis* H37Rv genome. Rv3265c shows homology to the *Escherichia coli* gene wbbL, which encodes a dTDP-Rha: α -D-GlcNAc-pyrophosphate polyprenol, α -3-L-rhamnosyltransferase. In *E. coli* this enzyme is involved in O-antigen synthesis, but in mycobacteria it is required for the rhamnosyl-containing linker unit responsible for the attachment of the cell wall polymer mycolyl-arabinogalactan to the peptidoglycan. The *M. tuberculosis* wbbL homologue, encoded by Rv3265c, was shown to be capable of restoring an *E. coli* K12 strain containing an insertionally inactivated wbbL to O-antigen positive. Likewise, the *E. coli* wbbL gene allowed 2-20/32 to grow at higher non-permissive temperatures. The rhamnosyltransferase activity of *M. tuberculosis* WbbL was demonstrated in 2-20/32 as was the loss of this transferase activity in 2-20/32 at elevated temperatures. The wbbL of the temperature-sensitive mutant contained a single-base change that converted what was a proline in mc2155 to a serine residue. Exposure of 2-20/32 to higher non-permissive temperatures resulted in bacteria that could not be recovered at the lower permissive temperatures.

Min, S. H., R. C. M. Simmen, et al. (2002). "Altered Levels of Growth-related and Novel Gene Transcripts in Reproductive and Other Tissues of Female Mice Overexpressing Spermidine/Spermine N1-Acetyltransferase (SSAT)." *J. Biol. Chem.* **277**(5): 3647-3657.

<http://www.jbc.org/cgi/content/abstract/277/5/3647>

Overexpression of SSAT (polyamine catabolic enzyme) in female mice results in impaired ovarian folliculogenesis and uterine hypoplasia. To identify the molecular basis for this, the gene expression profiles in uterus and ovary and for comparison, liver and kidney, from non-transgenic (NT) and SSAT transgenic (ST) mice were compared. The mRNA abundance for lipoprotein lipase and glyceraldehyde-3-phosphate dehydrogenase was elevated in all four ST (>NT) tissues. The translation initiation factor-3 subunit 5 mRNA, and transcripts related to endogenous murine

leukemia provirus (MLV-related) and murine retrovirus-related sequences (MuRRS) were decreased in ST tissues. A novel calmodulin-related mRNA was strongly induced in ST liver and kidney. SSAT overexpression was associated with increased levels of IGF-binding protein-2 (IGFBP-2) in the uterus and ovary, and a reduction in IGFBP-3 mRNA levels in the uterus. Exogenous spermidine and spermine elevated endogenous IGFBP-2 and SSAT mRNA abundance, whereas, putrescine stimulated IGFBP-2 mRNA abundance and transfected IGFBP-2 gene promoter activity in human (Hec-1-A) uterine cells. Sp1 and BTEB1 mRNAs that encode transcription factors for the IGFBP-2 gene also were induced in some ST tissues. The data suggest that SSAT and polyamines are important for the control of molecular pathways underlying reproductive tract tissue growth, phenotype, and function.

Mishra, D. P. and C. Shaha (2005). "Estrogen-induced Spermatogenic Cell Apoptosis Occurs via the Mitochondrial Pathway: ROLE OF SUPEROXIDE AND NITRIC OXIDE." *J. Biol. Chem.* **280**(7): 6181-6196.

<http://www.jbc.org/cgi/content/abstract/280/7/6181>

The detrimental effects of estrogen on testicular function provide a conceptual basis to examine the speculative link between increased exposure to estrogens and spermatogenic cell death. Using an in vitro model, we provide an understanding of the events leading to estrogen-induced apoptosis in cells of spermatogenic lineage. Early events associated with estrogen exposure were up-regulation of FasL and increased generation of H₂O₂, superoxide, and nitric oxide. The ability of anti-FasL antibodies to prevent several downstream biochemical changes and cell death induced by 17β-estradiol substantiates the involvement of the cell death receptor pathway. Evidence for the amplification of the death-inducing signals through mitochondria was obtained from the transient mitochondrial hyperpolarization observed after estradiol exposure resulting in cytochrome c release. A combination of nitric oxide and superoxide but not H₂O₂ was responsible for the mitochondrial hyperpolarization. Mn(III) tetrakis(4-benzoic acid)porphyrin chloride, an intracellular peroxynitrite scavenger, was able to reduce mitochondrial hyperpolarization and cell death. Although nitric oxide augmentation occurred through an increase in the expression of inducible nitric-oxide synthase, superoxide up-regulation was a product of estradiol metabolism. All of the above changes were mediated through an estrogen receptor-based mechanism because tamoxifen, the estrogen receptor modulator, was able to rescue the cells from estrogen-induced alterations. This study establishes the importance of the independent capability of cells of the spermatogenic lineage to respond to estrogens and most importantly suggests that low dose estrogens can potentially cause severe spermatogenic cellular dysfunction leading to impaired fertility even without interference of the hypothalamo-hypophyseal axis.

Mitoma, J., B. Petryniak, et al. (2003). "Extended Core 1 and Core 2 Branched O-Glycans Differentially Modulate Sialyl Lewis x-type L-selectin Ligand Activity." *J. Biol. Chem.* **278**(11): 9953-9961.

<http://www.jbc.org/cgi/content/abstract/278/11/9953>

It has been established that sialyl Lewis x in core 2 branched O-glycans serves as an E- and P-selectin ligand. Recently, it was discovered that 6-sulfosialyl Lewis x in extended core 1 O-glycans, NeuNAc[α]2[→]3Gal[β]1[→]4(Fuc[α]1[→]3(sulfo[→]6))GlcNAc[β]1[→]3Gal[β]1[→]3GalNAc[α]1[→]Ser/Thr, functions as an L-selectin ligand in high endothelial venules. Extended core 1 O-glycans can be synthesized when a core 1 extension enzyme is present. In this study, we first show that [β]1,3-N-acetylglucosaminyltransferase-3 ([β]3GlcNAcT-3) is almost exclusively responsible for core 1 extension among seven different

[beta]3GlcNAcTs and thus acts on core 1 O-glycans attached to PSGL-1. We found that transcripts encoding [beta]3GlcNAcT-3 were expressed in human neutrophils and lymphocytes but that their levels were lower than those of transcripts encoding core 2 [beta]1,6-N-acetylglucosaminyltransferase I (Core2GlcNAcT-I). Neutrophils also expressed transcripts encoding fucosyltransferase VII (FucT-VII) and Core2GlcNAcT-I, whereas lymphocytes expressed only small amounts of transcripts encoding FucT-VII. To determine the roles of sialyl Lewis x in extended core 1 O-glycans, Chinese hamster ovary (CHO) cells were stably transfected to express PSGL-1, FucT-VII, and either [beta]3GlcNAcT-3 or Core2GlcNAcT-I. Glycan structural analyses disclosed that PSGL-1 expressed in these transfected cells carried comparable amounts of sialyl Lewis x in extended core 1 and core 2 branched O-glycans. In a rolling assay, CHO cells expressing sialyl Lewis x in extended core 1 O-glycans supported a significant degree of shear-dependent tethering and rolling of neutrophils and lymphocytes, although less than CHO cells expressing sialyl Lewis x in core 2 branched O-glycans. These results indicate that sialyl Lewis x in extended core 1 O-glycans can function as an L-selectin ligand and is potentially involved in neutrophil adhesion on neutrophils bound to activated endothelial cells.

Mitsutake, S., T.-J. Kim, et al. (2004). "Ceramide Kinase Is a Mediator of Calcium-dependent Degranulation in Mast Cells." *J. Biol. Chem.* **279**(17): 17570-17577.

<http://www.jbc.org/cgi/content/abstract/279/17/17570>

Ceramide kinase (CERK) catalyzes the conversion of ceramide to ceramide 1-phosphate (C1P) and is known to be activated by calcium. Although several groups have examined the functions of CERK and its product C1P, the functions of C1P and CERK are not understood. We studied the RBL-2H3 cell line, a widely used model for mast cells, and found that CERK and C1P are required for activation of the degranulation process in mast cells. We found that C1P formation was enhanced during activation induced by IgE/antigen or by Ca²⁺ ionophore A23187. The formation of C1P required the intracellular elevation of Ca²⁺. We generated RBL-2H3 cells that stably express CERK, and when these cells were treated with A23187, a concomitant C1P formation was observed and degranulation increased 4-fold, compared with mock transfectants. The cell-permeable N-acetylsphingosine (C2-ceramide), a poor substrate of CERK, inhibited both the formation of C1P and degranulation, indicating that C1P formation was necessary for degranulation. Exogenous introduction of CERK into permeabilized RBL-2H3 cells caused degranulation. We identified a cytosolic localization of CERK that provides exposure to cytosolic Ca²⁺. Taken together, these results indicate that C1P formation is a necessary step in the degranulation pathway in RBL-2H3 cells.

Miyakawa-Naito, A., P. Uhlen, et al. (2003). "Cell Signaling Microdomain with Na,K-ATPase and Inositol 1,4,5-Trisphosphate Receptor Generates Calcium Oscillations." *J. Biol. Chem.* **278**(50): 50355-50361.

<http://www.jbc.org/cgi/content/abstract/278/50/50355>

Recent studies indicate novel roles for the ubiquitous ion pump, Na,K-ATPase, in addition to its function as a key regulator of intracellular sodium and potassium concentration. We have previously demonstrated that ouabain, the endogenous ligand of Na,K-ATPase, can trigger intracellular Ca²⁺ oscillations, a versatile intracellular signal controlling a diverse range of cellular processes. Here we report that Na,K-ATPase and inositol 1,4,5-trisphosphate (InsP3) receptor (InsP3R) form a cell signaling microdomain that, in the presence of ouabain, generates slow Ca²⁺ oscillations in renal cells. Using fluorescent resonance energy transfer (FRET) measurements, we detected a close spatial proximity between Na,K-ATPase and InsP3R.

Ouabain significantly enhanced FRET between Na,K-ATPase and InsP3R. The FRET effect and ouabain-induced Ca²⁺ oscillations were not observed following disruption of the actin cytoskeleton. Partial truncation of the NH₂ terminus of Na,K-ATPase catalytic {alpha}1-subunit abolished Ca²⁺ oscillations and downstream activation of NF- κ B. Ouabain-induced Ca²⁺ oscillations occurred in cells expressing an InsP3 sponge and were hence independent of InsP3 generation. Thus, we present a novel principle for a cell signaling microdomain where an ion pump serves as a receptor.

Modregger, J., A. A. Schmidt, et al. (2003). "Characterization of Endophilin B1b, a Brain-specific Membrane-associated Lysophosphatidic Acid Acyl Transferase with Properties Distinct from Endophilin A1." *J. Biol. Chem.* **278**(6): 4160-4167.

<http://www.jbc.org/cgi/content/abstract/278/6/4160>

We have characterized mammalian endophilin B1, a novel member of the endophilins and a representative of their B subgroup. The endophilins B show the same domain organization as the endophilins A, which contain an N-terminal domain responsible for lipid binding and lysophosphatidic acid acyl transferase activity, a central coiled-coil domain for oligomerization, a less conserved linker region, and a C-terminal Src homology 3 (SH3) domain. The endophilin B1 gene gives rise to at least three splice variants, endophilin B1a, which shows a widespread tissue distribution, and endophilins B1b and B1c, which appear to be brain-specific. Endophilin B1, like endophilins A, binds to palmitoyl-CoA, exhibits lysophosphatidic acid acyl transferase activity, and interacts with dynamin, amphiphysins 1 and 2, and huntingtin. However, in contrast to endophilins A, endophilin B1 does not bind to synaptojanin 1 and synapsin 1, and overexpression of its SH3 domain does not inhibit transferrin endocytosis. Consistent with this, immunofluorescence analysis of endophilin B1b transfected into fibroblasts shows an intracellular reticular staining, which in part overlaps with that of endogenous dynamin. Upon subcellular fractionation of brain and transfected fibroblasts, endophilin B1 is largely recovered in association with membranes. Together, our results suggest that the action of the endophilins is not confined to the formation of endocytic vesicles from the plasma membrane, with endophilin B1 being associated with, and presumably exerting a functional role at, intracellular membranes.

Monslow, J., J. D. Williams, et al. (2004). "Identification and Analysis of the Promoter Region of the Human Hyaluronan Synthase 2 Gene." *J. Biol. Chem.* **279**(20): 20576-20581.

<http://www.jbc.org/cgi/content/abstract/279/20/20576>

Hyaluronan (HA) is a linear glycosaminoglycan of the vertebrate extracellular matrix that is synthesized at the plasma membrane by the HA synthase (HAS) enzymes HAS1, -2 and -3. The regulation of HA synthesis has been implicated in a variety of extracellular matrix-mediated and pathological processes, including renal fibrosis. We have recently described the genomic structures of each of the human HAS genes. In the present study, we analyzed the HAS2 promoter region. In 5'-rapid amplification of cDNA ends analysis of purified mRNA from human renal epithelial proximal tubular cells, we detected an extended sequence for HAS2 exon 1, relocating the transcription initiation site 130 nucleotides upstream of the reference HAS2 mRNA sequence, GenBankTM accession number NM_005328. A luciferase reporter gene assay of nested fragments spanning the 5' terminus of NM_005328 demonstrated the constitutive promoter activity of sequences directly upstream of the repositioned transcription initiation site but not of the newly designated exonic nucleotides. Using reverse transcription-PCR, expression of this extended HAS2 mRNA was demonstrated in a variety of human cell types, and orthologous sequences were detected in mouse and rat kidney. Alignment of human, murine, and equine genomic DNA sequences upstream of the repositioned HAS2 exon 1 provided evidence for the

evolutionary conservation of specific transcription factor binding sites. The location of the HAS2 promoter will facilitate analysis of the transcriptional regulation of this gene in a variety of pathological contexts as well as in developmental models in which HAS2 null animals have an embryonic lethal phenotype.

Morinobu, A., Y. Kanno, et al. (2004). "Discrete Roles for Histone Acetylation in Human T Helper 1 Cell-specific Gene Expression." J. Biol. Chem. **279**(39): 40640-40646.

<http://www.jbc.org/cgi/content/abstract/279/39/40640>

To better understand the control of T helper (TH) 1-expressed genes, we compared and contrasted acetylation and expression for three key genes, IFNG, TBET, and IL18RAP and found them to be distinctly regulated. The TBET and the IFNG genes, but not the IL18RAP gene, showed preferential acetylation of histones H3 and H4 during TH1 differentiation. Analysis of acetylation of specific histone residues revealed that H3(Lys-9), H4(Lys-8), and H4(Lys-12) were preferentially modified in TH1 cells, suggesting a possible contribution of acetylation of these residues for induction of these genes. On the other hand, the acetylation of IL18RAP gene occurred both in TH1 and TH2 cells the similar kinetics and on the same with residues, demonstrating that selective histone acetylation was not universally the case for all TH1-expressed genes. Histone H3 acetylation of IFNG and TBET genes occurred with different kinetics, however, and was distinctively regulated by cytokines. Interleukin (IL)-12 and IL-18 enhanced the histone acetylation of the IFNG gene. By contrast, histone acetylation of the TBET gene was markedly suppressed by IL-4, whereas IL-12 and IL-18 had only modest effects suggesting that histone acetylation during TH1 differentiation is a process that is regulated by various factors at multiple levels. By treating Th2 cells with a histone deacetylase inhibitor, we restored histone acetylation of the IFNG and TBET genes, but it did not fully restore their expression in TH2 cells, again suggesting that histone acetylation explains one but not all the aspects of TH1-specific gene expression.

Morohashi, Y., N. Hatano, et al. (2002). "Molecular Cloning and Characterization of CALP/KChIP4, a Novel EF-hand Protein Interacting with Presenilin 2 and Voltage-gated Potassium Channel Subunit Kv4." J. Biol. Chem. **277**(17): 14965-14975.

<http://www.jbc.org/cgi/content/abstract/277/17/14965>

Presenilin (PS) genes linked to early-onset familial Alzheimer's disease encode polytopic membrane proteins that are presumed to constitute the catalytic subunit of [gamma]-secretase, forming a high molecular weight complex with other proteins. During our attempts to identify binding partners of PS2, we cloned CALP (calsenilin-like protein)/KChIP4, a novel member of calsenilin/KChIP protein family that interacts with the C-terminal region of PS. Upon co-expression in cultured cells, CALP was directly bound to and co-localized with PS2 in endoplasmic reticulum. Overexpression of CALP did not affect the metabolism or stability of PS complex, and [gamma]-cleavage of [beta]APP or Notch site 3 cleavage was not altered. However, co-expression of CALP and a voltage-gated potassium channel subunit Kv4.2 reconstituted the features of A-type K⁺ currents and CALP directly bound Kv4.2, indicating that CALP functions as KChIPs that are known as components of native Kv4 channel complex. Taken together, CALP/KChIP4 is a novel EF-hand protein interacting with PS as well as with Kv4 that may modulate functions of a subset of membrane proteins in brain.

Muradov, K. G., K. K. Boyd, et al. (2003). "The GAFa Domains of Rod cGMP-phosphodiesterase 6 Determine the Selectivity of the Enzyme Dimerization." *J. Biol. Chem.* **278**(12): 10594-10601.

<http://www.jbc.org/cgi/content/abstract/278/12/10594>

Retinal rod cGMP phosphodiesterase (PDE6 family) is the effector enzyme in the vertebrate visual transduction cascade. Unlike other known PDEs that form catalytic homodimers, the rod PDE6 catalytic core is a heterodimer composed of [alpha] and [beta] subunits. A system for efficient expression of rod PDE6 is not available. Therefore, to elucidate the structural basis for specific dimerization of rod PDE6, we constructed a series of chimeric proteins between PDE6[alpha][beta] and PDE5, which contain the N-terminal GAFa/GAFb domains, or portions thereof, of the rod enzyme. These chimeras were co-expressed in Sf9 cells in various combinations as His-, myc-, or FLAG-tagged proteins. Dimerization of chimeric PDEs was assessed using gel filtration and sucrose gradient centrifugation. The composition of formed dimeric enzymes was analyzed with Western blotting and immunoprecipitation. Consistent with the selectivity of PDE6 dimerization in vivo, efficient heterodimerization was observed between the GAF regions of PDE6[alpha] and PDE6[beta] with no significant homodimerization. In addition, PDE6[alpha] was able to form dimers with the cone PDE6[alpha]' subunit. Furthermore, our analysis indicated that the PDE6 GAFa domains contain major structural determinants for the affinity and selectivity of dimerization of PDE6 catalytic subunits. The key dimerization selectivity module of PDE6 has been localized to a small segment within the GAFa domains, PDE6[alpha]-59-74/PDE6[beta]-57-72. This study provides tools for the generation of the homodimeric [alpha][alpha] and [beta][beta] enzymes that will allow us to address the question of functional significance of the unique heterodimerization of rod PDE6.

Nagaoka, K., H. Nojima, et al. (2003). "Regulation of Blastocyst Migration, Apposition, and Initial Adhesion by a Chemokine, Interferon {gamma}-inducible Protein 10 kDa (IP-10), during Early Gestation." *J. Biol. Chem.* **278**(31): 29048-29056.

<http://www.jbc.org/cgi/content/abstract/278/31/29048>

For a pregnancy to be established, initial apposition and adhesion of the blastocyst to maternal endometrium must occur in a coordinated manner; however, a key factor(s) that mediates the trophoblast cell migration and attachment to the apical surface of the endometrium has not been identified. In this study, we examined the effect of an endometrial chemokine, interferon-{gamma}-inducible protein 10 kDa (IP-10), on conceptus migration to the endometrial epithelium. We first studied endometrial IP-10 mRNA expression, which was localized in the subepithelial stromal region, and detected the protein in the uterine flushing media during early pregnancy. Expression of IP-10 mRNA by the endometrium of cyclic animals was stimulated by the addition of a conceptus factor interferon-tau (IFN- τ). Immunofluorescent analysis revealed that IP-10 receptor, CXCR3, was localized in the trophoblast cells, to which biotinylated-recombinant caprine IP-10 (rcIP-10) bound. Chemotaxis assay indicated that rcIP-10 stimulated the migration of trophoblast cells, and the effects of rcIP-10 were neutralized by the pretreatment with an anti-IP-10 antibody. Adhesive activity of trophoblast cells to fibronectin was promoted by rcIP-10, and the effect was inhibited by the use of anti-IP-10 antibody. Further adhesion experiments demonstrated that binding of trophoblast cells to fibronectin was completely inhibited by a peptide of the Arg-Gly-Asp (RGD) sequence, which binds to integrins {alpha}{5}{beta}1, {alpha}V{beta}1, {alpha}V{beta}3, and {alpha}V{beta}5, whereas non-binding peptide containing Arg-Gly-Glu (RGE) had minimal effects. More importantly, rcIP-10 promoted the adhesion of trophoblast cells to primary cells isolated from endometrial epithelium. Furthermore, rcIP-10 stimulated the expression of integrin {alpha}5, {alpha}V, and {beta}3 subunit mRNA in trophoblast cells. These findings suggest that endometrial IP-10 regulates the establishment of apical interactions between trophoblast and epithelial cells during early gestation.

Nair, R. and C. Shaha (2003). "Diethylstilbestrol Induces Rat Spermatogenic Cell Apoptosis in Vivo through Increased Expression of Spermatogenic Cell Fas/FasL System." *J. Biol. Chem.* **278**(8): 6470-6481.

<http://www.jbc.org/cgi/content/abstract/278/8/6470>

The significant role that estrogens play in spermatogenesis has opened up an exciting area of research in male reproductive biology. The realization that estrogens are essential for proper maintenance of spermatogenesis, as well as growing evidence pointing to the deleterious effects of estrogen-like chemicals on male reproductive health, has made it imperative to dissect the role estrogens play in the male. Using a model estrogen, diethylstilbestrol (DES), to induce spermatogenic cell apoptosis in vivo in the male rat, we provide a new insight into an estrogen-dependent regulation of the Fas-FasL system specifically in spermatogenic cells. We show a distinct increase in Fas-FasL expression in spermatogenic cells upon exposure to diethylstilbestrol. This increase is confined to the spermatid population, which correlates with increased apoptosis seen in the haploid cells. Testosterone supplementation is able to prevent DES-induced Fas-FasL up-regulation and apoptosis in the spermatogenic cells. DES-induced germ cell apoptosis does not occur in Fas-deficient lpr mice. One other important finding is that spermatogenic cells are type II cells, as the increase in Fas-FasL expression in the spermatogenic cells is followed by the cleavage of caspase-8 to its active form, following which Bax translocates to the mitochondria and precipitates the release of cytochrome c that is accompanied by a drop in mitochondrial potential. Subsequent to this, activation of caspase-9 occurs that in turn activates caspase-3 leading to the cleavage of poly(ADP-ribose) polymerase. Taken together, the data indicate that estrogen-like chemicals can precipitate apoptotic death in spermatogenic cells by increasing the expression of spermatogenic cell Fas-FasL, thus initiating apoptosis in the same lineage of cells through the activation of the apoptotic pathway chosen by type II cells.

Ngo, T. T., M. K. Bennett, et al. (2002). "A Role for Cyclic AMP Response Element-binding Protein (CREB) but Not the Highly Similar ATF-2 Protein in Sterol Regulation of the Promoter for 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase." *J. Biol. Chem.* **277**(37): 33901-33905.

<http://www.jbc.org/cgi/content/abstract/277/37/33901>

Sterol regulatory element-binding proteins (SREBPs) activate promoters for key genes of metabolism to keep pace with the cellular demand for lipids. In each SREBP-regulated promoter, at least one ubiquitous co-regulatory factor that binds to a neighboring recognition site is also required for efficient gene induction. Some of these putative co-regulatory proteins are members of transcription factor families that all bind to the same DNA sequence elements in vitro and are often expressed in the same cells. These two observations have made it difficult to assign specific and redundant functions to the unique members of a specific gene family. We have used the chromatin immunoprecipitation (ChIP) technique coupled with a transient complementation assay in *Drosophila* SL2 cells to directly compare the ability of two members of the CREB/ATF family to function as co-regulatory proteins for SREBP-dependent activation of the HMG-CoA reductase promoter. Results from both of these experimental systems demonstrate that CREB is an efficient SREBP co-regulator but ATF-2 is not.

Nishino, K., N. Hattori, et al. (2004). "DNA Methylation-mediated Control of Sry Gene Expression in Mouse Gonadal Development." *J. Biol. Chem.* **279**(21): 22306-22313.

<http://www.jbc.org/cgi/content/abstract/279/21/22306>

DNA methylation at CpG sequences is involved in tissue-specific and developmentally regulated gene expression. The Sry (sex-determining region on the Y chromosome) gene encodes a master protein for initiating testis differentiation in mammals, and its expression is restricted to gonadal somatic cells at 10.5-12.5 days post-coitum (dpc) in the mouse. We found that in vitro methylation of the 5'-flanking region of the Sry gene caused suppression of reporter activity, implying that Sry gene expression could be regulated by DNA methylation-mediated gene silencing. Bisulfite restriction mapping and sodium bisulfite sequencing revealed that the 5'-flanking region of the Sry gene was hypermethylated in the 8.5-dpc embryos in which the Sry gene was not expressed. Importantly, this region was specifically hypomethylated in the gonad at 11.5 dpc, while the hypermethylated status was maintained in tissues that do not express the Sry gene. We concluded that expression of the Sry gene is under the control of an epigenetic mechanism mediated by DNA methylation.

Nishiya, T. and A. L. DeFranco (2004). "Ligand-regulated Chimeric Receptor Approach Reveals Distinctive Subcellular Localization and Signaling Properties of the Toll-like Receptors." J. Biol. Chem. **279**(18): 19008-19017.

<http://www.jbc.org/cgi/content/abstract/279/18/19008>

Toll-like receptors (TLRs) are sensors for the detection of invading infectious agents and can initiate innate immune responses. Because the innate immune system induces an appropriate defense against different pathogens, different TLR signaling domains may have unique properties that are responsible for eliciting distinctive responses to different types of pathogens. To test this hypothesis, we created ligand-regulated TLR chimeric receptors composed of the extracellular region of TLR4 and the transmembrane and cytoplasmic regions of other TLRs and expressed these chimeras in macrophages lacking endogenous TLR4. Interestingly, the chimeras between TLR4 and either TLR3, TLR7, or TLR9 were localized completely intracellularly whereas other chimeras were expressed on the cell surface. Lipopolysaccharide (LPS), a ligand for these chimeras, induced the activation of nuclear factor κ B and mitogen-activated protein kinases and the subsequent production of pro-inflammatory cytokines in macrophages expressing TLR4, TLR4/TLR5, or TLR4/TLR8 chimeras but not in macrophages expressing TLR4/TLR1, TLR4/TLR2, or TLR4/TLR6 chimeras. Co-expression of unresponsive chimeras in some combinations (chimeras with TLR1+TLR2 or TLR2+TLR6 but not TLR1+TLR6) resulted in LPS responsiveness, indicating functional complementarity. Furthermore, the pair of TLR2+TLR6 chimera required approximately 10-fold less LPS to induce the same responses compared with the TLR1+TLR2 pair. Finally, LPS induced effective interferon- β production and subsequent Stat1 phosphorylation in macrophages expressing full-length TLR4 but not other cell surface TLR chimeras. These results suggest that the functions of TLRs are diversified not only in their extracellular regions for ligand recognition but also in their transmembrane and cytoplasmic regions for subcellular localization and signaling properties.

Ohshima, T. and K. Shimotohno (2003). "Transforming Growth Factor- β -mediated Signaling via the p38 MAP Kinase Pathway Activates Smad-dependent Transcription through SUMO-1 Modification of Smad4." J. Biol. Chem. **278**(51): 50833-50842.

<http://www.jbc.org/cgi/content/abstract/278/51/50833>

Post-translational modifications such as ubiquitination, phosphorylation, and acetylation play important roles in the regulation of Smad-mediated functions. Here, we demonstrate that Smad4

is covalently modified by SUMO-1, which was characterized recently as a key modulator of many transcription factors. Sumoylation of Smad4 mainly occurs at lysine 159, located in the linker region, and facilitates Smad-dependent transcriptional activation. Furthermore, we show that the PIAS family proteins, PIAS1 and PIASx{beta}, function as E3 ligase factors for Smad4. Intriguingly, sumoylation of Smad4 was strongly enhanced by TGF- β -induced activation of the p38 MAP kinase pathway but not the Smad pathway. Activation of p38 not only stabilized PIASx{beta} protein but also enhanced PIASx{beta} gene expression, suggesting that PIAS-mediated sumoylation of Smad4 is regulated by the p38 MAP kinase pathway. These findings illustrate a novel regulatory mechanism by which Smad-dependent transcriptional activation cooperatively modulates Smad proteins through receptor-mediated phosphorylation and sumoylation.

Ohya, T., Y. Kawasaki, et al. (2002). "The DNA Polymerase Domain of polepsilon Is Required for Rapid, Efficient, and Highly Accurate Chromosomal DNA Replication, Telomere Length Maintenance, and Normal Cell Senescence in *Saccharomyces cerevisiae*." *J. Biol. Chem.* **277**(31): 28099-28108.

<http://www.jbc.org/cgi/content/abstract/277/31/28099>

Saccharomyces cerevisiae POL2 encodes the catalytic subunit of DNA polymerase [epsilon]. This study investigates the cellular functions performed by the polymerase domain of Pol2p and its role in DNA metabolism. The pol2-16 mutation has a deletion in the catalytic domain of DNA polymerase [epsilon] that eliminates its polymerase and exonuclease activities. It is a viable mutant, which displays temperature sensitivity for growth and a defect in elongation step of chromosomal DNA replication even at permissive temperatures. This mutation is synthetic lethal in combination with temperature-sensitive mutants or the 3'- to 5'-exonuclease-deficient mutant of DNA polymerase [delta] in a haploid cell. These results suggest that the catalytic activity of DNA polymerase [epsilon] participates in the same pathway as DNA polymerase [delta], and this is consistent with the observation that DNA polymerases [delta] and [epsilon] colocalize in some punctate foci on yeast chromatids during S phase. The pol2-16 mutant senesces more rapidly than wild type strain and also has shorter telomeres. These results indicate that the DNA polymerase domain of Pol2p is required for rapid, efficient, and highly accurate chromosomal DNA replication in yeast.

Okuda, T., M. Okamura, et al. (2002). "Single Nucleotide Polymorphism of the Human High Affinity Choline Transporter Alters Transport Rate." *J. Biol. Chem.* **277**(47): 45315-45322.

<http://www.jbc.org/cgi/content/abstract/277/47/45315>

High affinity choline uptake plays a critical role in the regulation of acetylcholine synthesis in cholinergic neurons. Recently, we succeeded in molecular cloning of the high affinity choline transporter (CHT1), which is specifically expressed in cholinergic neurons. Here we demonstrate the presence of functionally relevant, nonsynonymous single nucleotide polymorphism in the human CHT1 gene by comprehensive sequence analysis of the exons and the intron/exon boundaries including the transcription start site. The deduced amino acid change for the polymorphism is isoleucine to valine at amino acid 89 (I89V) located within the third transmembrane domain of the protein. The allele frequency of I89V was 6% for Ashkenazi Jews. Functional assessment of the I89V transporter in mammalian cell lines revealed a 40-50% decrease in Vmax for choline uptake rate compared with the wild type, whereas there was no alteration in the apparent affinities for choline, sodium, chloride, and the specific inhibitor hemicholinium-3. There also was no change in the specific hemicholinium-3 binding activity. The decreased choline uptake was not associated with the surface expression level of the protein as

assessed by biotinylation assay. These results suggest an impaired substrate translocation in the I89V transporter. The *Caenorhabditis elegans* ortholog of CHT1 has a valine residue at the corresponding position and a single replacement from valine to isoleucine caused a decrease in the choline uptake rate by 40%, suggesting that this hydrophobic residue is generally critical in the choline transport rate in CHT1. This polymorphism in the allelic CHT1 gene may represent a predisposing factor for cholinergic dysfunction.

Olosz, F. and T. R. Malek (2002). "Structural Basis for Binding Multiple Ligands by the Common Cytokine Receptor gamma -Chain." J. Biol. Chem. **277**(14): 12047-12052.

<http://www.jbc.org/cgi/content/abstract/277/14/12047>

The common [gamma]-chain ([gamma]c) that functions both in ligand binding and signal transduction is a shared subunit of the multichain receptors for interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15, and IL-21. The structural basis by which the ectodomain of [gamma]c contributes to binding six distinct cytokines is only partially defined. In the present study, epitope mapping of antagonistic anti-[gamma]c monoclonal antibodies led to the identification of Asn-128 of mouse [gamma]c that represents another potential contact residue that is required for binding IL-2, IL-7, and IL-15 but not IL-4. In addition, Tyr-103, Cys-161, Cys-210, and Cys-211, previously identified to contribute to binding IL-2 and IL-7, were also found to be involved in binding IL-4 and IL-15. Collectively, these data favor a model in which [gamma]c utilizes a common mechanism for its interactions with multiple cytokines, and the binding sites are largely overlapping but not identical. Asn-128 and Tyr-103 likely act as contact residues whereas Cys-161, Cys-210, and Gly-211 may stabilize the structure of the proposed ligand-interacting surface formed by the two extracytoplasmic domains.

Ortega, A., P. Ferrer, et al. (2003). "Down-regulation of Glutathione and Bcl-2 Synthesis in Mouse B16 Melanoma Cells Avoids Their Survival during Interaction with the Vascular Endothelium." J. Biol. Chem. **278**(41): 39591-39599.

<http://www.jbc.org/cgi/content/abstract/278/41/39591>

B16 melanoma (B16M) cells with high GSH content show high metastatic activity. However, the molecular mechanisms linking GSH to metastatic cell survival are unclear. The possible relationship between GSH and the ability of Bcl-2 to prevent cell death was studied in B16M cells with high (F10) and low (F1) metastatic potential. Analysis of a Bcl-2 family of genes revealed that B16M-F10 cells, as compared with B16M-F1 cells, overexpressed preferentially Bcl-2 ([~]5.7-fold). Hepatic sinusoidal endothelium-induced B16M-F10 cytotoxicity in vitro increased from [~]19% (controls) to [~]97% in GSH-depleted B16M-F10 cells treated with an antisense Bcl-2 oligodeoxynucleotide (Bcl-2-AS). L-Buthionine (S,R)-sulfoximine-induced GSH depletion or Bcl-2-AS decreased the metastatic growth of B16M-F10 cells in the liver. However, the combination of L-buthionine (S,R)-sulfoximine and Bcl-2-AS abolished metastatic invasion. Bcl-2-overexpressing B16M-F1/Tet-Bcl-2 and B16M-F10/Tet-Bcl-2 cells, as compared with controls, showed an increase in GSH content, no change in the rate of GSH synthesis, and a decrease in GSH efflux. Thus, Bcl-2 overexpression may increase metastatic cell resistance against oxidative/nitrosative stress by inhibiting release of GSH. In addition, Bcl-2 availability regulates the mitochondrial GSH (mtGSH)-dependent opening of the permeability transition pore complex. Death in B16M-F10 cells was sharply activated at mtGSH levels below 30% of controls values. However, this critical threshold increased to [~]60% of control values in Bcl-2-AS-treated B16M-F10 cells. GSH ester-induced replenishment of mtGSH levels (even under conditions of cytosolic GSH depletion) prevented cell death. Our results indicate that survival of B16M cells with high metastatic potential can be challenged by inhibiting their GSH and Bcl-2 synthesis.

Ortega, A. L., J. Carretero, et al. (2003). "Tumor Cytotoxicity by Endothelial Cells. IMPAIRMENT OF THE MITOCHONDRIAL SYSTEM FOR GLUTATHIONE UPTAKE IN MOUSE B16 MELANOMA CELLS THAT SURVIVE AFTER IN VITRO INTERACTION WITH THE HEPATIC SINUSOIDAL ENDOTHELIUM." *J. Biol. Chem.* **278**(16): 13888-13897.

<http://www.jbc.org/cgi/content/abstract/278/16/13888>

High GSH content associates with high metastatic activity in B16-F10 melanoma cells cultured to low density (LD B16M). GSH homeostasis was investigated in LD B16M cells that survive after adhesion to the hepatic sinusoidal endothelium (HSE). Invasive B16M (iB16M) cells were isolated using anti-Met-72 monoclonal antibodies and flow cytometry-coupled cell sorting. HSE-derived NO and H₂O₂ caused GSH depletion and a decrease in [gamma]-glutamylcysteine synthetase activity in iB16M cells. Overexpression of [gamma]-glutamylcysteine synthetase heavy and light subunits led to a rapid recovery of cytosolic GSH, whereas mitochondrial GSH (mtGSH) further decreased during the first 18 h of culture. NO and H₂O₂ damaged the mitochondrial system for GSH uptake (rates in iB16M were approximately 75% lower than in LD B16M cells). iB16M cells also showed a decreased activity of mitochondrial complexes II, III, and IV, less O₂ consumption, lower ATP levels, higher O₂ production, and lower mitochondrial membrane potential. In vitro growing iB16M cells maintained high viability (>98%) and repaired HSE-induced mitochondrial damages within 48 h. However, iB16M cells with low mtGSH levels were highly susceptible to TNF- α -induced oxidative stress and death. Therefore depletion of mtGSH levels may represent a critical target to challenge survival of invasive cancer cells.

Osada, M., S. Imaoka, et al. (2002). "NADPH-Cytochrome P-450 Reductase in the Plasma Membrane Modulates the Activation of Hypoxia-inducible Factor 1." *J. Biol. Chem.* **277**(26): 23367-23373.

<http://www.jbc.org/cgi/content/abstract/277/26/23367>

Hypoxia induces a group of physiologically important genes that include erythropoietin (EPO) and vascular endothelial growth factor (VEGF). Hypoxia-inducible factor 1 (HIF-1) was identified as a hypoxia-activated transcription factor; however, the molecular mechanisms that underlie hypoxia signal transduction in mammalian cells remain undefined. In this study, we found that a flavoprotein, NADPH-P450 reductase (NPR), could regulate the induction of EPO mRNA under hypoxic conditions. Hypoxic EPO mRNA induction in Hep3B cells was inhibited by diphenyleneiodonium chloride, which is an inhibitor of NADPH-dependent enzymes. NPR antisense cDNA was transfected into Hep3B cells, and NPR-deficient hepatocyte cells (NPR⁻ cells) were established. NPR⁻ cells lacked EPO induction under hypoxia, and HIF-1 α in NPR⁻ cells did not respond to either transcriptional activation or translocation to the nucleus based on electrophoretic mobility shift assays and reporter gene assay including hypoxia response element. In contrast, NPR overexpression in Hep3B cells enhanced the DNA binding activity of HIF-1 α by luciferase reporter gene assay. A study with HeLa S3 cells produced the same results. Furthermore, anti-NPR IgG inhibited EPO induction. EPO induction inhibited by diphenyleneiodonium chloride was recovered by bovine serum albumin-NADPH (a covalent binding complex of bovine serum albumin and NADPH) as well as NADPH. These results suggested that NPR located at the plasma membrane regulates EPO expression in hypoxia, including HIF-1 activation and translocation. We further studied the expression of NPR and VEGF mRNAs in human tumor tissues and found that the NPR mRNA levels were correlated with the VEGF mRNA levels, suggesting that NPR might be an important factor in the hypoxic induction of genes such as VEGF in vivo.

Pankonin, M. S., J. T. Gallagher, et al. (2005). "Specific Structural Features of Heparan Sulfate Proteoglycans Potentiate Neuregulin-1 Signaling." J. Biol. Chem. **280**(1): 383-388.

<http://www.jbc.org/cgi/content/abstract/280/1/383>

Neuregulins are a family of growth and differentiation factors that act through activation of cell-surface erbB receptor tyrosine kinases and have essential functions both during development and on the growth of cancer cells. One alternatively spliced neuregulin-1 form has a distinct heparin-binding immunoglobulin-like domain that enables it to adhere to heparan sulfate proteoglycans at key locations during development and substantially potentiates its activity. We examined the structural specificity needed for neuregulin-1-heparin interactions using a gel mobility shift assay together with an assay that measures the ability of specific oligosaccharides to block erbB receptor phosphorylation in L6 muscle cells. Whereas the N-sulfate group of heparin was most important, the 2-O-sulfate and 6-O-sulfate groups also contributed to neuregulin-1 binding in these two assays. Optimal binding to neuregulin-1 required eight or more heparin disaccharides; however, as few as two disaccharides were still able to bind neuregulin-1 to a lesser extent. The physiological importance of this specificity was shown both by chemical and siRNA treatment of cultured muscle cells. Pretreatment of muscle cells with chlorate that blocks all sulfation or with an siRNA that selectively blocks N-sulfation significantly reduced erbB receptor activation by neuregulin-1 but had no effect on the activity of neuregulin-1 that lacks the heparin-binding domain. These results suggest that the regulation of glycosaminoglycan sulfation is an important biological mechanism that can modulate both the localization and potentiation of neuregulin-1 signaling.

Papoucheva, E., A. Dumuis, et al. (2004). "The 5-Hydroxytryptamine(1A) Receptor Is Stably Palmitoylated, and Acylation Is Critical for Communication of Receptor with Gi Protein." J. Biol. Chem. **279**(5): 3280-3291.

<http://www.jbc.org/cgi/content/abstract/279/5/3280>

In the present study, we verified that the mouse 5-hydroxytryptamine(1A) (5-HT1A) receptor is modified by palmitic acid, which is covalently attached to the protein through a thioester-type bond. Palmitoylation efficiency was not modulated by receptor stimulation with agonists. Block of protein synthesis by cycloheximide resulted in a significant reduction of receptor acylation, suggesting that palmitoylation occurs early after synthesis of the 5-HT1A receptor. Furthermore, pulse-chase experiments demonstrated that fatty acids are stably attached to the receptor. Two conserved cysteine residues 417 and 420 located in the proximal C-terminal domain were identified as acylation sites by site-directed mutagenesis. To address the functional role of 5-HT1A receptor acylation, we have analyzed the ability of acylation-deficient mutants to interact with heterotrimeric Gi protein and to modulate downstream effectors. Replacement of individual cysteine residues (417 or 420) resulted in a significantly reduced coupling of receptor with Gi protein and impaired inhibition of adenylyl cyclase activity. When both palmitoylated cysteines were replaced, the communication of receptors with G α i subunits was completely abolished. Moreover, non-palmitoylated mutants were no longer able to inhibit forskolin-stimulated cAMP formation, indicating that palmitoylation of the 5-HT1A receptor is critical for the enabling of Gi protein coupling/effector signaling. The receptor-dependent activation of extracellular signal-regulated kinase was also affected by acylation-deficient mutants, suggesting the importance of receptor palmitoylation for the signaling through the G β γ -mediated pathway, in addition to the G α i-mediated signaling.

Patnaik, S. K., B. Potvin, et al. (2004). "LEC12 and LEC29 Gain-of-Function Chinese Hamster Ovary Mutants Reveal Mechanisms for Regulating VIM-2 Antigen Synthesis and E-selectin Binding." J.

Biol. Chem. **279**(48): 49716-49726.

<http://www.jbc.org/cgi/content/abstract/279/48/49716>

LEC12 and LEC29 are two gain-of-function Chinese hamster ovary glycosylation mutants that express the Fut9 gene encoding $\alpha(1,3)$ fucosyltransferase IX ($\alpha(1,3)$ Fuc-TIX). Both mutants express the Lewis X (LeX) determinant $\text{Gal}\beta(1,4)[\text{Fuc}\alpha(1,3)]\text{GlcNAc}$, and LEC12, but not LEC29 cells, also express the VIM-2 antigen $\text{SA}\alpha(2,3)\text{-Gal}\beta(1,4)\text{GlcNAc}\beta(1,3)\text{Gal}\beta(1,4)[\text{Fuc}\alpha(1,3)]\text{GlcNAc}$. Here we show that LEC29 cells transfected with a Fut9 cDNA express VIM-2, and thus LEC29 cells synthesize appropriate acceptors to generate the VIM-2 epitope. Semiquantitative reverse transcription-PCR showed that LEC12 has 10- to 20-fold less Fut9 gene transcripts than LEC29. However, Western analysis revealed that LEC12 has ~ 20 times more Fut9 protein than LEC29. The latter finding was consistent with our previous observation that LEC12 has ~ 40 times more in vitro $\alpha(1,3)$ Fuc-T activity than LEC29. The basis for the difference in Fut9 protein levels was found to lie in sequence differences in the 5'-untranslated regions (5'-UTR) of LEC12 and LEC29 Fut9 gene transcripts. Whereas reporter assays with the respective 5'-UTR regions linked to luciferase did not indicate a reduced translation efficiency caused by the LEC29 5'-UTR, transfected full-length LEC29 Fut9 cDNA or in vitro-synthesized full-length LEC29 Fut9 RNA gave less Fut9 protein than similar constructs with a LEC12 5'-UTR. This difference appears to be largely responsible for the reduced $\alpha(1,3)$ Fuc-TIX activity and lack of VIM-2 expression of LEC29 cells. This could be of physiological relevance, because LEC29 and parent Chinese hamster ovary cells transiently expressing a Fut9 cDNA were able to bind mouse E-selectin, although they did not express sialyl-LeX.

Paulussen, A., A. Raes, et al. (2002). "A Novel Mutation (T65P) in the PAS Domain of the Human Potassium Channel HERG Results in the Long QT Syndrome by Trafficking Deficiency." J. Biol. Chem. **277**(50): 48610-48616.

<http://www.jbc.org/cgi/content/abstract/277/50/48610>

The congenital long QT syndrome is a cardiac disease characterized by an increased susceptibility to ventricular arrhythmias. The clinical hallmark is a prolongation of the QT interval, which reflects a delay in repolarization caused by mutations in cardiac ion channel genes. Mutations in the HERG (human ether-a-go-go-related gene KCNH2) can cause a reduction in I_{Kr} , one of the currents responsible for cardiac repolarization. We describe the identification and characterization of a novel missense mutation T65P in the PAS (Per-Arnt-Sim) domain of HERG, resulting in defective trafficking of the protein to the cell membrane. Defective folding of the mutant protein could be restored by decreased cell incubation temperature and pharmacologically by cisapride and E-4031. When trafficking was restored by growing cells at 27 $^{\circ}\text{C}$, the kinetics of the mutated channel resembled that of wild-type channels although the rate of activation, deactivation, and recovery from inactivation were accelerated. No positive evidence for the formation of heterotetramers was obtained by co-expression of wild-type with mutant subunits at 37 $^{\circ}\text{C}$. As a consequence the clinical symptoms may be explained rather by haploinsufficiency than by dominant negative effects. This study is the first to relate a PAS domain mutation in HERG to a trafficking deficiency at body temperature, apart from effects on channel deactivation.

Pelicano, H., L. Feng, et al. (2003). "Inhibition of Mitochondrial Respiration: A NOVEL STRATEGY TO ENHANCE DRUG-INDUCED APOPTOSIS IN HUMAN LEUKEMIA CELLS BY A REACTIVE OXYGEN SPECIES-MEDIATED MECHANISM." J. Biol. Chem. **278**(39): 37832-37839.

<http://www.jbc.org/cgi/content/abstract/278/39/37832>

Cancer cells are under intrinsic increased oxidative stress and vulnerable to free radical-induced apoptosis. Here, we report a strategy to hinder mitochondrial electron transport and increase superoxide [IMG]f1.gif" BORDER="0"> radical generation in human leukemia cells as a novel mechanism to enhance apoptosis induced by anticancer agents. This strategy was first tested in a proof-of-principle study using rotenone, a specific inhibitor of mitochondrial electron transport complex I. Partial inhibition of mitochondrial respiration enhances electron leakage from the transport chain, leading to an increase in [IMG]f1.gif" BORDER="0"> generation and sensitization of the leukemia cells to anticancer agents whose action involve free radical generation. Using leukemia cells with genetic alterations in mitochondrial DNA and biochemical approaches, we further demonstrated that As₂O₃, a clinically active anti-leukemia agent, inhibits mitochondrial respiratory function, increases free radical generation, and enhances the activity of another [IMG]f2.gif" BORDER="0"> agent against cultured leukemia cells and primary leukemia cells isolated from patients. Our study shows that interfering mitochondrial respiration is a novel mechanism by which As₂O₃ increases generation of free radicals. This novel mechanism of action provides a biochemical basis for developing new drug combination strategies using As₂O₃ to enhance the activity of anticancer agents by promoting generation of free radicals.

Perrier, E., R. Perrier, et al. (2004). "Ca²⁺ Controls Functional Expression of the Cardiac K⁺ Transient Outward Current via the Calcineurin Pathway." *J. Biol. Chem.* **279**(39): 40634-40639.

<http://www.jbc.org/cgi/content/abstract/279/39/40634>

The transient outward K⁺ current (I_{to}) modulates transmembrane Ca²⁺ influx into cardiomyocytes, which, in turn, might act on I_{to}. Here, we investigated whether Ca²⁺ modifies functional expression of I_{to}. Whole-cell I_{to} were recorded using the patch clamp technique in single right ventricular myocytes isolated from adult rats and incubated for 24 h at 37 {degrees}C in a serum-free medium containing various Ca²⁺ concentrations ([Ca²⁺]_o). Increasing the [Ca²⁺]_o from 0.5 to 1.0 and 2.5 mM produced a gradual decrease in I_{to} density without change in current kinetics. Quantitative reverse transcriptase-PCR showed that a decrease of the Kv4.2 mRNA could account for this decrease. In the acetoxymethyl ester form of 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA-AM)-loaded myocytes (a permeant Ca²⁺ chelator), I_{to} density increased significantly when cells were exposed for 24 h to either 1 or 2.5 mM [Ca²⁺]_o. Moreover, 24-h exposure to the Ca²⁺ channel agonist, Bay K8644, in 1 mM [Ca²⁺]_o induced a decrease in I_{to} density, whereas the Ca²⁺ channel antagonist, nifedipine, blunted I_{to} decrease in 2.5 mM [Ca²⁺]_o. The decrease of I_{to} in 2.5 mM [Ca²⁺]_o was also prevented by co-incubation with either the calmodulin inhibitor W7 or the calcineurin inhibitors FK506 or cyclosporin A. Furthermore, in myocytes incubated for 24 h with 2.5 mM [Ca²⁺]_o, calcineurin activity was significantly increased compared with 1 mM [Ca²⁺]_o. Our data suggest that modulation of [Ca²⁺]_i via L-type Ca²⁺ channels, which appears to involve the Ca²⁺/calmodulin-regulated protein phosphatase calcineurin, down-regulates the functional expression of I_{to}. This effect might be involved in many physiological and pathological modulations of I_{to} channel expression in cardiac cells, as well other cell types.

Piechotta, K., J. Lu, et al. (2002). "Cation Chloride Cotransporters Interact with the Stress-related Kinases Ste20-related Proline-Alanine-rich Kinase (SPAK) and Oxidative Stress Response 1 (OSR1)." *J. Biol. Chem.* **277**(52): 50812-50819.

<http://www.jbc.org/cgi/content/abstract/277/52/50812>

Cells respond to stress stimuli by mounting specific responses. During osmotic and oxidative stress, cation chloride cotransporters, e.g. Na-K-2Cl and K-Cl cotransporters, are activated to maintain fluid/ion homeostasis. Here we report the interaction of the stress-related serine-threonine kinases Ste20-related proline-alanine-rich kinase (SPAK) and oxidative stress response 1 (OSR1) with the cotransporters KCC3, NKCC1, and NKCC2 but not KCC1 and KCC4. The interaction was identified using yeast two-hybrid assays and confirmed via glutathione S-transferase pull-down experiments. Evidence for in vivo interaction was established by co-immunoprecipitation of SPAK from mouse brain with anti-NKCC1 antibody. The interacting region of both kinases comprises the last 100 amino acids of the protein. The SPAK/OSR1 binding motif on the cotransporters consists of nine residues, starting with an (R/K)FX(V/I) sequence followed by five additional residues that are essential for binding but for which no consensus was found. Immunohistochemical analysis of choroid plexus epithelium revealed co-expression of NKCC1 and SPAK on the apical membrane. In contrast, in choroid plexus epithelium from NKCC1 null mice, SPAK immunostaining was found in the cytoplasm. We conclude that several cation chloride co-transporters interact with SPAK and/or OSR1, and we hypothesize that this interaction might play a role during the initiation of the cellular stress response.

Pinkoski, M. J., N. M. Droin, et al. (2002). "Tumor Necrosis Factor alpha Up-regulates Non-lymphoid Fas-ligand following Superantigen-induced Peripheral Lymphocyte Activation." *J. Biol. Chem.* **277**(44): 42380-42385.

<http://www.jbc.org/cgi/content/abstract/277/44/42380>

Members of the tumor necrosis factor (TNF) and TNF receptor families play important roles in inducing apoptosis and mediating the inflammatory response. Activated T lymphocytes can trigger the expression of Fas-ligand on non-lymphoid tissue, such as intestinal epithelial cells (IEC), and this, in turn, can induce apoptosis in the T cells. Here, we examine the role of TNF[alpha] in this feedback regulation. Injection of TNF[alpha] into mice caused a rapid up-regulation of Fas-ligand mRNA in IEC. TNF[alpha]-induced activation of the Fas-ligand promoter in IEC requires NF-[kappa]B as this was blocked by an I-[kappa]B[alpha]M super-repressor and by mutation of an NF-[kappa]B site in the Fas-ligand promoter. Activation of T cells by antigen induced Fas-ligand expression in IEC in vivo in wild type, but not in TNF[alpha]-/- or TNFR1-/- mice. These results define a novel pathway wherein TNF[alpha], produced by activated T cells in the intestine, induce Fas-ligand expression in IEC. This is the first observation that one member of the TNF superfamily mediates the regulation of another family member and represents a potential feedback mechanism controlling lymphocyte infiltration and inflammation in the small intestine.

Ponimaskin, E. G., M. Heine, et al. (2002). "The 5-Hydroxytryptamine(4a) Receptor Is Palmitoylated at Two Different Sites, and Acylation Is Critically Involved in Regulation of Receptor Constitutive Activity." *J. Biol. Chem.* **277**(4): 2534-2546.

<http://www.jbc.org/cgi/content/abstract/277/4/2534>

We have reported recently that the mouse 5-hydroxytryptamine(4a) (5-HT4(a)) receptor undergoes dynamic palmitoylation (Ponimaskin, E. G., Schmidt, M. F., Heine, M., Bickmeyer, U., and Richter, D. W. (2001) *Biochem. J.* 353, 627-663). In the present study, conserved cysteine residues 328/329 in the carboxyl terminus of the 5-HT4(a) receptor were identified as potential acylation sites. In contrast to other palmitoylated G-protein-coupled receptors, the additional cysteine residue 386 positioned close to the COOH-terminal end of the receptor was also found to be palmitoylated. Using pulse and pulse-chase labeling techniques, we demonstrated that palmitoylation of individual cysteines is a reversible process and that agonist stimulation of the 5-

HT4(a) receptor independently increases the rate of palmitate turnover for both acylation sites. Analysis of acylation-deficient mutants revealed that non-palmitoylated 5-HT4(a) receptors were indistinguishable from the wild type in their ability to interact with Gs, to stimulate the adenylyl cyclase activity and to activate cyclic nucleotide-sensitive cation channels after agonist stimulation. The most distinctive finding of the present study was the ability of palmitoylation to modulate the agonist-independent constitutive 5-HT4(a) receptor activity. We demonstrated that mutation of the proximal palmitoylation site (Cys328 [right-arrow] Ser/Cys329 [right-arrow] Ser) significantly increases the capacity of receptors to convert from the inactive (R) to the active (R*) form in the absence of agonist. In contrast, the rate of isomerization from R to R* for the Cys386 [right-arrow] Ser as well as for the triple, non-palmitoylated mutant (Cys328 [right-arrow] Ser/Cys329 [right-arrow] Ser/Cys386 [right-arrow] Ser) was similar to that obtained for the wild type.

Priest, J. W. and S. L. Hajduk (2003). "Trypanosoma brucei Cytochrome c1 Is Imported into Mitochondria Along an Unusual Pathway." *J. Biol. Chem.* **278**(17): 15084-15094.

<http://www.jbc.org/cgi/content/abstract/278/17/15084>

In most eukaryotic organisms, cytochrome c1 is encoded in the nucleus, translated on cytosolic ribosomes, and directed to its final destination in the mitochondrial inner membrane by a bipartite, cleaved, amino-terminal presequence. However, in the kinetoplastids and euglenoids, the cytochrome c1 protein has been shown to lack a cleaved presequence; a single methionine is removed from the amino terminus upon maturation, and the sequence upstream of the heme-binding site is generally shorter than that of the other eukaryotic homologs. We have used a newly developed mitochondrial protein import assay system from *Trypanosoma brucei* to demonstrate that the *T. brucei* cytochrome c1 protein is imported along a non-conservative pathway similar to that described for the inner membrane carrier proteins of other organisms. This pathway requires external ATP and an external protein receptor but is not absolutely dependent on a membrane potential or on ATP hydrolysis in the mitochondrial matrix. We propose the cytochrome c1 import in *T. brucei* is a two-step process first involving a membrane potential independent translocation across the outer mitochondrial membrane followed by heme attachment and a membrane potential-dependent insertion into the inner membrane.

Pruitt, K., A. S. Ulku, et al. (2005). "RAS mediated loss of the pro-apoptotic response protein par-4 is mediated by DNA hypermethylation through RAF-independent and RAF-dependent signaling cascades in epithelial cells." *J. Biol. Chem.*: M503083200.

<http://www.jbc.org/cgi/content/abstract/M503083200v1>

The apoptosis promoting protein, Par-4, has been shown to be downregulated in Ras-transformed NIH 3T3 fibroblasts through the Raf/MEK/ERK mitogen-activated protein kinase (MAPK) pathway. Since mutations of the ras gene are most often found in tumors of epithelial origin, we explored the signaling pathways utilized by oncogenic Ras to downregulate Par-4 in RIE-1 and ROSE epithelial cells. We determined that constitutive activation of the Raf, phosphatidylinositol 3-kinase (PI3K), or Ral guanine nucleotide exchange factor effector pathway alone was not sufficient to downregulate Par-4 in RIE-1 or ROSE cells. However, treatment of Ras-transformed RIE-1 or ROSE cells with the MEK inhibitors U0126 or PD98059 increased Par-4 protein expression. Thus, while oncogenic Ras utilizes the Raf/MEK/ERK pathway to down modulate Par-4 in both fibroblasts and epithelial cells, Ras activation of an additional signaling pathway(s) is required to achieve the same outcome in epithelial cells. Methylation-specific PCR showed the Par-4 promoter is methylated in Ras transformed cells through a MEK-dependent pathway and treatment with the DNA methyltransferase inhibitor, azadeoxycytidine, restored Par-

4 mRNA transcript and protein levels suggesting that the mechanism for Ras-mediated downregulation of Par-4 is by promoter methylation. Support for this possibility is provided by our observation that Ras transformation was associated with upregulated expression of the Dnmt1 and Dnmt3 DNA methyltransferases. Finally, ectopic Par-4 expression significantly reduced Ras-mediated growth in soft agar but not morphological transformation highlighting the importance of Par-4 downregulation in specific aspects of Ras-mediated transformation of epithelial cells.

Pulliainen, A. T., S. Haataja, et al. (2003). "Molecular Basis of H₂O₂ Resistance Mediated by Streptococcal Dpr. DEMONSTRATION OF THE FUNCTIONAL INVOLVEMENT OF THE PUTATIVE FERROXIDASE CENTER BY SITE-DIRECTED MUTAGENESIS IN STREPTOCOCCUS SUI." J. Biol. Chem. **278**(10): 7996-8005.

<http://www.jbc.org/cgi/content/abstract/278/10/7996>

H₂O₂ is an unavoidable cytotoxic by-product of aerobic life. Dpr, a recently discovered member of the Dps protein family, provides a means for catalase-negative bacteria to tolerate H₂O₂. Potentially, Dpr could bind free intracellular iron and thus inhibit the Fenton chemistry-catalyzed formation of toxic hydroxyl radicals (H₂O₂ + Fe²⁺ [right-arrow] {middle dot}OH + -OH + Fe³⁺). We explored the in vivo function of Dpr in the catalase- and NADH peroxidase-negative pig and human pathogen *Streptococcus suis*. We show that: (i) a Dpr allelic exchange knockout mutant was hypersensitive (~106-fold) to H₂O₂, (ii) Dpr incorporated iron in vivo, (iii) a putative ferroxidase center was present in Dpr, (iv) single amino acid substitutions D74A or E78A to the putative ferroxidase center abolished the in vivo iron incorporation, and (v) the H₂O₂ hypersensitive phenotype was complemented by wild-type Dpr or by a membrane-permeating iron chelator, but not by the site-mutated forms of Dpr. These results demonstrate that the putative ferroxidase center of Dpr is functionally active in iron incorporation and that the H₂O₂ resistance is mediated by Dpr in vivo by its iron binding activity.

Rafiee, P., Y. Shi, et al. (2003). "Cellular Redistribution of Inducible Hsp70 Protein in the Human and Rabbit Heart in Response to the Stress of Chronic Hypoxia: ROLE OF PROTEIN KINASES." J. Biol. Chem. **278**(44): 43636-43644.

<http://www.jbc.org/cgi/content/abstract/278/44/43636>

Many infants who undergo cardiac surgery have a congenital cyanotic defect where the heart is chronically perfused with hypoxemic blood. Infant hearts adapt to chronic hypoxemia by activation of intracellular protein kinase signal transduction pathways. However, the involvement of heat shock protein 70 in adaptation to chronic hypoxemia and its role in protein kinase signaling pathways is unknown. We determined expression of message and subcellular protein distribution for inducible (Hsp70i) and constitutive heat shock protein 70 (Hsc70) in chronically hypoxic and normoxic infant human and rabbit hearts and their relationship to protein kinases. In chronically hypoxic human and rabbit hearts message levels for Hsp70i were elevated 4- to 5-fold compared with normoxic hearts, Hsp70i protein was redistributed from the particulate to the cytosolic fraction. In normoxic infants Hsp70i protein was distributed almost equally between the cytosolic and particulate fractions. Hsc70 message and subcellular distribution of Hsc70 protein were unaffected by chronic hypoxia. We then determined if protein kinases influence Hsp70i protein subcellular distribution. In rabbit hearts SB203580 and chelerythrine reduced Hsp70i message levels, whereas SB203580, chelerythrine, and curcumin reversed the subcellular redistribution of Hsp70i protein caused by chronic hypoxia, with no effect in normoxic hearts, indicating regulation of Hsp70i message and subcellular distribution of Hsp70i protein in chronically hypoxic rabbit hearts is influenced by protein kinase C and mitogen-activated protein kinases, specifically p38 MAPK and JNK. We conclude the Hsp70 signal transduction pathway plays an important role in

adaptation of infant human and rabbit hearts to chronic hypoxemia.

Rahman, A. S., J. Hotherhall, et al. (2005). "Tandemly Duplicated Acyl Carrier Proteins, Which Increase Polyketide Antibiotic Production, Can Apparently Function Either in Parallel or in Series." J. Biol. Chem. **280**(8): 6399-6408.

<http://www.jbc.org/cgi/content/abstract/280/8/6399>

Polyketide biosynthesis involves the addition of subunits commonly derived from malonate or methylmalonate to a starter unit such as acetate. Type I polyketide synthases are multifunctional polypeptides that contain one or more modules, each of which normally contains all the enzymatic domains for a single round of extension and modification of the polyketide backbone. Acyl carrier proteins (ACP(s)) hold the extender unit to which the starter or growing chain is added. Normally there is one ACP for each ketosynthase module. However, there are an increasing number of known examples of tandemly repeated ACP domains, whose function is as yet unknown. For the doublet and triplet ACP domains in the biosynthetic pathway for the antibiotic mupirocin from *Pseudomonas fluorescens* NCIMB10586 we have inactivated ACP domains by inframe deletion and amino acid substitution of the active site serine. By deletion analysis each individual ACP from a cluster can provide a basic but reduced activity for the pathway. In the doublet cluster, substitution analysis indicates that the pathway may follow two parallel routes, one via each of the ACPs, thus increasing overall pathway flow. In the triplet cluster, substitution in ACP5 blocked the pathway. Thus ACP5 appears to be arranged "in series" to ACP6 and ACP7. Thus although both the doublet and triplet clusters increase antibiotic production, the mechanisms by which they do this appear to be different and depend specifically on the biosynthetic stage involved. The function of some ACPs may be determined by their location in the protein rather than absolute enzymic activity.

Ramakrishnan, S. N., P. Lau, et al. (2005). "Rev-erb{beta} Regulates the Expression of Genes Involved in Lipid Absorption in Skeletal Muscle Cells: EVIDENCE FOR CROSS-TALK BETWEEN ORPHAN NUCLEAR RECEPTORS AND MYOKINES." J. Biol. Chem. **280**(10): 8651-8659.

<http://www.jbc.org/cgi/content/abstract/280/10/8651>

Rev-erb{beta} is an orphan nuclear receptor that selectively blocks trans-activation mediated by the retinoic acid-related orphan receptor- α (ROR α). ROR α has been implicated in the regulation of high density lipoprotein cholesterol, lipid homeostasis, and inflammation. Rev-erb{beta} and ROR α are expressed in similar tissues, including skeletal muscle; however, the pathophysiological function of Rev-erb{beta} has remained obscure. We hypothesize from the similar expression patterns, target genes, and overlapping cognate sequences of these nuclear receptors that Rev-erb{beta} regulates lipid metabolism in skeletal muscle. This lean tissue accounts for >30% of total body weight and 50% of energy expenditure. Moreover, this metabolically demanding tissue is a primary site of glucose disposal, fatty acid oxidation, and cholesterol efflux. Consequently, muscle has a significant role in insulin sensitivity, obesity, and the blood-lipid profile. We utilize ectopic expression in skeletal muscle cells to understand the regulatory role of Rev-erb{beta} in this major mass peripheral tissue. Exogenous expression of a dominant negative version of mouse Rev-erb{beta} decreases the expression of many genes involved in fatty acid/lipid absorption (including Cd36, and Fabp-3 and -4). Interestingly, we observed a robust induction (>15-fold) in mRNA expression of interleukin-6, an "exercise-induced myokine" that regulates energy expenditure and inflammation. Furthermore, we observed the dramatic repression (>20-fold) of myostatin mRNA, another myokine that is a negative regulator of muscle hypertrophy and hyperplasia that impacts on body fat accumulation. This study implicates Rev-erb{beta} in the control of lipid and energy homeostasis in skeletal

muscle. In conclusion, we speculate that selective modulators of Rev-erb{beta} may have therapeutic utility in the treatment of dyslipidemia and regulation of muscle growth.

Rees, M. I., K. Harvey, et al. (2003). "Isoform Heterogeneity of the Human Gephyrin Gene (GPHN), Binding Domains to the Glycine Receptor, and Mutation Analysis in Hyperekplexia." J. Biol. Chem. **278**(27): 24688-24696.

<http://www.jbc.org/cgi/content/abstract/278/27/24688>

Gephyrin (GPHN) is an organizational protein that clusters and localizes the inhibitory glycine (GlyR) and GABAA receptors to the microtubular matrix of the neuronal postsynaptic membrane. Mice deficient in gephyrin develop a hereditary molybdenum cofactor deficiency and a neurological phenotype that mimics startle disease (hyperekplexia). This neuromotor disorder is associated with mutations in the GlyR {alpha}1 and {beta} subunit genes (GLRA1 and GLRB). Further genetic heterogeneity is suspected, and we hypothesized that patients lacking mutations in GLRA1 and GLRB might have mutations in the gephyrin gene (GPHN). In addition, we adopted a yeast two-hybrid screen, using the GlyR {beta} subunit intracellular loop as bait, in an attempt to identify further GlyR-interacting proteins implicated in hyperekplexia. Gephyrin cDNAs were isolated, and subsequent RT-PCR analysis from human tissues demonstrated the presence of five alternatively spliced GPHN exons concentrated in the central linker region of the gene. This region generated 11 distinct GPHN transcript isoforms, with 10 being specific to neuronal tissue. Mutation analysis of GPHN exons in hyperekplexia patients revealed a missense mutation (A28T) in one patient causing an amino acid substitution (N10Y). Functional testing demonstrated that GPHNN10Y does not disrupt GlyR-gephyrin interactions or collybistin-induced cell-surface clustering. We provide evidence that GlyR-gephyrin binding is dependent on the presence of an intact C-terminal MoeA homology domain. Therefore, the N10Y mutation and alternative splicing of GPHN transcripts do not affect interactions with GlyRs but may affect other interactions with the cytoskeleton or gephyrin accessory proteins.

Reglier-Poupet, H., C. Frehel, et al. (2003). "Maturation of Lipoproteins by Type II Signal Peptidase Is Required for Phagosomal Escape of *Listeria monocytogenes*." J. Biol. Chem. **278**(49): 49469-49477.

<http://www.jbc.org/cgi/content/abstract/278/49/49469>

Lipoproteins of Gram-positive bacteria are involved in a broad range of functions such as substrate binding and transport, antibiotic resistance, cell signaling, or protein export and folding. Lipoproteins are also known to initiate both innate and adaptive immune responses. However, their role in the pathogenicity of intracellular microorganisms is yet poorly understood. In *Listeria monocytogenes*, a Gram-positive facultative intracellular human pathogen, surface proteins have important roles in the interactions of the microorganism with the host cells. Among the putative surface proteins of *L. monocytogenes*, lipoproteins constitute the largest family. Here, we addressed the role of the signal peptidase (SPase II), responsible for the maturation of lipoproteins in listerial pathogenesis. We identified a gene, *lsp*, encoding a SPase II in the genome of *L. monocytogenes* and constructed a {Delta}*lsp* chromosomal deletion mutant. The mutant strain fails to process several lipoproteins demonstrating that *lsp* encodes a genuine SPase II. This defect is accompanied by a reduced efficiency of phagosomal escape during infection of eucaryotic cells, and leads to an attenuated virulence. We show that *lsp* gene expression is strongly induced when bacteria are still entrapped inside phagosomes of infected macrophages. The data presented establish, thus, that maturation of lipoproteins is critical for efficient phagosomal escape of *L. monocytogenes*, a process temporally controlled by the regulation of *lsp* production in infected cells.

Richer, J. K., B. M. Jacobsen, et al. (2002). "Differential Gene Regulation by the Two Progesterone Receptor Isoforms in Human Breast Cancer Cells." J. Biol. Chem. **277**(7): 5209-5218.

<http://www.jbc.org/cgi/content/abstract/277/7/5209>

The PR-A and PR-B isoforms of progesterone receptors (PR) have different physiological functions, and their ratio varies widely in breast cancers. To determine whether the two PR regulate different genes, we used human breast cancer cell lines engineered to express one or the other isoform. Cells were treated with progesterone in triplicate, time-separated experiments, allowing statistical analyses of microarray gene expression data. Of 94 progesterone-regulated genes, 65 are uniquely regulated by PR-B, 4 uniquely by PR-A, and only 25 by both. Almost half the genes encode proteins that are membrane-bound or involved in membrane-initiated signaling. We also find an important set of progesterone-regulated genes involved in mammary gland development and/or implicated in breast cancer. This first, large scale study of PR gene regulation has important implications for the measurement of PR in breast cancers and for the many clinical uses of synthetic progestins. It suggests that it is important to distinguish between the two isoforms in breast cancers and that isoform-specific genes can be used to screen for ligands that selectively modulate the activity of PR-A or PR-B. Additionally, use of natural target genes, rather than "consensus" response elements, for transcription studies should improve our understanding of steroid hormone action.

Robichaud, G. A., M. Nardini, et al. (2004). "Human Pax-5 C-terminal Isoforms Possess Distinct Transactivation Properties and Are Differentially Modulated in Normal and Malignant B Cells." J. Biol. Chem. **279**(48): 49956-49963.

<http://www.jbc.org/cgi/content/abstract/279/48/49956>

The transcription factor Pax-5 occupies a central role in B cell differentiation and has been implicated in the development of B cell lymphoma. The transcriptional activation function of Pax-5 requires an intact N-terminal DNA-binding domain and is strongly influenced by the C-terminal transactivation domain. We report the identification and characterization of five human Pax-5 isoforms, which occur through the alternative splicing of exons that encode for the C-terminal transactivation domain. These isoforms arise from the inclusion or exclusion of exon 7, exon 8, and/or exon 9. Three of the Pax-5 isoforms generate novel protein sequences rich in proline, serine, and threonine amino acids that are the hallmarks of transactivation domains. The Pax-5 isoforms are expressed in peripheral blood mononuclear cells, cancerous and non-cancerous B cell lines, as well as in primary B cell lymphoma tissue. Electrophoretic mobility shift assays demonstrate that the isoforms possess specific DNA binding activity and recognize the PAX-5 consensus binding sites. In reporter assays using the CD19 promoter, the transactivation properties of the various isoforms were significantly influenced by the changes in the C-terminal protein sequence. Finally, we demonstrate, for the first time, that human Pax-5 isoform expression is modulated by specific signaling pathways in B lymphocytes.

Rumberger, J. M., T. Wu, et al. (2003). "Role of Hexosamine Biosynthesis in Glucose-mediated Up-regulation of Lipogenic Enzyme mRNA Levels: EFFECTS OF GLUCOSE, GLUTAMINE, AND GLUCOSAMINE ON GLYCEROPHOSPHATE DEHYDROGENASE, FATTY ACID SYNTHASE, AND ACETYL-CoA CARBOXYLASE mRNA LEVELS." J. Biol. Chem. **278**(31): 28547-28552.

<http://www.jbc.org/cgi/content/abstract/278/31/28547>

Glucose uptake into adipose and liver cells is known to up-regulate mRNA levels for various lipogenic enzymes such as fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC). To determine whether the hexosamine biosynthesis pathway (HBP) mediates glucose regulation of mRNA expression, we treated primary cultured adipocytes for 18 h with insulin (25 ng/ml) and either glucose (20 mM) or glucosamine (2 mM). A ribonuclease protection assay was used to quantitate mRNA levels for FAS, ACC, and glycerol-3-P dehydrogenase (GPDH). Treatment with insulin and various concentrations of D-glucose increased mRNA levels for FAS (280%), ACC (93%), and GPDH (633%) in a dose-dependent manner (ED₅₀ 8-16 mM). Mannose similarly elevated mRNA levels, but galactose and fructose were only partially effective. L-glucose had no effect. Omission of glutamine from the culture medium markedly diminished the stimulatory effect of glucose on mRNA expression. Since glutamine is a crucial amide donor in hexosamine biosynthesis, we interpret these data to mean that glucose flux through the HBP is linked to regulation of lipogenesis through control of gene expression. Further evidence for hexosamine regulation was obtained using glucosamine, which is readily transported into adipocytes where it directly enters the HBP. Glucosamine was 15-30 times more potent than glucose in elevating FAS, ACC, and GPDH mRNA levels (ED₅₀ [~]0.5 mM). In summary: 1) GPDH, FAS, and ACC mRNA levels are upregulated by glucose; 2) glucose-induced up-regulation requires glutamine; and 3) mRNA levels for lipogenic enzymes are up-regulated by glucosamine. Hyperglycemia is the hallmark of diabetes mellitus and leads to insulin resistance, impaired glucose metabolism, and dyslipidemia. We postulate that disease pathophysiology may have a common underlying factor, excessive glucose flux through the HBP.

Sancho, R., N. Marquez, et al. (2004). "Imperatorin Inhibits HIV-1 Replication through an Sp1-dependent Pathway." *J. Biol. Chem.* **279**(36): 37349-37359.

<http://www.jbc.org/cgi/content/abstract/279/36/37349>

Coumarins and structurally related compounds have been recently shown to present anti-human immunodeficiency virus, type 1 (HIV-1) activity. Among them, the dietary furanocoumarin imperatorin is present in citrus fruits, in culinary herbs, and in some medicinal plants. In this study we report that imperatorin inhibits either vesicular stomatitis virus-pseudotyped or gp160-enveloped recombinant HIV-1 infection in several T cell lines and in HeLa cells. These recombinant viruses express luciferase as a marker of viral replication. Imperatorin did not inhibit the reverse transcription nor the integration steps in the viral cell cycle. Using several 5' long terminal repeat-HIV-1 constructs where critical response elements were either deleted or mutated, we found that the transcription factor Sp1 is critical for the inhibitory activity of imperatorin induced by both phorbol 12-myristate 13-acetate and HIV-1 Tat. Moreover in transient transfections imperatorin specifically inhibited phorbol 12-myristate 13-acetate-induced transcriptional activity of the Gal4-Sp1 fusion protein. Since Sp1 is also implicated in cell cycle progression we further studied the effect of imperatorin on cyclin D1 gene transcription and protein expression and in HeLa cell cycle progression. We found that imperatorin strongly inhibited cyclin D1 expression and arrested the cells at the G1 phase of the cell cycle. These results highlight the potential of Sp1 transcription factor as a target for natural anti-HIV-1 compounds such as furanocoumarins that might have a potential therapeutic role in the management of AIDS.

Satake, H., H. Y. Chen, et al. (2003). "Genes Modulated by Expression of GD3 Synthase in Chinese Hamster Ovary Cells. EVIDENCE THAT THE Tis21 GENE IS INVOLVED IN THE INDUCTION OF GD3 9-O-ACETYLATION." *J. Biol. Chem.* **278**(10): 7942-7948.

<http://www.jbc.org/cgi/content/abstract/278/10/7942>

9-O-Acetylation is a common sialic acid modification, expressed in a developmentally regulated and tissue/cell type-specific manner. The relevant 9-O-acetyltransferase(s) have not been isolated or cloned; nor have mechanisms for their regulation been elucidated. We previously showed that transfection of the GD3 synthase (ST8Sia-I) gene into Chinese hamster ovary (CHO)-K1 cells gave expression of not only the disialoganglioside GD3 but also 9-O-acetyl-GD3. We now use differential display PCR between wild type CHO-K1 cells and clones stably expressing GD3 synthase (CHO-GD3 cells) to detect any increased expression of other genes and explore the possible induction of a 9-O-acetyltransferase. The four CHO mRNAs showing major up-regulation were homologous to VCAM-1, Tis21, the KC-protein-like protein, and a functionally unknown type II transmembrane protein. A moderate increase in expression of the FxC1 and SPR-1 genes was also seen. Interestingly, these are different from genes observed by others to be up-regulated after transfection of GD3 synthase into a neuroblastoma cell line. We also isolated a CHO-GD3 mutant lacking 9-O-acetyl-GD3 following chemical mutagenesis (CHO-GD3-OAc[-]). Analysis of the above differential display PCR-derived genes in these cells showed that expression of Tis21 was selectively reduced. Transfection of a mouse Tis21 cDNA into the CHO-GD3-OAc[-] mutant cells restored 9-O-acetyl-GD3 expression. Since the only major gangliosides expressed by CHO-GD3 cells are GD3 and 9-O-acetyl-GD3 (in addition to GM3, the predominant ganglioside type in wild-type CHO-K1 cells), we conclude that GD3 enhances its own 9-O-acetylation via induction of Tis21. This is the first known nuclear inducible factor for 9-O-acetylation and also the first proof that 9-O-acetylation can be directly regulated by GD3 synthase. Finally, transfection of CHO-GD3-OAc[-] mutant cells with ST6Gal-I induced 9-O-acetylation specifically on sialylated N-glycans, in a manner similar to wild-type cells. This indicates separate machineries for 9-O-acetylation on [alpha]2-8-linked sialic acids of gangliosides and on [alpha]2-6-linked sialic acids on N-glycans.

Schmidt, C., B. Li, et al. (2003). "Random Mutagenesis of the M3 Muscarinic Acetylcholine Receptor Expressed in Yeast: IDENTIFICATION OF POINT MUTATIONS THAT "SILENCE" A CONSTITUTIVELY ACTIVE MUTANT M3 RECEPTOR AND GREATLY IMPAIR RECEPTOR/G PROTEIN COUPLING." *J. Biol. Chem.* **278**(32): 30248-30260.

<http://www.jbc.org/cgi/content/abstract/278/32/30248>

The M3 muscarinic receptor is a prototypical member of the class I family of G protein-coupled receptors (GPCRs). To facilitate studies on the structural mechanisms governing M3 receptor activation, we generated an M3 receptor-expressing yeast strain (*Saccharomyces cerevisiae*) that requires agonist-dependent M3 receptor activation for cell growth. By using receptor random mutagenesis followed by a genetic screen in yeast, we initially identified a point mutation at the cytoplasmic end of transmembrane domain (TM) VI (Q490L) that led to robust agonist-independent M3 receptor signaling in both yeast and mammalian cells. To explore further the molecular mechanisms by which point mutations can render GPCRs constitutively active, we subjected a region of the Q490L mutant M3 receptor that included TM V-VII to random mutagenesis. We then applied a yeast genetic screen to identify second-site mutations that could suppress the activating effects of the Q490L mutation and restore wild-type receptor-like function to the Q490L mutant receptor. This analysis led to the identification of 12 point mutations that allowed the Q490L mutant receptor to function in a fashion similar to the wild-type receptor. These amino acid substitutions mapped to two distinct regions of the M3 receptor, the exofacial segments of TM V and VI and the cytoplasmic ends of TM V-VII. Strikingly, in the absence of the activating Q490L mutation, all recovered point mutations severely reduced the efficiency of receptor/G protein coupling, indicating that the targeted residues play important roles in receptor activation and/or receptor/G protein coupling. This strategy should be generally applicable to identify sites in GPCRs that are critically involved in receptor function.

Schwarzer, C., T. E. Machen, et al. (2004). "NADPH Oxidase-dependent Acid Production in Airway Epithelial Cells." *J. Biol. Chem.* **279**(35): 36454-36461.

<http://www.jbc.org/cgi/content/abstract/279/35/36454>

The purpose of this study was to determine the role of NADPH oxidase in H⁺ secretion by airway epithelia. In whole cell patch clamp recordings primary human tracheal epithelial cells (hTE) and the human serous gland cell line Calu-3 expressed a functionally similar zincblockable plasma membrane H⁺ conductance. However, the rate of H⁺ secretion of confluent epithelial monolayers measured in Ussing chambers was 9-fold larger in hTE compared with Calu-3. In hTE H⁺ secretion was blocked by mucosal ZnCl₂ and the NADPH oxidase blockers acetovanillone and 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), whereas these same blockers had no effect in Calu-3. We determined levels of transcripts for the NADPH oxidase transmembrane isoforms (Nox1 through -5, Duox1 and -2, and p22phox) and found Duox1, -2, and p22phox to be highly expressed in hTE, as well as the intracellular subunits p40phox, p47phox, and p67phox. In contrast, Calu-3 lacked transcripts for Duox1, p40phox, and p47phox. Anti-Duox antibody staining resulted in prominent apical staining in hTE but no significant staining in Calu-3. When treated with amiloride to block the Na⁺/H⁺ exchanger, intracellular pH in hTE acidified at significantly higher rates than in Calu-3, and treatment with AEBSF blocked acidification. These data suggest a role for an apically located Duox-based NADPH oxidase during intracellular H⁺ production and H⁺ secretion, but not in H⁺ conduction.

Smith, S. B., R. Gasa, et al. (2003). "Neurogenin3 and Hepatic Nuclear Factor 1 Cooperate in Activating Pancreatic Expression of Pax4." *J. Biol. Chem.* **278**(40): 38254-38259.

<http://www.jbc.org/cgi/content/abstract/278/40/38254>

During fetal development, paired/homeodomain transcription factor Pax4 controls the formation of the insulin-producing {beta} cells and the somatostatin-producing {delta} cells in the islets of Langerhans in the pancreas. Targeting of Pax4 expression to the islet lineage in the fetal pancreas depends on a short sequence located [~]2 kb upstream of the transcription initiation site of the PAX4 gene. This short sequence contains binding sites for homeodomain transcription factors PDX1 and hepatic nuclear factor (HNF)1, nuclear receptor HNF4{alpha}, and basic helix-loop-helix factor Neurogenin3. In the current study we demonstrate that the HNF1{alpha} and Neurogenin3 binding sites are critical for activity of the region through synergy between the two proteins. Synergy involves a physical interaction between the factors and requires the activation domains of both factors. Furthermore, exogenous expression of Neurogenin3 is sufficient to induce expression of the endogenous pax4 gene in the mouse pancreatic ductal cell line mPAC, which already expresses HNF1{alpha}, whereas expression of both Neurogenin3 and HNF1{alpha} are necessary to activate the pax4 gene in the fibroblast cell line NIH3T3. These data demonstrate how Neurogenin3 and HNF1{alpha} activate the pax4 gene during the cascade of gene expression events that control pancreatic endocrine cell development.

Sorger, D., K. Athenstaedt, et al. (2004). "A Yeast Strain Lacking Lipid Particles Bears a Defect in Ergosterol Formation." *J. Biol. Chem.* **279**(30): 31190-31196.

<http://www.jbc.org/cgi/content/abstract/279/30/31190>

Lipid particles of the yeast *Saccharomyces cerevisiae* are storage compartments for triacylglycerols (TAG) and steryl esters (STE). Four gene products, namely the TAG synthases Dga1p and Lro1p, and the STE synthases Are1p and Are2p contribute to storage lipid synthesis.

A yeast strain lacking the four respective genes is devoid of lipid particles thus providing a valuable tool to study the physiological role of storage lipids and lipid particles. Using a *dga1lro1are1are2* quadruple mutant transformed with plasmids bearing inducible *DGA1*, *LRO1*, or *ARE2* we demonstrate that TAG synthesis contributes more efficiently to lipid particle proliferation than synthesis of STE. Moreover, we show that proteins typically located to lipid particles in wild type such as Erg1p, Erg6p, Erg7p, and Ayr1p are refined to microsomal fractions of the *dga1lro1are1are2* quadruple mutant. This result confirms the close relationship between lipid particles and endoplasmic reticulum. Most interestingly, the amount of the squalene epoxidase Erg1p, which is dually located in lipid particles and endoplasmic reticulum of wild type, is decreased in the quadruple mutant, whereas amounts of other lipid particle proteins tested were not reduced. This decrease is not caused by down-regulation of *ERG1* transcription but by the low stability of Erg1p in the quadruple mutant. Because a similar effect was also observed in *are1are2* mutants this finding can be mainly attributed to the lack of STE. The quadruple mutant, however, was more sensitive to terbinafine, an inhibitor of Erg1p, than the *are1are2* strain suggesting that the presence of TAG and/or intact lipid particles has an additional protective effect. In a strain lacking the two STE synthases, *Are1p* and *Are2p*, incorporation of ergosterol into the plasma membrane was reduced, although the total cellular amount of free ergosterol was higher in the mutant than in wild type. Thus, an esterification/deacylation mechanism appears to contribute to the supply of ergosterol to the plasma membrane.

Tabuchi, A., H. Sakaya, et al. (2002). "Involvement of an Upstream Stimulatory Factor as Well as cAMP-responsive Element-binding Protein in the Activation of Brain-derived Neurotrophic Factor Gene Promoter I." *J. Biol. Chem.* **277**(39): 35920-35931.

<http://www.jbc.org/cgi/content/abstract/277/39/35920>

The use of different brain-derived neurotrophic factor (BDNF) gene promoters results in the differential production of 5'-alternative transcripts, suggesting versatile functions of BDNF in neurons. Among four BDNF promoters I, II, III, and IV (BDNF-PI, -PII, -PIII, and -PIV), BDNF-PI was markedly activated, as well as BDNF-PIII, by Ca^{2+} signals evoked via neuronal activity. However, little is known about the mechanisms for the transcriptional activation of BDNF-PI. Using rat cortical neurons in culture, we assigned the promoter sequences responsible for the Ca^{2+} signal-mediated activation of BDNF-PI and found that the Ca^{2+} -responsive elements were located in two separate (distal and proximal) regions and that the DNA sequences in the proximal region containing cAMP-responsive element (CRE), which is overlapped by the upstream stimulatory factor (USF)-binding element, were largely responsible for the activation of BDNF-PI. CRE-binding protein (CREB) family transcription factors and USF1/USF2 bind to this overlapping site, depending upon their preferred sequences which also control the magnitude of the activation. Overexpression of dominant negative CREB or USF reduced the BDNF-PI activation. These findings support that not only CREB but also USF1/USF2 contributes to Ca^{2+} signal-mediated activation of BDNF-PI through the recognition of an overlapping CRE and USF-binding element.

Tagami, S., J.-i. Inokuchi, et al. (2002). "Ganglioside GM3 Participates in the Pathological Conditions of Insulin Resistance." *J. Biol. Chem.* **277**(5): 3085-3092.

<http://www.jbc.org/cgi/content/abstract/277/5/3085>

Gangliosides are known as modulators of transmembrane signaling by regulating various receptor functions. We have found that insulin resistance induced by tumor necrosis factor- $[\alpha]$ (TNF- $[\alpha]$) in 3T3-L1 adipocytes was accompanied by increased GM3 ganglioside expression caused by elevating GM3 synthase activity and its mRNA. We also demonstrated that TNF-

[alpha] simultaneously produced insulin resistance by uncoupling insulin receptor activity toward insulin receptor substrate-1 (IRS-1) and suppressing insulin-sensitive glucose transport. Pharmacological depletion of GM3 in adipocytes by an inhibitor of glucosylceramide synthase prevented the TNF-[alpha]-induced defect in insulin-dependent tyrosine phosphorylation of IRS-1 and also counteracted the TNF-[alpha]-induced serine phosphorylation of IRS-1. Moreover, when the adipocytes were incubated with exogenous GM3, suppression of tyrosine phosphorylation of insulin receptor and IRS-1 and glucose uptake in response to insulin stimulation was observed, demonstrating that GM3 itself is able to mimic the effects of TNF on insulin signaling. We used the obese Zucker fa/fa rat and ob/ob mouse, which are known to overproduce TNF-[alpha] mRNA in adipose tissues, as typical models of insulin resistance. We found that the levels of GM3 synthase mRNA in adipose tissues of these animals were significantly higher than in their lean counterparts. Taken together, the increased synthesis of cellular GM3 by TNF may participate in the pathological conditions of insulin resistance in type 2 diabetes.

Tang, S. and Z.-M. Zheng (2002). "Kaposi's Sarcoma-associated Herpesvirus K8 Exon 3 Contains Three 5'-Splice Sites and Harbors a K8.1 Transcription Start Site." *J. Biol. Chem.* **277**(17): 14547-14556.

<http://www.jbc.org/cgi/content/abstract/277/17/14547>

Kaposi's sarcoma-associated herpesvirus (KSHV) K8 and K8.1 open reading frames are juxtaposed and span from nucleotide (nt) 74850 to 76695 of the virus genome. A K8 pre-mRNA overlaps the entire K8.1 coding region, and alternative splicing of KSHV K8 and K8.1 pre-mRNAs each produces three isoforms ([alpha], [beta], and [gamma]) of the mRNAs. We have mapped the 5' end of the K8.1 RNA in butyrate-induced KSHV-positive JSC-1 cells to nt 75901 in the KSHV genome and have shown that exon 3 of the K8 pre-mRNA in JSC-1 cells covers most part of the intron 3 defined previously and has three 5'-splice sites (ss), respectively, at nt 75838, 76155, and 76338. Selection of the nt 75838 5'-ss dictates the K8 mRNA production and overwhelms the RNA processing. Alternative selection of other two 5'-ss is feasible and leads to production of two additional bicistronic mRNAs, K8/K8.1[alpha] and -[beta]. However, the novel bicistronic K8/K8.1 mRNAs translated a little K8 and no detectable K8.1 proteins in 293 cells. Data suggest that production of the K8/K8.1 mRNAs may be an essential way to control K8 mRNAs, especially K8[alpha], to a threshold at RNA processing level.

Tanner, J. A., R. M. Watt, et al. (2003). "The Severe Acute Respiratory Syndrome (SARS) Coronavirus NTPase/Helicase Belongs to a Distinct Class of 5' to 3' Viral Helicases." *J. Biol. Chem.* **278**(41): 39578-39582.

<http://www.jbc.org/cgi/content/abstract/278/41/39578>

The putative NTPase/helicase protein from severe acute respiratory syndrome coronavirus (SARS-CoV) is postulated to play a number of crucial roles in the viral life cycle, making it an attractive target for anti-SARS therapy. We have cloned, expressed, and purified this protein as an N-terminal hexahistidine fusion in *Escherichia coli* and have characterized its helicase and NTPase activities. The enzyme unwinds double-stranded DNA, dependent on the presence of a 5' single-stranded overhang, indicating a 5' to 3' polarity of activity, a distinct characteristic of coronaviridae helicases. We provide the first quantitative analysis of the polynucleic acid binding and NTPase activities of a Nidovirus helicase, using a high throughput phosphate release assay that will be readily adaptable to the future testing of helicase inhibitors. All eight common NTPs and dNTPs were hydrolyzed by the SARS helicase in a magnesium-dependent reaction, stimulated by the presence of either single-stranded DNA or RNA. The enzyme exhibited a preference for ATP, dATP, and dCTP over the other NTP/dNTP substrates. Homopolynucleotides

significantly stimulated the ATPase activity (15-25-fold) with the notable exception of poly(G) and poly(dG), which were non-stimulatory. We found a large variation in the apparent strength of binding of different homopolynucleotides, with dT24 binding over 10 times more strongly than dA24 as observed by the apparent K_m .

Tardif, K. D., K. Mori, et al. (2004). "Hepatitis C Virus Suppresses the IRE1-XBP1 Pathway of the Unfolded Protein Response." J. Biol. Chem. **279**(17): 17158-17164.

<http://www.jbc.org/cgi/content/abstract/279/17/17158>

Hepatitis C virus (HCV) gene expression disrupts normal endoplasmic reticulum (ER) functions and induces ER stress. ER stress results from the accumulation of unfolded or misfolded proteins in the ER; cells can alleviate this stress by degrading or refolding these proteins. The IRE1-XBP1 pathway directs both protein refolding and degradation in response to ER stress. Like IRE1-XBP1, other branches of the ER stress response mediate protein refolding. However, IRE1-XBP1 can also specifically activate protein degradation. We show here that XBP1 expression is elevated in cells carrying HCV subgenomic replicons, but XBP1 trans-activating activity is repressed. This prevents the IRE1-XBP1 transcriptional induction of EDEM (ER degradation-enhancing $\{\alpha\}$ -mannosidase-like protein). The mRNA expression of EDEM is required for the degradation of misfolded proteins. Consequently, misfolded proteins are stable in cells expressing HCV replicons. HCV may suppress the IRE1-XBP1 pathway to stimulate the synthesis of its viral proteins. IRE1 $\{\alpha\}$ -null MEFs, a cell line with a defective IRE1-XBP1 pathway, show elevated levels of HCV IRES-mediated translation. Therefore, HCV may suppress the IRE1-XBP1 pathway to not only promote HCV expression but also to contribute to the persistence of the virus in infected hepatocytes.

Tian, B., D. E. Nowak, et al. (2005). "Identification of Direct Genomic Targets Downstream of the Nuclear Factor- $\{\kappa\}$ B Transcription Factor Mediating Tumor Necrosis Factor Signaling." J. Biol. Chem. **280**(17): 17435-17448.

<http://www.jbc.org/cgi/content/abstract/280/17/17435>

Tumor necrosis factor (TNF) is a pro-inflammatory cytokine that controls expression of inflammatory genetic networks. Although the nuclear factor- $\{\kappa\}$ B (NF- $\{\kappa\}$ B) pathway is crucial for mediating cellular TNF responses, the complete spectrum of NF- $\{\kappa\}$ B-dependent genes is unknown. In this study, we used a tetracycline-regulated cell line expressing an NF- $\{\kappa\}$ B inhibitor to systematically identify NF- $\{\kappa\}$ B-dependent genes. A microarray data set generated from a time course of TNF stimulation in the presence or absence of NF- $\{\kappa\}$ B signaling was analyzed. We identified 50 unique genes that were regulated by TNF ($P(F) < 0.001$) and demonstrated a change in signal intensity of $\{+/-\}$ 3-fold relative to control. Of these, 28 were NF- $\{\kappa\}$ B-dependent, encoding proteins involved in diverse cellular activities. Quantitative real-time PCR assays of eight characterized NF- $\{\kappa\}$ B-dependent genes and five genes not previously known to be NF- $\{\kappa\}$ B-dependent (Gro- $\{\beta\}$ and $\{\gamma\}$, I $\{\kappa\}$ B $\{\epsilon\}$, interleukin (IL)-7R, and Naf-1) were used to determine whether they were directly or indirectly NF- $\{\kappa\}$ B regulated. Expression of constitutively active enhanced green fluorescent $\{\middle dot\}$ NF- $\{\kappa\}$ B/Rel A fusion protein transactivated all but IL-6 and IL-7R in the absence of TNF stimulation. Moreover, TNF strongly induced all 12 genes in the absence of new protein synthesis. High probability NF- $\{\kappa\}$ B sites in novel genes were predicted by binding site analysis and confirmed by electrophoretic mobility shift assay. Chromatin immunoprecipitation assays show the endogenous I $\{\kappa\}$ B $\{\alpha\}/\{\epsilon\}$, Gro- $\{\beta\}/\{\gamma\}$, and Naf-1 promoters directly bound NF- $\{\kappa\}$ B/Rel A in TNF-stimulated cells. Together, these studies systematically identify the direct NF- $\{\kappa\}$ B-dependent gene network

downstream of TNF signaling, extending our knowledge of biological processes regulated by this pathway.

- Torres, B., G. Porras, et al. (2003). "Regulation of the mhp Cluster Responsible for 3-(3-Hydroxyphenyl)propionic Acid Degradation in *Escherichia coli*." J. Biol. Chem. **278**(30): 27575-27585.

<http://www.jbc.org/cgi/content/abstract/278/30/27575>

The mhp gene cluster from *Escherichia coli* constitutes a model system to study bacterial degradation of 3-(3-hydroxyphenyl)propionic acid (3HPP). In this work the regulation of the inducible mhp catabolic genes has been studied by genetic and biochemical approaches. The Pr and Pa promoters, which control the expression of the divergently transcribed mhpR regulatory gene and mhp catabolic genes, respectively, show a peculiar arrangement leading to transcripts that are complementary at their 5'-ends. By using Pr-lacZ and Pa-lacZ translational fusions and gel retardation assays, we have shown that the mhpR gene product behaves as a 3HPP-dependent activator of the Pa promoter, being the expression from Pr constitutive and MhpR-independent. DNase I footprinting experiments and mutational analysis mapped an MhpR-protected region, centered at position -58 with respect to the Pa transcription start site, which is indispensable for MhpR binding and in vivo activation of the Pa promoter. Superimposed in the specific MhpR-mediated regulation of the Pa promoter, we have observed a strict catabolite repression control carried out by the cAMP receptor protein (CRP) that allows expression of the mhp catabolic genes when the preferred carbon source (glucose) is not available and 3HPP is present in the medium. Gel retardation assays revealed that the specific activator, MhpR, is essential for the binding of the second activator, CRP, to the Pa promoter. Such peculiar synergistic transcription activation has not yet been observed in other aromatic catabolic pathways, and the MhpR activator becomes the first member of the IclR family of transcriptional regulators that is indispensable for recruiting CRP to the target promoter.

- Tremper-Wells, B. and M. L. Vallano (2005). "Nuclear Calpain Regulates Ca²⁺-dependent Signaling via Proteolysis of Nuclear Ca²⁺/Calmodulin-dependent Protein Kinase Type IV in Cultured Neurons." J. Biol. Chem. **280**(3): 2165-2175.

<http://www.jbc.org/cgi/content/abstract/280/3/2165>

Accumulating evidence indicates that calpains can reside in or translocate to the cell nucleus, but their functions in this compartment remain poorly understood. Dissociated cultures of cerebellar granule cells (GCs) demonstrate improved long-term survival when their growth medium is supplemented with depolarizing agents that stimulate Ca²⁺ influx and activate calmodulin-dependent signaling cascades, notably 20 mM KCl. We previously observed Ca²⁺-dependent down-regulation of Ca²⁺/calmodulin-dependent protein kinase (CaMK) type IV, which was attenuated by calpain inhibitors, in GCs supplemented with 20 mM KCl (Tremper-Wells, B., Mathur, A., Beaman-Hall, C. M., and Vallano, M. L. (2002) *J. Neurochem.* **81**, 314-324). CaMKIV is highly enriched in the nucleus and thought to be critical for improved survival. Here, we demonstrate by immunolocalization/confocal microscopy and subcellular fractionation that the regulatory and catalytic subunits of m-calpain are enriched in GC nuclei, including GCs grown in medium containing 5 mM KCl. Calpain-mediated proteolysis of CaMKIV is selective, as several other nuclear and non-nuclear calpain substrates were not degraded under chronic depolarizing culture conditions. Depolarization and Ca²⁺-dependent down-regulation of CaMKIV were associated with significant alterations in other components of the Ca²⁺-CaMKIV signaling cascade: the ratio of phosphorylated to total cAMP response element-binding protein (a downstream CaMKIV substrate) was reduced by [~]10-fold, and the amount of CaMK kinase (an

upstream activator of CaMKIV) protein and mRNA was significantly reduced. We hypothesize that calpain-mediated CaMKIV proteolysis is an autoregulatory feedback response to sustained activation of a Ca²⁺-CaMKIV signaling pathway, resulting from growth of cultures in medium containing 25 mM KCl. This study establishes nuclear m-calpain as a regulator of CaMKIV and associated signaling molecules under conditions of sustained Ca²⁺ influx.

Tuli, R., S. Tuli, et al. (2003). "Transforming Growth Factor- β -mediated Chondrogenesis of Human Mesenchymal Progenitor Cells Involves N-cadherin and Mitogen-activated Protein Kinase and Wnt Signaling Cross-talk." *J. Biol. Chem.* **278**(42): 41227-41236.

<http://www.jbc.org/cgi/content/abstract/278/42/41227>

The multilineage differentiation potential of adult tissue-derived mesenchymal progenitor cells (MPCs), such as those from bone marrow and trabecular bone, makes them a useful model to investigate mechanisms regulating tissue development and regeneration, such as cartilage. Treatment with transforming growth factor- β (TGF- β) superfamily members is a key requirement for the in vitro chondrogenic differentiation of MPCs. Intracellular signaling cascades, particularly those involving the mitogen-activated protein (MAP) kinases, p38, ERK-1, and JNK, have been shown to be activated by TGF- β s in promoting cartilage-specific gene expression. MPC chondrogenesis in vitro also requires high cell seeding density, reminiscent of the cellular condensation requirements for embryonic mesenchymal chondrogenesis, suggesting common chondro-regulatory mechanisms. Prompted by recent findings of the crucial role of the cell adhesion protein, N-cadherin, and Wnt signaling in condensation and chondrogenesis, we have examined here their involvement, as well as MAP kinase signaling, in TGF- β 1-induced chondrogenesis of trabecular bone-derived MPCs. Our results showed that TGF- β 1 treatment initiates and maintains chondrogenesis of MPCs through the differential chondro-stimulatory activities of p38, ERK-1, and to a lesser extent, JNK. This regulation of MPC chondrogenic differentiation by the MAP kinases involves the modulation of N-cadherin expression levels, thereby likely controlling condensation-like cell-cell interaction and progression to chondrogenic differentiation, by the sequential up-regulation and progressive down-regulation of N-cadherin. TGF- β 1-mediated MAP kinase activation also controls WNT-7A gene expression and Wnt-mediated signaling through the intracellular β -catenin-TCF pathway, which likely regulates N-cadherin expression and subsequent N-cadherin-mediated cell-adhesion complexes during the early steps of MPC chondrogenesis.

Tynan, F. E., N. A. Borg, et al. (2005). "The high resolution structures of highly bulged viral epitopes bound to the major histocompatibility class I: Implications for T-cell receptor engagement and T-cell immunodominance." *J. Biol. Chem.*: M503060200.

<http://www.jbc.org/cgi/content/abstract/M503060200v1>

Although HLA class I alleles can bind epitopes up to 14 amino acids in length, little is known about the immunogenicity or the responding T-cell repertoire against such determinants. Here we describe a HLA-B*3508-restricted CTL response to a 13-mer viral epitope (LPEPLPQGQLTAY). The rigid, centrally-bulged epitope generates a biased T-cell response. Only the N-terminal face of the peptide bulge is critical for recognition by the dominant clonotype, SB27. The SB27 public TcR associates slowly onto the bulged pMHC complex, suggesting significant remodelling upon engagement. The broad antigen binding cleft of HLA-B*3508 represents a critical feature for engagement of the public TcR, as the narrower binding cleft of HLA-B*3501LPEPLPQGQLTAY, which differs from HLA-B*3508 by a single amino acid polymorphism (Arg 156'Leu), interacts poorly with the dominant TcR. Biased TcR usage in this CTL response appears to reflect a dominant role of the prominent pMHC-I surface.

Uchimura, K., K. Kadomatsu, et al. (2002). "Functional Analysis of the Chondroitin 6-Sulfotransferase Gene in Relation to Lymphocyte Subpopulations, Brain Development, and Oversulfated Chondroitin Sulfates." *J. Biol. Chem.* **277**(2): 1443-1450.

<http://www.jbc.org/cgi/content/abstract/277/2/1443>

Chondroitin 6-sulfotransferase (C6ST) catalyzes the transfer of sulfate to position 6 of the N-acetylgalactosamine residue of chondroitin. To obtain direct evidence regarding the function of C6ST and its product, chondroitin 6-sulfate, in vivo, we isolated the mouse C6ST gene (C6st) and generated mice deficient in this gene (C6st^{-/-}) by embryonic stem cell technology. C6st^{-/-} mice were born at approximately the expected frequency and were viable through adulthood. In the spleen of C6st^{-/-} mice, the level of chondroitin 6-sulfate became almost undetectable. Analyses of these knockout mice provided insights into the biosynthesis of oversulfated chondroitin sulfates in mice; chondroitin sulfate D in the brain of null mice and the cartilage and telencephalon of null embryos disappeared, whereas the chondroitin sulfate E level in the spleen and brain of the null mice was unchanged. Despite the disappearance of chondroitin sulfate D structure, brain development was normal in the C6st^{-/-} mice. Further analysis revealed that the number of CD62L⁺CD44^{low} T lymphocytes corresponding to naive T lymphocytes in the spleen of 5-6-week-old C6st^{-/-} mice was significantly decreased, whereas those in other secondary lymphoid organs were unchanged. This finding suggested that chondroitin 6-sulfate plays a role in the maintenance of naive T lymphocytes in the spleen of young mice.

Unnikrishnan, I., S. Miller, et al. (2003). "Multiple Positive and Negative Elements Involved in the Regulation of Expression of GSY1 in *Saccharomyces cerevisiae*." *J. Biol. Chem.* **278**(29): 26450-26457.

<http://www.jbc.org/cgi/content/abstract/278/29/26450>

GSY1 is one of the two genes encoding glycogen synthase in *Saccharomyces cerevisiae*. Both the GSY1 message and the protein levels increased as cells approached stationary phase. A combination of deletion analysis and site-directed mutagenesis revealed a complex promoter containing multiple positive and negative regulatory elements. Expression of GSY1 was dependent upon the presence of a TATA box and two stress response elements (STREs). Expression was repressed by Mig1, which mediates responses to glucose, and Rox1, which mediates responses to oxygen. Characterization of the GSY1 promoter also revealed a novel negative element. This element, N1, can repress expression driven by either an STRE or a heterologous element, the UAS of CYC1. Repression by N1 is dependent on the number of these elements that are present, but is independent of their orientation. N1 repressed expression when placed either upstream or downstream of the UAS, although the latter position is more effective. Gel shift analysis detected a factor that appears to bind to the N1 element. The complexity of the GSY1 promoter, which includes two STREs and three distinct negative elements, was surprising. This complexity may allow GSY1 to respond to a wide range of environmental stresses.

Valcourt, U., J. Gouttenoire, et al. (2002). "Functions of Transforming Growth Factor-beta Family Type I Receptors and Smad Proteins in the Hypertrophic Maturation and Osteoblastic Differentiation of Chondrocytes." *J. Biol. Chem.* **277**(37): 33545-33558.

<http://www.jbc.org/cgi/content/abstract/277/37/33545>

We investigated the effects of bone morphogenetic protein (BMP)-2, a member of the transforming growth factor-[beta] superfamily, on the regulation of the chondrocyte phenotype, and we identified signaling molecules involved in this regulation. BMP-2 triggers three concomitant responses in mouse primary chondrocytes and chondrocytic MC615 cells. First, BMP-2 stimulates expression or synthesis of type II collagen. Second, BMP-2 induces expression of molecular markers characteristic of pre- and hypertrophic chondrocytes, such as Indian hedgehog, parathyroid hormone/parathyroid hormone-related peptide receptor, type X collagen, and alkaline phosphatase. Third, BMP-2 induces osteocalcin expression, a specific trait of osteoblasts. Constitutively active forms of transforming growth factor-[beta] family type I receptors and Smad proteins were overexpressed to address their role in this process. Activin receptor-like kinase (ALK)-1, ALK-2, ALK-3, and ALK-6 were able to reproduce the hypertrophic maturation of chondrocytes induced by BMP-2. In addition, ALK-2 mimicked further the osteoblastic differentiation of chondrocytes induced by BMP-2. In the presence of BMP-2, Smad1, Smad5, and Smad8 potentiated the hypertrophic maturation of chondrocytes, but failed to induce osteocalcin expression. Smad6 and Smad7 impaired chondrocytic expression and osteoblastic differentiation induced by BMP-2. Thus, our results indicate that Smad-mediated pathways are essential for the regulation of the different steps of chondrocyte and osteoblast differentiation and suggest that additional Smad-independent pathways might be activated by ALK-2.

Valineva, T., J. Yang, et al. (2005). "The Transcriptional Co-activator Protein p100 Recruits Histone Acetyltransferase Activity to STAT6 and Mediates Interaction between the CREB-binding Protein and STAT6." *J. Biol. Chem.* **280**(15): 14989-14996.

<http://www.jbc.org/cgi/content/abstract/280/15/14989>

STAT6 is a critical regulator of transcription for interleukin-4 (IL-4)-induced genes. Activation of gene expression involves recruitment of coactivator proteins that function as bridging factors connecting sequence-specific transcription factors to the basal transcription machinery, and as chromatin-modifying enzymes. Coactivator proteins CBP/p300 have been implicated in regulation of transcription in all STATs. CBP is also required for STAT6-mediated gene activation, but the underlying molecular mechanisms are still elusive. In this study we investigated the mechanisms by which STAT6 recruits CBP and chromatin-modifying activities to the promoter. Our results indicate that while STAT1-interacted directly with CBP, the interaction between STAT6 and CBP was found to be mediated through p100 protein, a coactivator protein that has previously been shown to stimulate the transcription of IL-4-induced genes. The staphylococcal nuclease-like (SN)-domains of p100 directly interacted with amino acids 1099-1758 of CBP, while p100 did not associate with SRC-1, another coactivator of STAT6. p100 was found to recruit histone acetyltransferase (HAT) activity to STAT6 in vivo. Chromatin immunoprecipitation studies demonstrated that p100 increases the STAT6-p100-CBP ternary complex formation in the human Ig{epsilon} promoter. p100 also increased the amount of acetylated histone H4 at the Ig{epsilon} promoter, and siRNAs directed against p100 effectively inhibited Ig{epsilon} reporter gene expression. Our results suggest that p100 has an important role in the assembly of STAT6 transcriptosome, and that p100 stimulates IL-4-dependent transcription by mediating interaction between STAT6 and CBP and recruiting chromatin modifying activities to STAT6-responsive promoters.

van der Slot, A. J., A.-M. Zuurmond, et al. (2003). "Identification of PLOD2 as Telopeptide Lysyl Hydroxylase, an Important Enzyme in Fibrosis." *J. Biol. Chem.* **278**(42): 40967-40972.

<http://www.jbc.org/cgi/content/abstract/278/42/40967>

The hallmark of fibrotic processes is an excessive accumulation of collagen. The deposited

collagen shows an increase in pyridinoline cross-links, which are derived from hydroxylated lysine residues within the telopeptides. This change in cross-linking is related to irreversible accumulation of collagen in fibrotic tissues. The increase in pyridinoline cross-links is likely to be the result of increased activity of the enzyme responsible for the hydroxylation of the telopeptides (telopeptide lysyl hydroxylase, or TLH). Although the existence of TLH has been postulated, the gene encoding TLH has not been identified. By analyzing the genetic defect of Bruck syndrome, which is characterized by a pyridinoline deficiency in bone collagen, we found two missense mutations in exon 17 of PLOD2, thereby identifying PLOD2 as a putative TLH gene. Subsequently, we investigated fibroblasts derived from fibrotic skin of systemic sclerosis (SSc) patients and found that PLOD2 mRNA is highly increased indeed. Furthermore, increased pyridinoline cross-link levels were found in the matrix deposited by SSc fibroblasts, demonstrating a clear link between mRNA levels of the putative TLH gene (PLOD2) and the hydroxylation of lysine residues within the telopeptides. These data underscore the significance of PLOD2 in fibrotic processes.

Vanden Abeele, F., M. Roudbaraki, et al. (2003). "Store-operated Ca²⁺ Current in Prostate Cancer Epithelial Cells. ROLE OF ENDOGENOUS Ca²⁺ TRANSPORTER TYPE 1." J. Biol. Chem. **278**(17): 15381-15389.

<http://www.jbc.org/cgi/content/abstract/278/17/15381>

Ca²⁺ influx via store-operated channels (SOCs) following stimulation of the plasma membrane receptors is the key event controlling numerous processes in nonexcitable cells. The human transient receptor potential vanilloid type 6 channel, originally termed Ca²⁺ transporter type 1 (CaT1) protein, is one of the promising candidates for the role of endogenous SOC, although investigations of its functions have generated considerable controversy. In order to assess the role of CaT1 in generating endogenous store-operated Ca²⁺ current (ISOC) in the lymph node carcinoma of the prostate (LNCaP) human prostate cancer epithelial cell line, we manipulated its endogenous levels by means of antisense hybrid depletion or pharmacological up-regulation (antiandrogen treatment) combined with functional evaluation of ISOC. Antisense hybrid depletion of CaT1 decreased ISOC in LNCaP cells by ~50%, whereas enhancement of CaT1 levels by 60% in response to Casodex treatment potentiated ISOC by 30%. The functional characteristics of ISOC in LNCaP cells were similar in many respects to those reported for heterologously expressed CaT1, although 2-aminoethoxydiphenyl borate sensitivity and lack of constitutive current highlighted notable departures. Our results suggest that CaT1 is definitely involved in ISOC, but it may constitute only a part of the endogenous SOC, which in general may be a heteromultimeric channel composed of homologous CaT1 and other transient receptor potential subunits.

Vannahme, C., N. Smyth, et al. (2002). "Characterization of SMOC-1, a Novel Modular Calcium-binding Protein in Basement Membranes." J. Biol. Chem. **277**(41): 37977-37986.

<http://www.jbc.org/cgi/content/abstract/277/41/37977>

We have isolated the novel gene SMOC-1 that encodes a secreted modular protein containing an EF-hand calcium-binding domain homologous to that in BM-40. It further consists of two thyroglobulin-like domains, a follistatin-like domain and a novel domain. Recombinant expression in human cells showed that SMOC-1 is a glycoprotein with a calcium-dependent conformation. Results from Northern blots, reverse transcriptase-PCR, and immunoblots revealed a widespread expression in many tissues. Immunofluorescence studies with an antiserum directed against recombinant human SMOC-1 demonstrated a basement membrane localization of the protein and additionally its presence in other extracellular matrices. Immunogold electron microscopy

confirmed the localization of SMOC-1 within basement membranes in kidney and skeletal muscle as well as its expression in the zona pellucida surrounding the oocyte.

Vargas, M. R., M. Pehar, et al. (2005). "Fibroblast growth factor-1 induces heme oxygenase-1 via nuclear factor erythroid 2-related factor 2 (Nrf2) in spinal cord astrocytes: Consequences for motor neuron survival." J. Biol. Chem.: M501920200.

<http://www.jbc.org/cgi/content/abstract/M501920200v1>

Fibroblast growth factor-1 (FGF-1) is highly expressed in motor neurons and can be released in response to sub-lethal cell injury. Because FGF-1 potently activates astroglia and exerts a direct neuroprotection after spinal cord injury or axotomy, we examined whether it regulated the expression of inducible and cytoprotective heme oxygenase-1 (HO-1) enzyme in astrocytes. FGF-1 induced the expression of HO-1 in cultured rat spinal cord astrocytes, which was dependent on FGF receptor activation and prevented by cycloheximide. FGF-1 also induced Nrf2 mRNA and protein levels and prompted its nuclear translocation. HO-1 induction was abolished by transfection of astrocytes with a dominant-negative mutant Nrf2, indicating that FGF-1 regulates HO-1 expression through Nrf2. FGF-1 also modified the expression of other antioxidant genes regulated by Nrf2. Both Nrf2 and HO-1 levels were increased and co-localized with reactive astrocytes in the degenerating lumbar spinal cord of rats expressing the amyotrophic lateral sclerosis (ALS)-linked SOD1 G93A mutation. Overexpression of Nrf2 in astrocytes increased survival of co-cultured embryonic motor neurons and prevented motor neuron apoptosis mediated by nerve growth factor through p75 neurotrophin receptor. Taken together, these results emphasize the key role of astrocytes in determining motor neuron fate in ALS.

Wang, G., A. Woods, et al. (2004). "RhoA/ROCK Signaling Suppresses Hypertrophic Chondrocyte Differentiation." J. Biol. Chem. **279**(13): 13205-13214.

<http://www.jbc.org/cgi/content/abstract/279/13/13205>

Coordinated proliferation and differentiation of growth plate chondrocytes is required for normal growth and development of the endochondral skeleton, but little is known about the intracellular signal transduction pathways regulating these processes. We have investigated the roles of the GTPase RhoA and its effector kinases ROCK1/2 in hypertrophic chondrocyte differentiation. RhoA, ROCK1, and ROCK2 are expressed throughout chondrogenic differentiation. RhoA overexpression in chondrogenic ATDC5 cells results in increased proliferation and a marked delay of hypertrophic differentiation, as shown by decreased induction of alkaline phosphatase activity, mineralization, and expression of the hypertrophic markers collagen X, bone sialoprotein, and matrix metalloproteinase 13. These effects are accompanied by activation of cyclin D1 transcription and repression of the collagen X promoter by RhoA. In contrast, inhibition of Rho/ROCK signaling by the pharmacological inhibitor Y27632 inhibits chondrocyte proliferation and accelerates hypertrophic differentiation. Dominant-negative RhoA also inhibits induction of the cyclin D1 promoter by parathyroid hormone-related peptide. Finally, Y27632 treatment partially rescues the effects of RhoA overexpression. In summary, we identify the RhoA/ROCK signaling pathway as a novel and important regulator of chondrocyte proliferation and differentiation.

Wang, X.-D., J. Shou, et al. (2004). "Notch1-expressing Cells Are Indispensable for Prostatic Branching Morphogenesis during Development and Re-growth Following Castration and Androgen

Replacement." J. Biol. Chem. **279**(23): 24733-24744.

<http://www.jbc.org/cgi/content/abstract/279/23/24733>

Notch expression is frequently associated with progenitor cells, and its function is crucial for development. Our recent work showing that Notch1 is selectively expressed in basal epithelial cells of the prostate and higher Notch1 expression during development suggests that Notch1-expressing cells may define progenitor cells in the prostate. To test this hypothesis, we have generated a transgenic mouse line in which the Notch1-expressing cells can be ablated in a controlled manner. Specific targeting was achieved by expressing the bacterial nitroreductase, an enzyme that catalyzes its substrate into a cytotoxin capable of inducing apoptosis, under the Notch1 promoter. Cell death in transgenic prostate was confirmed by histological analyses including terminal dUTP nick-end labeling and caspase 3 immunocytochemical staining. We evaluated the consequences of ablation of Notch1-expressing cells in two systems, organ culture of early postnatal prostates and re-growth of prostate in castrated mice triggered by hormone replacement. Our data show that elimination of Notch1-expressing cells inhibited the branching morphogenesis, growth, and differentiation of early postnatal prostate in culture and impaired prostate re-growth triggered by hormone replacement in castrated mice. Furthermore, we found that Notch1 expression following castration and hormone replacement was concomitant with known basal cell markers p63 and cytokeratin 14 and was high in the proliferative human prostate epithelial cells. Taken together, these data suggest that Notch1-expressing cells define the progenitor cells in the prostatic epithelial cell lineage, which are indispensable for prostatic development and re-growth.

Wang, Y. L., K. A. Frauwirth, et al. (2002). "Thiazolidinedione Activation of Peroxisome Proliferator-activated Receptor gamma Can Enhance Mitochondrial Potential and Promote Cell Survival." J. Biol. Chem. **277**(35): 31781-31788.

<http://www.jbc.org/cgi/content/abstract/277/35/31781>

Thiazolidinediones (TZDs) are widely used for treatment of type 2 diabetes mellitus. Peroxisome proliferator-activated receptor [gamma] (PPAR[gamma]) is the molecular target of TZDs and is believed to mediate the apoptotic effects of this class of drugs in a variety of cell types, including B and T lymphocytes. The finding that TZDs induce lymphocyte death has raised concerns regarding whether TZDs might further impair immune functions in diabetics. To address this issue, we investigated the roles of PPAR[gamma] and TZDs in lymphocyte survival. PPAR[gamma] was up-regulated upon T cell activation. As previously reported, PPAR[gamma] agonists induced T cell death in a dose-dependent manner. However, the concentrations of TZD needed to cause T cell death were above those needed to induce PPAR[gamma]-dependent transcription. Surprisingly, at concentrations that induce optimal transcriptional activation, TZD activation of PPAR[gamma] protected cells from apoptosis following growth factor withdrawal. The survival-enhancing effects depended on both the presence and activation of PPAR[gamma]. Measurements of mitochondrial potential revealed that PPAR[gamma] activation enhanced the ability of cells to maintain their mitochondrial potential. These data indicate that activation of PPAR[gamma] with TZDs can promote cell survival and suggest that PPAR[gamma] activation may potentially augment the immune responses of diabetic patients.

Warren, C. E., A. Krizus, et al. (2002). "The *Caenorhabditis elegans* Gene, *gly-2*, Can Rescue the N-Acetylglucosaminyltransferase V Mutation of *Lec4* Cells." J. Biol. Chem. **277**(25): 22829-22838.

<http://www.jbc.org/cgi/content/abstract/277/25/22829>

UDP-N-acetylglucosamine:[alpha]-6-D-mannoside [beta]-1,6-N-acetylglucosaminyltransferase V (GlcNAc-TV) is a regulator of polylactosamine-containing N-glycans and is causally involved in T cell regulation and tumor metastasis. The *Caenorhabditis elegans* genome contains a single orthologous gene, *gly-2*, that is transcribed and encodes a 669-residue type II membrane protein that is 36.7% identical to mammalian GlcNAc-TV (Mgat-5). Recombinant GLY-2 possessed GlcNAc-TV activity when assayed in vitro, and protein truncations demonstrated that the N-terminal boundary of the catalytic domain is Ile-138. *gly-2* complemented the *Phaseolus vulgaris* leucoagglutinin binding defect of Chinese hamster ovary Lec4 cells, whereas GLY-2(L116R), an equivalent mutation to that which causes the Lec4A phenotype, could not. We conclude that the worm gene is functionally interchangeable with the mammalian form. GlcNAc-TV activity was detected in wild-type animals but not those homozygous for a deletion allele of *gly-2*. Activity was restored in mutant animals by an extrachromosomal array that encompassed the *gly-2* gene. Green fluorescent protein reporter transgenes driven by the *gly-2* promoter were expressed by developing embryos from the late comma stage onward, present in a complex subset of neurons in larvae and, in addition, the spermathecal and pharyngeal-intestinal valves and certain vulval cells of adults. However, no overt phenotypes were observed in animals homozygous for deletion alleles of *gly-2*.

Wehage, E., J. Eisfeld, et al. (2002). "Activation of the Cation Channel Long Transient Receptor Potential Channel 2 (LTRPC2) by Hydrogen Peroxide. A SPLICE VARIANT REVEALS A MODE OF ACTIVATION INDEPENDENT OF ADP-RIBOSE." *J. Biol. Chem.* **277**(26): 23150-23156.

<http://www.jbc.org/cgi/content/abstract/277/26/23150>

LTRPC2 is a cation channel recently reported to be activated by adenosine diphosphate-ribose (ADP-ribose) and NAD. Since ADP-ribose can be formed from NAD and NAD is elevated during oxidative stress, we studied whole cell currents and increases in the intercellular free calcium concentration ($[Ca^{2+}]_i$) in long transient receptor potential channel 2 (LTRPC2)-transfected HEK 293 cells after stimulation with hydrogen peroxide (H_2O_2). Cation currents carried by monovalent cations and Ca^{2+} were induced by H_2O_2 (5 mM in the bath solution) as well as by intracellular ADP-ribose (0.3 mM in the pipette solution) but not by NAD (1 mM). H_2O_2 -induced currents developed slowly after a characteristic delay of 3-6 min and receded after wash-out of H_2O_2 . $[Ca^{2+}]_i$ was rapidly increased by H_2O_2 in LTRPC2-transfected cells as well as in control cells; however, in LTRPC2-transfected cells, H_2O_2 evoked a second delayed rise in $[Ca^{2+}]_i$. A splice variant of LTRPC2 with a deletion in the C terminus (amino acids 1292-1325) was identified in neutrophil granulocytes. This variant was stimulated by H_2O_2 as the wild type. However, it did not respond to ADP-ribose. We conclude that activation of LTRPC2 by H_2O_2 is independent of ADP-ribose and that LTRPC2 may mediate the influx of Na^+ and Ca^{2+} during oxidative stress, such as the respiratory burst in granulocytes.

Whiteman, S. C., A. Bianco, et al. (2003). "Human Rhinovirus Selectively Modulates Membranous and Soluble Forms of Its Intercellular Adhesion Molecule-1 (ICAM-1) Receptor to Promote Epithelial Cell Infectivity." *J. Biol. Chem.* **278**(14): 11954-11961.

<http://www.jbc.org/cgi/content/abstract/278/14/11954>

Human rhinoviruses are responsible for many upper respiratory tract infections. 90% of rhinoviruses utilize intercellular adhesion molecule-1 (ICAM-1) as their cellular receptor, which also plays a critical role in recruitment of immune effector cells. Two forms of this receptor exist; membrane-bound (mICAM-1) and soluble ICAM-1 (sICAM-1). The soluble receptor may be produced independently from the membrane-bound form or it may be the product of proteolytic cleavage of mICAM-1. The ratio of airway epithelial cell expression of mICAM-1 to the sICAM-1

form may influence cell infectivity and outcome of rhinovirus infection. We therefore investigated the effect of rhinovirus on expression of both ICAM-1 receptors in normal human bronchial epithelial cells. We observed separate distinct messenger RNA transcripts coding for mICAM-1 and sICAM-1 in these cells, which were modulated by virus. Rhinovirus induced mICAM-1 expression on epithelial cells while simultaneously down-regulating sICAM-1 release, with consequent increase in target cell infectivity. The role of protein tyrosine kinases was investigated as a potential mechanistic pathway. Rhinovirus infection induced rapid phosphorylation of intracellular tyrosine kinase, which may be critical in up-regulation of mICAM-1. Elucidation of the underlying molecular mechanisms involved in differential modulation of both ICAM-1 receptors may lead to novel therapeutic strategies.

Winn, R. A., L. Marek, et al. (2005). "Restoration of Wnt-7a expression reverses non-small cell lung cancer cell transformation through frizzled-9 mediated growth inhibition and promotion of cellular differentiation." J. Biol. Chem.: M409392200.

<http://www.jbc.org/cgi/content/abstract/M409392200v1>

The Wnt signaling pathway is critical in normal development and mutation of specific components is frequently observed in carcinomas of diverse origins. The potential involvement of this pathway in lung tumorigenesis, however, has not been established. In this study, analysis of multiple Wnt mRNAs in non-small cell lung cancer (NSCLC) cell lines and primary lung tumors revealed markedly decreased Wnt-7a expression when compared to normal short term bronchial epithelial cell lines and normal uninvolved lung tissue. Wnt-7a transfection in NSCLC cell lines reversed cellular transformation, decreased anchorage independent growth, and induced epithelial differentiation as demonstrated by soft agar and three dimensional cell culture assays in a subset of the NSCLC cell lines. The action of Wnt-7a correlated with the expression of the specific Wnt receptor Fzd-9, and transfection of Fzd-9 into a Wnt-7a-insensitive NSCLC cell line established Wnt-7a sensitivity. Moreover, Wnt-7a was present in Fzd-9 immunoprecipitates, indicating a direct interaction of Wnt-7a and Fzd-9. In NSCLC cells, Wnt-7a and Fzd-9 induced both cadherin and Sprouty 4 expression, and stimulated the JNK pathway, but not b-catenin/TCF-activity. In addition, transfection of gain-of-function JNK strongly inhibited anchorage-independent growth. Thus, this study demonstrates that Wnt-7a and Fzd-9 signaling through activation of the JNK pathway induces cadherin proteins and the receptor tyrosine kinase inhibitor, Sprouty 4, and represents a novel tumor suppressor pathway in lung cancer that is required for maintenance of epithelial differentiation and inhibition of transformed cell growth in a subset of human NSCLC cancers.

Wright, G., J. J. Higgin, et al. (2003). "Activation of the Prolyl Hydroxylase Oxygen-sensor Results in Induction of GLUT1, Heme Oxygenase-1, and Nitric-oxide Synthase Proteins and Confers Protection from Metabolic Inhibition to Cardiomyocytes." J. Biol. Chem. **278**(22): 20235-20239.

<http://www.jbc.org/cgi/content/abstract/278/22/20235>

Recently an oxygen-sensing/transducing mechanism has been identified as a family of O₂-dependent prolyl hydroxylase domain-containing enzymes (PHD). In normoxia, PHD hydroxylates a specific proline residue that directs the degradation of constitutively synthesized hypoxia-inducible factor-1{ α }. During hypoxia, the cessation of hydroxylation of this proline results in less degradation and thus increases hypoxia-inducible factor-1{ α } protein levels. In this study we have examined the consequences of activating the PHD oxygen-sensing pathway in cultured neonatal myocytes using ethyl-3,4 dihydroxybenzoate and dimethyloxalylglycine, inhibitors that, similar to hypoxia, inhibit this family of O₂-dependent PHD enzymes. Increased glucose uptake and enhanced glycolytic metabolism are classical cellular responses to hypoxia. Ethyl-3,4

dihydroxybenzoate treatment of cardiomyocyte cultures for 24 h increased [3H]deoxy-4-glucose uptake concurrent with an induction of GLUT1 protein. In addition, ethyl-3,4 dihydroxybenzoate, dimethyloxalylglycine, and hypoxia treatments were found to induce protein levels of nitric oxide synthase-2 and heme oxygenase-1, two important cardioregulatory proteins whose expression in response to hypoxic conditions is poorly understood. In conjunction with these changes in gene expression, activation of the PHD oxygen-sensing mechanism was found to preserve myocyte viability in the face of metabolic inhibition with cyanide and 2-deoxyglucose. These results point to a key role for the PHD pathway in the phenotypic changes that are observed in a hypoxic myocyte and may suggest a strategy to pharmacologically induce protection in heart.

Xiao, J., P. Jethanandani, et al. (2003). "Regulation of α 7 Integrin Expression during Muscle Differentiation." *J. Biol. Chem.* **278**(50): 49780-49788.

<http://www.jbc.org/cgi/content/abstract/278/50/49780>

Expression of the laminin-binding α 7 integrin is tightly regulated during myogenic differentiation, reflecting required functions that range from cell motility to formation of stable myotendinous junctions. However, the exact mechanism controlling α 7 expression in a tissue- and differentiation-specific manner is poorly understood. This report provides evidence that α 7 gene expression during muscle differentiation is regulated by the c-Myc transcription factor. In myoblasts, α 7 is expressed at basal levels, but following conversion to myotubes the expression of the integrin is strongly elevated. The increased α 7 mRNA and protein levels following myogenic differentiation are inversely correlated with c-Myc expression. Transfection of myoblasts with the c-Myc transcription factor down-regulated α 7 expression, whereas overexpression of Madmyc, a dominant-negative c-Myc chimera, induced elevated α 7 expression. Functional analysis with site-specific deletions identified a specific double E-box sequence in the upstream promoter region (-2.0 to -2.6 kb) that is responsible for c-Myc-induced suppression of α 7 expression. DNA-protein binding assays and supershift analysis revealed that c-Myc forms a complex with this double E-box sequence. Our results suggest that the interaction of c-Myc with this promoter region is an important regulatory element controlling α 7 integrin expression during muscle development and myotendinous junction formation.

Xu, G., L.-x. Pan, et al. (2002). "Regulation of the Farnesoid X Receptor (FXR) by Bile Acid Flux in Rabbits." *J. Biol. Chem.* **277**(52): 50491-50496.

<http://www.jbc.org/cgi/content/abstract/277/52/50491>

We investigated the roles of hydrophobic deoxycholic acid (DCA) and hydrophilic ursocholic acid (UCA) in the regulation of the orphan nuclear farnesoid X receptor (FXR) in vivo. Rabbits with bile fistula drainage (removal of the endogenous bile acid pool), rabbits with bile fistula drainage and replacement with either DCA or UCA, and intact rabbits fed 0.5% cholic acid (CA) (enlarged endogenous bile acid pool) were studied. After bile fistula drainage, cholesterol 7 α -hydroxylase (CYP7A1) mRNA and activity levels increased, FXR-mediated transcription was decreased, and FXR mRNA and nuclear protein levels declined. Replacing the enterohepatic bile acid pool with DCA restored FXR mRNA and nuclear protein levels and activated FXR-mediated transcription as evidenced by the increased expression of its target genes, SHP and BSEP, and decreased CYP7A1 mRNA level and activity. Replacing the bile acid pool with UCA also restored FXR mRNA and nuclear protein levels but did not activate FXR-mediated transcription, because the SHP mRNA level and CYP7A1 mRNA level and activity were unchanged. Feeding CA to intact rabbits expanded the bile acid pool enriched with the FXR high affinity ligand, DCA. FXR-mediated transcription became activated as shown by increased SHP and BSEP mRNA levels and decreased CYP7A1 mRNA level and activity but did not change FXR mRNA or nuclear

protein levels. Thus, both hydrophobic and hydrophilic bile acids are effective in maintaining FXR mRNA and nuclear protein levels. However, the activating ligand (DCA) in the enterohepatic flux is necessary for FXR-mediated transcriptional regulation, which leads to down-regulation of CYP7A1.

Yamagata, H., K. Yonesu, et al. (2002). "TGTCACA Motif Is a Novel cis-Regulatory Enhancer Element Involved in Fruit-specific Expression of the cucumisin Gene." *J. Biol. Chem.* **277**(13): 11582-11590.

<http://www.jbc.org/cgi/content/abstract/277/13/11582>

Cucumisin, a subtilisin-like serine protease, is expressed at high levels in the fruit of melon (*Cucumis melo* L.) and accumulates in the juice. We investigated roles of the promoter regions and DNA-protein interactions in fruit-specific expression of the cucumisin gene. In transient expression analysis, a chimeric gene construct containing a 1.2-kb cucumisin promoter fused to a [β]-glucuronidase (GUS) reporter gene was expressed in fruit tissues at high levels, but the promoter activities in leaves and stems were very low. Deletion analysis indicated that a positive regulatory region is located between nucleotides [-]234 and [-]214 relative to the transcriptional initiation site. Gain-of-function experiments revealed that this 20-bp sequence conferred fruit specificity and contained a regulatory enhancer. Gel mobility shift experiments demonstrated the presence of fruit nuclear factors that interact with the cucumisin promoter. A typical G-box (GACACGTGTC) present in the 20-bp sequence did not bind fruit protein, but two possible cis-elements, an I-box-like sequence (AGATATGATAAAA) and an odd base palindromic TGTCACA motif, were identified in the promoter region between positions [-]254 and [-]215. The I-box-like sequence bound more tightly to fruit nuclear protein than the TGTCACA motif. The I-box-like sequence functions as a negative regulatory element, and the TGTCACA motif is a novel enhancer element necessary for fruit-specific expression of the cucumisin gene. Specific nucleotides responsible for the binding of fruit nuclear protein in these two elements were also determined.

Yamamura, H., S. Ugawa, et al. (2004). "Capsazepine Is a Novel Activator of the δ Subunit of the Human Epithelial Na⁺ Channel." *J. Biol. Chem.* **279**(43): 44483-44489.

<http://www.jbc.org/cgi/content/abstract/279/43/44483>

The amiloride-sensitive epithelial Na⁺ channel (ENaC) regulates Na⁺ homeostasis into cells and across epithelia. So far, four homologous subunits of mammalian ENaC have been isolated and are denoted as α , β , γ , and δ . The chemical agents acting on ENaC are, however, largely unknown, except for amiloride and benzamil as ENaC inhibitors. In particular, there are no agonists currently known that are selective for ENaC δ , which is mainly expressed in the brain. Here we demonstrate that capsazepine, a competitive antagonist for transient receptor potential vanilloid subfamily 1, potentiates the activity of human ENaC $\delta\beta\gamma$ (hENaC $\delta\beta\gamma$) heteromultimer expressed in *Xenopus* oocytes. The inward currents at a holding potential of -60 mV in hENaC $\delta\beta\gamma$ -expressing oocytes were markedly enhanced by the application of capsazepine (≥ 1 μ M), and the capsazepine-induced current was mostly abolished by the addition of 100 μ M amiloride. The stimulatory effects of capsazepine on the inward current were concentration-dependent with an EC₅₀ value of 8 μ M. Neither the application of other vanilloid compounds (capsaicin, resiniferatoxin, and olvanil) nor a structurally related compound (dopamine) modulated the inward current. Although hENaC δ homomer was also significantly activated by capsazepine, unexpectedly, capsazepine had no effect on hENaC α and caused a slight decrease on the hENaC $\alpha\beta\gamma$ current. In

conclusion, capsazepine acts on ENaC{delta} and acts together with protons. Other vanilloids tested do not have any effect. These findings identify capsazepine as the first known chemical activator of ENaC{delta}.

Yan, C., H. Wang, et al. (2003). "Repression of 92-kDa Type IV Collagenase Expression by MTA1 Is Mediated through Direct Interactions with the Promoter via a Mechanism, Which Is Both Dependent on and Independent of Histone Deacetylation." J. Biol. Chem. **278**(4): 2309-2316.

<http://www.jbc.org/cgi/content/abstract/278/4/2309>

Although the expression of the metastases-associated gene MTA1 correlates with tumor metastases, its role in regulating type IV collagenase expression is unknown. Enforced MTA1 expression in HT1080 cells reduced basal and 12-myristate 13-acetate-induced 92-kDa type IV collagenase (MMP-9) protein/mRNA levels. DNase I hypersensitivity and PstI accessibility assays revealed multiple regions of the MMP-9 promoter ([-]650/[-]450 and [-]120/+1), showing reduced hypersensitivity in the MTA1-expressing cells. Chromatin immunoprecipitation assays demonstrated MTA1 binding to the distal region, which spans several regulatory cis elements. Co-immunoprecipitation and chromatin immunoprecipitation assay experiments revealed histone deacetylase 2 (HDAC2)-MTA1 protein-protein interactions and the MTA1-dependent recruitment of HDAC2 to the distal MMP-9 promoter region, yielding diminished histone H3/H4 acetylation. However, HDAC2 binding and H3/H4 acetylation at the proximal MMP-9 region were unaffected by MTA1 expression. Furthermore, trichostatin treatment only partially relieved MTA1-repressed MMP-9 expression, indicating a HDAC-insensitive component possibly involving the nucleosome-remodeling Mi2 activity, which was recruited to the promoter by MTA1. In summary, (a) MMP-9 adds to a short list of MTA1-regulated genes, which so far only includes c-myc and pS2, and (b) MTA1 binds to the MMP-9 promoter, thereby repressing expression of this type IV collagenase via histone-dependent and independent mechanisms.

Yang, T. and B. W. Poovaiah (2002). "A Calmodulin-binding/CGCG Box DNA-binding Protein Family Involved in Multiple Signaling Pathways in Plants." J. Biol. Chem. **277**(47): 45049-45058.

<http://www.jbc.org/cgi/content/abstract/277/47/45049>

We reported earlier that the tobacco early ethylene-responsive gene NtER1 encodes a calmodulin-binding protein (Yang, T., and Poovaiah, B. W. (2000) *J. Biol. Chem.* **275**, 38467-38473). Here we demonstrate that there is one NtER1 homolog as well as five related genes in Arabidopsis. These six genes are rapidly and differentially induced by environmental signals such as temperature extremes, UVB, salt, and wounding; hormones such as ethylene and abscisic acid; and signal molecules such as methyl jasmonate, H₂O₂, and salicylic acid. Hence, they were designated as AtSR1-6 (Arabidopsis thaliana signal-responsive genes). Ca²⁺/calmodulin binds to all AtSRs, and their calmodulin-binding regions are located on a conserved basic amphiphilic [alpha]-helical motif in the C terminus. AtSR1 targets the nucleus and specifically recognizes a novel 6-bp CGCG box (A/C/G)CGCG(G/T/C). The multiple CGCG cis-elements are found in promoters of genes such as those involved in ethylene signaling, abscisic acid signaling, and light signal perception. The DNA-binding domain in AtSR1 is located on the N-terminal 146 bp where all AtSR1-related proteins share high similarity but have no similarity to other known DNA-binding proteins. The calmodulin-binding nuclear proteins isolated from wounded leaves exhibit specific CGCG box DNA binding activities. These results suggest that the AtSR gene family encodes a family of calmodulin-binding/DNA-binding proteins involved in multiple signal transduction pathways in plants.

Yasukawa, K., D. Sawamura, et al. (2002). "Dominant and Recessive Compound Heterozygous Mutations in Epidermolysis Bullosa Simplex Demonstrate the Role of the Stutter Region in Keratin Intermediate Filament Assembly." *J. Biol. Chem.* **277**(26): 23670-23674.

<http://www.jbc.org/cgi/content/abstract/277/26/23670>

Keratin intermediate filaments are important cytoskeletal structural proteins involved in maintaining cell shape and function. Mutations in the epidermal keratin genes, keratin 5 or keratin 14 lead to the disruption of keratin filament assembly, resulting in an autosomal dominant inherited blistering skin disease, epidermolysis bullosa simplex (EBS). We investigated a large EBS kindred who exhibited a markedly heterogeneous clinical presentation and detected two distinct keratin 5 mutations in the proband, the most severely affected. One missense mutation (E170K) in the highly conserved helix initiation peptide sequence of the 1A rod domain was found in all the affected family members. In contrast, the other missense mutation (E418K) was found only in the proband. The E418K mutation was located in the stutter region, an interruption in the heptad repeat regularity, whose function as yet remains unclear. We hypothesized that this mutated stutter allele was clinically silent when combined with the wild type allele but aggravates the clinical severity of EBS caused by the E170K mutation on the other allele. To confirm this in vitro, we transfected mutant keratin 5 cDNA into cultured cells. Although only 12.7% of the cells transfected with the E170K mutation alone showed disrupted keratin filament aggregations, significantly more cells (30.0%) cotransfected with both E170K and E418K mutations demonstrated keratin aggregation ($p < 0.05$). These transfection assay results corresponded to the heterogeneous clinical findings of the EBS patient in this kindred. We have identified the first case of both compound heterozygous dominant (E170K) and recessive (E418K) mutations in any keratin gene and confirmed the significant involvement of the stutter region in the assembly and organization of the keratin intermediate filament network in vitro.

Yoshimura, Y., M. Tani, et al. (2004). "Molecular Cloning and Functional Analysis of Zebrafish Neutral Ceramidase." *J. Biol. Chem.* **279**(42): 44012-44022.

<http://www.jbc.org/cgi/content/abstract/279/42/44012>

Almost all observations on the functions of neutral ceramidase have been carried out at cellular levels but not at an individual level. Here, we report the molecular cloning of zebrafish neutral ceramidase (znCD) and its functional analysis during embryogenesis. We isolated a cDNA clone encoding znCD by 5' and 3' rapid amplification of cDNA ends-PCR. It possessed an open reading frame of 2,229 base pairs encoding 743 amino acids. A possible signal/anchor sequence near the N terminus and four potential O-glycosylation and eight potential N-glycosylation sites were found in the putative sequence. The enzyme activity at neutral pH increased markedly after transformation of Chinese hamster CHOP and zebrafish BRF41 cells with the cDNA. The overexpressed enzyme was found to be distributed in endoplasmic reticulum/Golgi compartments as well as the plasma membranes. The antisense morpholino oligonucleotide (AMO), which was designed based on the sequence of znCD mRNA, successfully blocked the translation of znCD in a wheat germ in vitro translation system. The knockdown of znCD with AMO led to an increase in the number of zebrafish embryos with severe morphological and cellular abnormalities such as abnormal morphogenesis in the head and tail, pericardiac edema, defect of blood cell circulation, and an increase of apoptotic cells, especially in the head and neural tube regions, at 36 h post-fertilization. The ceramide level in AMO-injected embryos increased significantly compared with that in control embryos. Simultaneous injection of both AMO and synthetic znCD mRNA into one-cell-stage embryos rescued znCD activity and blood cell circulation. These results indicate that znCD is essential for the metabolism of ceramide and the early development of zebrafish.

Young, N. M., J.-R. Brisson, et al. (2002). "Structure of the N-Linked Glycan Present on Multiple Glycoproteins in the Gram-negative Bacterium, *Campylobacter jejuni*." *J. Biol. Chem.* **277**(45): 42530-42539.

<http://www.jbc.org/cgi/content/abstract/277/45/42530>

Mass spectrometry investigations of partially purified *Campylobacter jejuni* protein PEB3 showed it to be partially modified with an Asn-linked glycan with a mass of 1406 Da and composed of one hexose, five N-acetylhexosamines and a species of mass 228 Da, consistent with a trideoxydiacetamidohexose. By means of soybean lectin affinity chromatography, a mixture of glycoproteins was obtained from a glycine extract, and two-dimensional gel proteomics analysis led to the identification of at least 22 glycoproteins, predominantly annotated as periplasmic proteins. Glycopeptides were prepared from the glycoprotein mixture by Pronase digestion and gel filtration. The structure of the glycan was determined by using nano-NMR techniques to be GalNAc-[alpha]1,4-GalNAc-[alpha]1,4-[Glc[beta]1,3]-GalNAc-[alpha]1,4-GalNAc-[alpha]1,4-GalNAc-[alpha]1,3-Bac-[beta]1,N-Asn-Xaa, where Bac is bacillosamine, 2,4-diacetamido-2,4,6-trideoxyglucopyranose. Protein glycosylation was abolished when the *pglB* gene was mutated, providing further evidence that the enzyme encoded by this gene is responsible for formation of the glycopeptide N-linkage. Comparison of the *pgl* locus with that of *Neisseria meningitidis* suggested that most of the homologous genes are probably involved in the biosynthesis of bacillosamine.

Yu, W., N. R. Murray, et al. (2003). "Role of Cyclooxygenase 2 in Protein Kinase C beta II-mediated Colon Carcinogenesis." *J. Biol. Chem.* **278**(13): 11167-11174.

<http://www.jbc.org/cgi/content/abstract/278/13/11167>

Elevated expression of protein kinase C [beta]II (PKC[beta]II) is an early promotive event in colon carcinogenesis (Gokmen-Polar, Y., Murray, N. R., Velasco, M. A., Gatalica, Z., and Fields, A. P. (2001) *Cancer Res.* 61, 1375-1381). Expression of PKC[beta]II in the colon of transgenic mice leads to hyperproliferation and increased susceptibility to colon carcinogenesis due, at least in part, to repression of transforming growth factor beta type II receptor (TGF-[beta]RII) expression (Murray, N. R., Davidson, L. A., Chapkin, R. S., Gustafson, W. C., Schattenberg, D. G., and Fields, A. P. (1999) *J. Cell Biol.*, 145, 699-711). Here we report that PKC[beta]II induces the expression of cyclooxygenase type 2 (Cox-2) in rat intestinal epithelial (RIE) cells in vitro and in transgenic PKC[beta]II mice in vivo. Cox-2 mRNA increases more than 10-fold with corresponding increases in Cox-2 protein and PGE2 production in RIE/PKC[beta]II cells. PKC[beta]II activates the Cox-2 promoter by 2- to 3-fold and stabilizes Cox-2 mRNA by at least 4-fold. The selective Cox-2 inhibitor Celecoxib restores expression of TGF-[beta]RII both in vitro and in vivo and restores TGF[beta]-mediated transcription in RIE/PKC[beta]II cells. Likewise, the [omega]-3 fatty acid eicosapentaenoic acid (EPA), which inhibits PKC[beta]II activity and colon carcinogenesis, causes inhibition of Cox-2 protein expression, re-expression of TGF-[beta]RII, and restoration of TGF-[beta]1-mediated transcription in RIE/PKC[beta]II cells. Our data demonstrate that PKC[beta]II promotes colon cancer, at least in part, through induction of Cox-2, suppression of TGF-[beta] signaling, and establishment of a TGF-[beta]-resistant, hyperproliferative state in the colonic epithelium. Our data define a procarcinogenic PKC[beta]II [right-arrow] Cox-2 [right-arrow] TGF-[beta] signaling axis within the colonic epithelium, and provide a molecular mechanism by which dietary [omega]-3 fatty acids and nonsteroidal antiinflammatory agents such as Celecoxib suppress colon carcinogenesis.

Zhang, D., R. C. M. Simmen, et al. (2002). "Secretory Leukocyte Protease Inhibitor Mediates Proliferation of Human Endometrial Epithelial Cells by Positive and Negative Regulation of Growth-associated Genes." *J. Biol. Chem.* **277**(33): 29999-30009.

<http://www.jbc.org/cgi/content/abstract/277/33/29999>

Secretory leukocyte protease inhibitor (SLPI) inhibits chymotrypsin, trypsin, elastase, and cathepsin G. This protein also exhibits proliferative effects, although little is known about the molecular mechanisms underlying this activity. We have generated SLPI-ablated epithelial sublines by stably transfecting the Ishikawa human endometrial cell line with an antisense human SLPI RNA expression vector. We demonstrate a positive correlation between cellular SLPI production and proliferation. We further show that Ishikawa sublines expressing low to undetectable SLPI have correspondingly increased and decreased expression, respectively, of transforming growth factor- β 1 and cyclin D1 genes, relative to parental cells. SLPI selectively increased cyclin D1 gene expression, with the effect occurring in part at the level of promoter activity. Cellular SLPI levels negatively influenced the anti-proliferative and pro-apoptotic insulin-like growth factor-binding protein-3 expression. We also identified lysyl oxidase, a phenotypic inhibitor of the ras oncogenic pathway and a tumor suppressor, as SLPI-repressed gene, whose expression is up-regulated by transforming growth factor- β 1. Our results suggest that SLPI acts at the node(s) of at least three major interacting growth inhibitory pathways. Because expression of SLPI is generally high in epithelial cells exhibiting abnormal proliferation such as in carcinomas, SLPI may define a novel pathway by which cellular growth is modulated.

Zhang, R.-Z., P. Sabatelli, et al. (2002). "Effects on Collagen VI mRNA Stability and Microfibrillar Assembly of Three COL6A2 Mutations in Two Families with Ullrich Congenital Muscular Dystrophy." *J. Biol. Chem.* **277**(46): 43557-43564.

<http://www.jbc.org/cgi/content/abstract/277/46/43557>

We recently reported a severe deficiency in collagen type VI, resulting from recessive mutations of the COL6A2 gene, in patients with Ullrich congenital muscular dystrophy. Their parents, who are all carriers of one mutant allele, are unaffected, although heterozygous mutations in collagen VI caused Bethlem myopathy. Here we investigated the consequences of three COL6A2 mutations in fibroblasts from patients and their parents in two Ullrich families. All three mutations lead to nonsense-mediated mRNA decay. However, very low levels of undegraded mutant mRNA remained in patient B with compound heterozygous mutations at the distal part of the triple-helical domain, resulting in deposition of abnormal microfibrils that cannot form extensive networks. This observation suggests that the C-terminal globular domain is not essential for triple-helix formation but is critical for microfibrillar assembly. In all parents, the COL6A2 mRNA levels are reduced to 57-73% of the control, but long term collagen VI matrix depositions are comparable with that of the control. The almost complete absence of abnormal protein and near-normal accumulation of microfibrils in the parents may account for their lack of myopathic symptoms.

Zhang, Y. J., W. K. O'Neal, et al. (2002). "Identification of Dynein Heavy Chain 7 as an Inner Arm Component of Human Cilia That Is Synthesized but Not Assembled in a Case of Primary Ciliary Dyskinesia." *J. Biol. Chem.* **277**(20): 17906-17915.

<http://www.jbc.org/cgi/content/abstract/277/20/17906>

Although the basic structure of the axoneme has been highly conserved throughout evolution, the varied functions of specialized axonemes require differences in structure and regulation. Cilia

lining the respiratory tract propel mucus along airway surfaces, providing a critical function to the defense mechanisms of the pulmonary system, yet little is known of their molecular structure. We have identified and cloned a dynein heavy chain that is a component of the inner dynein arm. Bronchial epithelial cells were obtained from normal donors and from a patient with primary ciliary dyskinesia (PCD) whose cilia demonstrated an absence of inner dynein arms by electron microscopy. Cilia from normal and PCD cells were compared by gel electrophoresis, and mass spectrometry was used to identify DNAH7 as a protein absent in PCD cilia. The full-length DNAH7 cDNA was cloned and shares 68% similarity with an inner arm dynein heavy chain from *Drosophila*. DNAH7 was induced during ciliated cell differentiation, and immunohistochemistry demonstrated the presence of DNAH7 in normal cilia. In cilia from PCD cells, DNAH7 was undetectable, whereas intracellular DNAH7 was clearly present. These studies identify DNAH7 as an inner arm component of human cilia that is synthesized but not assembled in a case of PCD.

Zhao, K., M. Liu, et al. (2005). "The Global Transcriptional Response of *Escherichia coli* to Induced σ^{32} Protein Involves σ^{32} Regulon Activation Followed by Inactivation and Degradation of σ^{32} in Vivo." *J. Biol. Chem.* **280**(18): 17758-17768.

<http://www.jbc.org/cgi/content/abstract/280/18/17758>

σ^{32} is the first alternative σ factor discovered in *Escherichia coli* and can direct transcription of many genes in response to heat shock stress. To define the physiological role of σ^{32} , we have used transcription profiling experiments to identify, on a genome-wide basis, genes under the control of σ^{32} in *E. coli* by moderate induction of a plasmid-borne *rpoH* gene under defined, steady-state growth conditions. Together with a bioinformatics approach, we successfully confirmed genes known previously to be directly under the control of σ^{32} and also assigned many additional genes to the σ^{32} regulon. In addition, to understand better the functional relevance of the increased amount of σ^{32} to changes in the transcriptional level of σ^{32} -dependent genes, we measured the protein level of σ^{32} both before and after induction by a newly developed quantitative Western blot method. At a normal constant growth temperature (37 °C), we found that the σ^{32} protein level rapidly increased, plateaued, and then gradually decreased after induction, indicating σ^{32} can be regulated by genes in its regulon and that the mechanisms of σ^{32} synthesis, inactivation, and degradation are not strictly temperature-dependent. The decrease in the transcriptional level of σ^{32} -dependent genes occurs earlier than the decrease in full-length σ^{32} in the wild type strain, and the decrease in the transcriptional level of σ^{32} -dependent genes is greatly diminished in a Δ DnaK strain, suggesting that DnaK can act as an anti- σ factor to functionally inactivate σ^{32} and thus reduce σ^{32} -dependent transcription in vivo.

Zheng, M. and P. J. McKeown-Longo (2002). "Regulation of HEF1 Expression and Phosphorylation by TGF- β 1 and Cell Adhesion." *J. Biol. Chem.* **277**(42): 39599-39608.

<http://www.jbc.org/cgi/content/abstract/277/42/39599>

Transforming growth factor- β 1 (TGF- β 1) is a multipotential cytokine, which regulates remodeling of tissue extracellular matrix during early tumorigenesis and wound healing. Human enhancer of filamentation-1 (HEF1), a multifunctional docking protein, is involved in integrin-based signaling, which affects cell motility, growth, and apoptosis. Our studies reveal that TGF- β 1 is a potent inducer of HEF1 gene transcription in human dermal fibroblasts. TGF- β 1 promoted HEF1 expression in a dose-dependent manner and resulted in a 16-fold increase in HEF1 protein level. TGF- β 1 had no effect on the stability of either HEF1 protein or mRNA. The TGF- β 1-induced HEF1 expression was independent of cell adhesion and resistant to cytoskeleton disruption. TGF- β 1 increased levels of both p105 and p115 HEF1 in adherent

fibroblasts. Digestion with specific phosphatases indicated that the p115HEF1 resulted from serine/threonine phosphorylation of p105HEF1. The appearance of the p115HEF1 as well as tyrosine phosphorylation of p105HEF1 required cell adhesion and/or an organized cytoskeleton. An in vitro kinase assay indicated that p105HEF1 was a substrate for Src. PP1, a specific Src kinase inhibitor, was able to block adhesion-dependent tyrosine phosphorylation of p105HEF1. These findings suggest that TGF- β 1 regulates HEF1 gene expression and that HEF1 phosphorylation is dependent on cell adhesion and Src kinase activity.

Zimmermann, K., K. Ahrens, et al. (2002). "Targeted Disruption of the GAS41 Gene Encoding a Putative Transcription Factor Indicates That GAS41 Is Essential for Cell Viability." *J. Biol. Chem.* **277**(21): 18626-18631.

<http://www.jbc.org/cgi/content/abstract/277/21/18626>

The glioma-amplified sequence (GAS) 41 protein has been proposed to be a transcription factor. To investigate its functional role in vivo, we attempted to knock out the GAS41 gene by targeted disruption in the chicken pre-lymphoid cell line DT40. Heterozygous GAS41+/- cell lines generated by the first round of homologous recombination express approximately half the normal level of GAS41 mRNA. However, a homozygous GAS41-/- cell line with both GAS41 alleles disrupted was not obtained following the second round of transfection, indicating that the GAS41 gene is essential for cell viability. Indeed, homozygous GAS41-/- cell lines with two disrupted GAS41 alleles can be generated following substitution of the endogenous gene by stable integration of GAS41 cDNA controlled by a tetracycline-regulated CMV promoter. Inactivation of this promoter by tetracycline withdrawal results in rapid depletion of GAS41, causing a significant decrease in RNA synthesis and subsequently cell death. Thus, our results indicate that GAS41 is required for RNA transcription.

J. Biomol. Tech. (3)

Haqqi, T., X. Zhao, et al. (2002). "Sequencing in the presence of betaine: Improvement in sequencing of the localized repeat sequence regions." *J. Biomol. Tech.* **13**(4): 265-271.

<http://jbt.highwire.org/cgi/content/abstract/13/4/265>

The presence of several copies of the same class of repetitive element in DNA templates increases the probability of ambiguous base calling caused by band compression artifacts in the BigDye (Applied Biosystems, Foster City, CA) terminator cycle sequencing method. The presence of di-, tri-, and tetranucleotide repeats and short tandem repeats, which is widespread in the genome, poses a daunting task in sequencing laboratories, where a variety of DNA templates are submitted for sequencing. These base anomalies arise mainly as a result of the formation of secondary structures, including hairpins, and intramolecular base pairing between guanine and cytosine bases on the template strand. A common approach to the optimization of such sequencing reactions is either to replace the guanine with a base analog (such as deoxyinosine 5'-triphosphate [dITP] or 7-deaza-deoxyguanosine 5'-triphosphate [dGTP]) or to add a denaturant (such as dimethylsulfoxide [DMSO]) to the reaction mixture to overcome the undesired sequencing artifacts. Additives sometimes are ineffective for sequencing templates with GC-rich regions and repeat sequences. Herein we describe the effectiveness of

(carboxymethyl)trimethylammonium (betaine) inner salt as an additive in the reaction mixture for reducing band compressions. The results presented show that betaine outperformed DMSO in sequencing through the localized regions containing GC-rich base pairs, guanine stretches, or TGC-type repeats in several DNA templates.

Vandenbergh, D. J., K. Heron, et al. (2003). "Simple Tests to Detect Errors in High-Throughput Genotype Data in the Molecular Laboratory." J. Biomol. Tech. **14**(1): 9-16.

<http://jbt.highwire.org/cgi/content/abstract/14/1/9>

With the advent of high-density DNA marker data sets for the mouse and other model systems, 100 or more genotypes are routinely generated from large groups of mice. Issues of the accuracy and reliability of the genotyping are extremely important but often not addressed until genetic analysis is conducted. Simple tests that rely on the robust predictions arising from Mendelian genetics can be made quickly in the molecular laboratory as the data are generated, and require only a spreadsheet program. In this report, genotype data from 392 mice tested at 96 marker sites were analyzed for errors that are typical when handling large volumes of data generated in a repetitive process. The testing consisted of: (1) repeating the genotyping of approximately 1% of the samples; (2) examining the deviation from the expected segregation ratio (1:2:1) on a marker-by-marker basis; and (3) testing the correlation of the genotype at one marker with that at neighboring genetic markers on a chromosome. These three steps allowed analysis at the level of the microtiter plate, where errors are most likely to occur. A set of 96 dinucleotide repeat markers that are polymorphic between the C57BL/6J and DBA/2J mouse strains and can be multiplexed is reported for use in other genotyping projects.

Wiebe, G. J., R. Pershad, et al. (2003). "DNA SEQUENCING RESEARCH GROUP (DSRG) 2003--A GENERAL SURVEY OF CORE DNA SEQUENCING FACILITIES." J. Biomol. Tech. **14**(3): 231-235.

<http://jbt.highwire.org/cgi/content/abstract/14/3/231>

DNA sequencing core facilities serve as centralized resources within both academic and commercial institutions, providing expertise in the area of DNA analysis. The composition and configuration of these facilities continue to evolve in response to new developments in instrumentation and methodology. The goal of the 2003 DNA Sequencing Research Group (DSRG) survey was to identify recent changes in staffing, funding, instrumentation, services, and customer relations. Responses to 58 survey questions from 30 participants are presented to offer a look at the current typical DNA core sequencing facility. The results from this study will serve as a resource for institutions to benchmark their shared core laboratories, and to give facility directors an opportunity to compare and contrast their respective services and experiences.

J. Bone Joint Surg. Am. (1)

Yang, S.-Y., S. Nasser, et al. (2005). "Human Periprosthetic Tissues Implanted in Severe Combined Immunodeficient Mice Respond to Gene Transfer of a Cytokine Inhibitor." J. Bone Joint Surg.

Am. **87**(5): 1088-1097.

<http://www.ejbs.org/cgi/content/abstract/87/5/1088>

Background: Periprosthetic tissue formation and local inflammation that are associated with wear debris contribute to the pathogenesis of aseptic loosening of a prosthesis. This study evaluated a retrovirus-mediated gene therapy with use of a novel xenograft-based animal model. **Methods:** Human periprosthetic tissues obtained from patients during revision arthroplasty performed because of aseptic loosening of a prosthetic joint were transplanted into the left quadriceps and paravertebral muscles of severe combined immunodeficient (SCID) mice. The engrafted tissues were recovered seven, fifteen, or thirty days after implantation for histological and molecular analyses. The periprosthetic tissues were incubated with retroviruses encoding for human interleukin-1 receptor antagonist (hIL-1Ra) or bacteria β -galactosidase (LacZ) at 37°C for three hours prior to implantation to evaluate their responses to gene modification. **Results:** The human periprosthetic tissues were well accepted in SCID mice for up to thirty days, with angiogenesis occurring in the majority of the implanted tissue sections. The histological appearance was consistent between the recovered graft tissue and the original donor tissue. Strong expression of interleukin-1, tumor necrosis factor, and interleukin-6 was detected in the xenografts with use of immunohistochemical stains. Histological analysis revealed that interleukin-1 receptor antagonist gene modification significantly decreased the total number of inflammatory cells ($p < 0.01$) in engrafted human tissue containing implant wear debris. Real-time reverse transcription-polymerase chain reaction and immunohistochemical staining showed declining expression levels of interleukin-1 and tumor necrosis factor following interleukin-1 receptor antagonist gene transfer in comparison with LacZ-transduced or virus-free controls. **Conclusions:** Human periprosthetic tissue can survive in the SCID mouse host for up to thirty days and responds to the interleukin-1 receptor antagonist gene transfer with the amelioration of inflammation. **Clinical Relevance:** The human periprosthetic tissue-SCID mouse chimera has been characterized in this study as a useful model to explore the properties of human periprosthetic tissue in vivo, laying the foundation for potential clinical application of gene therapy in aseptic loosening.

J. Cell Biol. (2)

Koli, K., F. Wempe, et al. (2004). "Disruption of LTBP-4 function reduces TGF- β activation and enhances BMP-4 signaling in the lung." J. Cell Biol. **167**(1): 123-133.

<http://www.jcb.org/cgi/content/abstract/167/1/123>

Disruption of latent TGF- β binding protein (LTBP)-4 expression in the mouse leads to abnormal lung development and colorectal cancer. Lung fibroblasts from these mice produced decreased amounts of active TGF- β , whereas secretion of latent TGF- β was significantly increased. Expression and secretion of TGF- β 2 and - β 3 increased considerably. These results suggested that TGF- β activation but not secretion would be severely impaired in LTBP-4 $-/-$ fibroblasts. Microarrays revealed increased expression of bone morphogenic protein (BMP)-4 and decreased expression of its inhibitor gremlin. This finding was accompanied by enhanced expression of BMP-4 target genes, inhibitors of differentiation 1 and 2, and increased deposition of fibronectin-rich extracellular matrix. Accordingly, increased expression of BMP-4 and decreased expression of gremlin were observed in mouse lung. Transfection of LTBP-4 rescued the $-/-$ fibroblast phenotype, while LTBP-1 was inefficient.

Treatment with active TGF- β 1 rescued BMP-4 and gremlin expression to wild-type levels. Our results indicate that the lack of LTBP-4-mediated targeting and activation of TGF- β 1 leads to enhanced BMP-4 signaling in mouse lung.

Zimmermann, K. C., J.-E. Ricci, et al. (2002). "The role of ARK in stress-induced apoptosis in *Drosophila* cells." *J. Cell Biol.* **156**(6): 1077-1087.

<http://www.jcb.org/cgi/content/abstract/156/6/1077>

The molecular mechanisms of apoptosis are highly conserved throughout evolution. The homologs of genes essential for apoptosis in *Caenorhabditis elegans* and *Drosophila melanogaster* have been shown to be important for apoptosis in mammalian systems. Although a homologue for CED-4/apoptotic protease-activating factor (Apaf)-1 has been described in *Drosophila*, its exact function and the role of the mitochondrial pathway in its activation remain unclear. Here, we used the technique of RNA interference to dissect apoptotic signaling pathways in *Drosophila* cells. Inhibition of the *Drosophila* CED-4/Apaf-1-related killer (ARK) homologue resulted in pronounced inhibition of stress-induced apoptosis, whereas loss of ARK did not protect the cells from Reaper- or Grim-induced cell death. Reduction of DIAP1 induced rapid apoptosis in these cells, whereas the inhibition of DIAP2 expression did not but resulted in increased sensitivity to stress-induced apoptosis; apoptosis in both cases was prevented by inhibition of ARK expression. Cells in which cytochrome c expression was decreased underwent apoptosis induced by stress stimuli, Reaper or Grim. These results demonstrate the central role of ARK in stress-induced apoptosis, which appears to act independently of cytochrome c. Apoptosis induced by Reaper or Grim can proceed via a distinct pathway, independent of ARK.

J. Cell Sci. (16)

Abell, A. N., A. M. DeCathelineau, et al. (2004). "Rac2D57N, a dominant inhibitory Rac2 mutant that inhibits p38 kinase signaling and prevents surface ruffling in bone-marrow-derived macrophages." *J. Cell Sci.* **117**(2): 243-255.

<http://jcs.biologists.org/cgi/content/abstract/117/2/243>

Rac2 is a Rho GTPase that is expressed in cells of hematopoietic origin, including neutrophils and macrophages. We recently described an immunodeficient patient with severe, recurrent bacterial infections that had a point mutation in one allele of the Rac2 gene, resulting in the substitution of aspartate 57 with asparagine. To ascertain further the effects of Rac2D57N in leukocytes, Rac2D57N was expressed in primary murine bone-marrow-derived macrophages (cells that we show express approximately equal amounts of Rac1 and Rac2). Rac2D57N expression in macrophages inhibited membrane ruffling. Rac2D57N expression inhibited the formation of macropinosomes, demonstrating a functional effect of the loss of surface membrane dynamics. Surprisingly, Rac2D57N induced an elongated, spread morphology but did not affect microtubule networks. Rac2D57N also inhibited lipopolysaccharide-stimulated p38 kinase activation. Examination of guanine nucleotide binding to recombinant Rac2D57N revealed reduced dissociation of GDP and association of GTP. Coimmunoprecipitation studies of Rac2D57N with RhoGDI α and Tiam1 demonstrated increased binding of Rac2D57N to these upstream regulators of Rac signaling relative to the wild type. Enhanced binding of

Rac2D57N to its upstream regulators would inhibit Rac-dependent effects on actin cytoskeletal dynamics and p38 kinase signaling.

Debacq-Chainiaux, F., C. Borlon, et al. (2005). "Repeated exposure of human skin fibroblasts to UVB at subcytotoxic level triggers premature senescence through the TGF- β 1 signaling pathway." *J. Cell Sci.* **118**(4): 743-758.

<http://jcs.biologists.org/cgi/content/abstract/118/4/743>

Premature senescence of human diploid fibroblasts (HDFs) can be induced by exposures to a variety of oxidative stress and DNA damaging agents. In this study we developed a robust model of UVB-induced premature senescence of skin HDFs. After a series of 10 subcytotoxic (non-proapoptotic) exposures to UVB at 250 mJ/cm², the so-called biomarkers of senescence were markedly expressed: growth arrest, senescence-associated β -galactosidase activity, senescence-associated gene overexpression, deletion in mitochondrial DNA. A set of 44 stress- and senescence-associated genes were found to be differentially expressed in this model, among which clusterin/apolipoprotein J (apo J) and transforming growth factor- β 1 (TGF- β 1). Transfection of apo J cDNA provided protection against premature senescence-inducing doses of UVB and other stressful agents. Neutralizing antibodies against TGF- β 1 or its receptor II (T β RII) sharply attenuated the senescence-associated features, suggesting a role for TGF- β 1 in UVB-induced premature senescence. Both the latent and active forms of TGF- β 1 were increased with time after the last UVB stress. Proteasome inhibition was ruled out as a potential mechanism of UVB-induced stress-induced premature senescence (SIPS). This model represents an alternative in vitro model in photoaging research for screening potential anti-photoaging compounds.

Fortes, F. S. A., I. L. Pecora, et al. (2004). "Modulation of intercellular communication in macrophages: possible interactions between GAP junctions and P2 receptors." *J. Cell Sci.* **117**(20): 4717-4726.

<http://jcs.biologists.org/cgi/content/abstract/117/20/4717>

Gap junctions are connexin-formed channels that play an important role in intercellular communication in most cell types. In the immune system, specifically in macrophages, the expression of connexins and the establishment of functional gap junctions are still controversial issues. Macrophages express P2X7 receptors that, once activated by the binding of extracellular ATP, lead to the opening of transmembrane pores permeable to molecules of up to 900 Da. There is evidence suggesting an interplay between gap junctions and P2 receptors in different cell systems. Thus, we used ATP-sensitive and -insensitive J774.G8 macrophage cell lines to investigate this interplay. To study junctional communication in J774-macrophage-like cells, we assessed cell-to-cell communication by microinjecting Lucifer Yellow. Confluent cultures of ATP-sensitive J774 cells (ATP-s cells) are coupled, whereas ATP-insensitive J774 cells (ATP-i cells), derived by overexposing J774 cells to extracellular ATP until they do not display the phenomenon of ATP-induced permeabilization, are essentially uncoupled. Western-blot and reverse-transcription polymerase chain reaction assays revealed that ATP-s and ATP-i cells express connexin43 (Cx43), whereas only ATP-s cells express the P2X7 receptor. Accordingly, ATP-i cells did not display any detectable ATP-induced current under whole-cell patch-clamp recordings. Using immunofluorescence microscopy, Cx43 reactivity was found at the cell surface and in regions of cell-cell contact of ATP-s cells, whereas, in ATP-i cells, Cx43 immunoreactivity was only present in cytosolic compartments. Using confocal microscopy, it is shown here that, in ATP-s cells as well as in peritoneal macrophages, Cx43 and P2X7 receptors are co-localized to the membrane of ATP-s cells and peritoneal macrophages.

Jurado, S., J. Sanchez-Prieto, et al. (2003). "Differential expression of NO-sensitive guanylyl cyclase subunits during the development of rat cerebellar granule cells: regulation via N-methyl-D-aspartate receptors." J. Cell Sci. **116**(15): 3165-3175.

<http://jcs.biologists.org/cgi/content/abstract/116/15/3165>

In primary cultures of rat cerebellar granule cells with a functional network of glutamatergic neurons, the expression pattern of the different subunits of nitric-oxide (NO)-sensitive guanylyl cyclase changes during cell differentiation. These cells express the {alpha}1, {alpha}2 and {beta}1 subunits of NO-sensitive guanylyl cyclase and synthesize cyclic guanosine monophosphate (cGMP) in response to exogenous or endogenous nitric oxide. In this study, we determined the protein content of the {alpha}1 and {beta}1 subunits and quantified {alpha}1, {alpha}2 and {beta}1 mRNA by reverse transcription coupled to a polymerase chain reaction (RT-PCR). Expression of the {beta}1 subunit increased with the degree of cell differentiation, although most marked changes occurred at the {alpha} subunit level. In cells freshly isolated from rat pups on postnatal day 7 (P7) the most abundant {alpha} subunit was {alpha}1, while {alpha}2 appeared as the predominant subunit of this type in cultured cells. N-methyl-D-aspartate (NMDA) receptor stimulation in 7- or 14-day-cultured cells led to the upregulation of guanylyl cyclase subunit mRNAs; {alpha}2 mRNA levels undergoing most significant change. This enhanced subunit expression was accompanied by an increase in the amount of cGMP synthesized in response to NO. Thus, it seems that {alpha}2 subunits are increasingly expressed as granule cells mature. The presence of this subunit in the guanylyl cyclase heterodimer facilitates its localization at synaptic membranes, where the enzyme acts as a sensor for NO formed by the postsynaptic protein 95 (PSD-95)-associated neuronal NO synthase.

Kaneko, Y., K. Kitazato, et al. (2004). "Integrin-linked kinase regulates vascular morphogenesis induced by vascular endothelial growth factor." J. Cell Sci. **117**(3): 407-415.

<http://jcs.biologists.org/cgi/content/abstract/117/3/407>

Integrin-linked kinase (ILK) is one of the signaling moieties that interact with the cytoplasmic domains of integrin {beta}1 and {beta}3 subunits. Integrin-mediated outside-in signals cooperate with vascular endothelial growth factor (VEGF) receptor to promote morphological changes, cell proliferation and motility in endothelial cells. In this report we demonstrate that VEGF-induced vessel morphogenesis of human umbilical vein endothelial cells (HUVEC) was inhibited by the transfection of a dominant negative, kinase-deficient ILK (ILK-KD), as well as by treatment with the phosphatidylinositol 3-kinase inhibitor LY294002. VEGF induced phosphorylation of protein kinase B (PKB/Akt), a regulator of cell survival and apoptosis, on serine 473, but not on threonine 308, in an ILK-dependent manner. Furthermore, transfection of antisense ILK (ILK-AS) blocked the survival effect of VEGF in annexin-V binding assays, and a VEGF-mediated decrease in caspase activity was reversed by both ILK-KD and ILK-AS as measured by a homogeneous caspase-3/7 assay. We also demonstrate that both chemotactic migration and cell proliferation of HUVEC induced by VEGF were suppressed by the inhibition of ILK. We conclude that ILK plays an important role in vascular morphogenesis mediated by VEGF.

Khodarev, N. N., J. Yu, et al. (2003). "Tumour-endothelium interactions in co-culture: coordinated changes of gene expression profiles and phenotypic properties of endothelial cells." J. Cell Sci. **116**(6): 1013-1022.

<http://jcs.biologists.org/cgi/content/abstract/116/6/1013>

Tumour angiogenesis is a complex process based upon a sequence of interactions between tumour cells and endothelial cells. To model tumour/endothelial-cell interactions, we co-cultured U87 human glioma cells with human umbilical vein endothelial cells (HUVECs). U87 cells induced an 'activated' phenotype in HUVECs, including an increase in proliferation, migration and net-like formation. Activation was observed in co-cultures where cells were in direct contact and physically separated, suggesting an important role for soluble factor(s) in the phenotypic and genotypic changes observed. Expressional profiling of tumour-activated endothelial cells was evaluated using cDNA arrays and confirmed by quantitative PCR. Matching pairs of receptors/ligands were found to be coordinately expressed, including TGF β RII with TGF β 3, FGFRII and cysteine-rich fibroblast growth factor receptor (CRF-1) with FGF7 and FGF12, CCR1, CCR3, CCR5 with RANTES and calcitonin receptor-like gene (CALCRL) with adrenomedullin. Consistent with cDNA array data, immunohistochemical staining of expressed proteins revealed the upregulation of Tie-2 receptor in vitro and in vivo. Our data suggest that tumour-induced activation of quiescent endothelial cells involves the expression of angiogenesis-related receptors and the induction of autocrine growth loops. We suggest that tumour cells release growth factors that induce endothelial cells to express specific ligands and their cognate receptors coordinately.

Lafleur, M. A., M. M. Handsley, et al. (2002). "Endothelial tubulogenesis within fibrin gels specifically requires the activity of membrane-type-matrix metalloproteinases (MT-MMPs)." *J. Cell Sci.* **115**(17): 3427-3438.

<http://jcs.biologists.org/cgi/content/abstract/115/17/3427>

Macro- and microvascular endothelial cells (EC) formed tubular structures when cultured within a 3D fibrin matrix, a process that was enhanced by vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), hepatocyte growth factor/scatter factor (HGF/SF) and an angiogenic cocktail composed of nine angiogenic factors. Endothelial tubulogenesis was also increased in co-culture with tumour cells such as U87 glioma cells, but not with non-tumorigenic cell types such as Madin-Darby canine kidney (MDCK) epithelial cells. VEGF/FGF-2-stimulated tube formation was dependent on metalloproteinase function [it is inhibited by the addition of tissue inhibitor of metalloproteinases-2 (TIMP-2)], whereas aprotinin, E64 [trans-epoxysuccinyl-L-leucylamido (4-guanidino)-butane] and pepstatin had no effect. In addition, TIMP-4 also inhibited tubulogenesis, but TIMP-1 or the C-terminal haemopexin domain of matrix metalloproteinase-2 (MMP-2) (PEX) and an anti-MMP-2 function-blocking antibody were unable to block tube formation. This suggests that MMP-2 and other soluble MMPs are not essential for tubulogenesis in fibrin gels, instead TIMP-1-insensitive MMPs, such as members of the membrane type-MMPs (MT-MMP) sub-group (MT1-, MT2-, MT3- or MT5-MMP), are required for this process. Further support for a role for MT1-MMP in endothelial tubulogenesis is that recombinant Y36G N-terminal TIMP-2 mutant protein, which retains an essentially unaltered apparent inhibition constant (K_{iapp}) for several MMPs compared to wild-type N-TIMP-2 but is a 40-fold poorer inhibitor of MT1-MMP, was unable to block tubulogenesis. Furthermore, when EC were cultured within fibrin gels, the mRNA levels of several MMPs (including MT1-MMP, MT2-MMP, MT3-MMP and MMP-2) increased during tubulogenesis. Therefore MT-MMPs and specifically MT1-MMP are likely candidates for involvement during endothelial tubulogenesis within a fibrin matrix, and thus their blockade may be a viable strategy for inhibition of angiogenesis.

Lu, D. P., V. Chandrakanthan, et al. (2004). "Trophic signals acting via phosphatidylinositol-3 kinase are required for normal pre-implantation mouse embryo development." *J. Cell Sci.* **117**(8): 1567-1576.

<http://jcs.biologists.org/cgi/content/abstract/117/8/1567>

The growth and survival of the preimplantation mammalian embryo may be regulated by several autocrine trophic factors that have redundant or overlapping actions. One of the earliest trophic factors to be produced is embryo-derived platelet-activating factor (1-O-alky-2-acetyl-sn-glycerol-3-phosphocholine). The addition of platelet-activating factor to embryo culture media exerted a trophic effect, but structurally related lipids (3-O-alky-2-acetyl-sn-glycerol-1-phosphocholine, 1-O-alky-sn-glycerol-3-phosphocholine, octadecyl-phosphocholine) had no effect. Platelet-activating factor induced a pertussis toxin-sensitive $[Ca^{2+}]_i$ transient in two-cell embryos that did not occur in platelet-activating factor-receptor null (Pafr^{-/-}) genotype embryos. Fewer Pafr^{-/-} mouse zygotes developed to the blastocyst stage in vitro compared with Pafr^{+/+} zygotes ($P < 0.02$), those that developed to blastocysts had fewer cells ($P < 0.001$) and more cells with fragmented nuclei ($P < 0.001$). The inhibition of 1-O-phosphatidylinositol 3-kinase (LY294002 (3 μ M and 15 μ M) and wortmannin (10 nM and 50 nM)) caused a dose-dependent inhibition of platelet-activating factor-induced $[Ca^{2+}]_i$ transients ($P < 0.001$). The two-cell embryo expressed 1-O-phosphatidylinositol 3-kinase catalytic subunits p110 $\{\alpha\}$, $\{\beta\}$, $\{\gamma\}$ and $\{\delta\}$, and regulatory subunits p85 $\{\alpha\}$ and $\{\beta\}$. LY294002 and wortmannin each caused a significant reduction in the proportion of embryos developing to the morula and blastocyst stages in vitro, reduced the number of cells within each blastocyst, and significantly increased the proportion of cells in blastocysts with fragmented nuclei. The results indicate that embryo-derived platelet-activating factor (and other embryotrophic factors) act through its membrane receptor to enhance embryo survival through a 1-O-phosphatidylinositol 3-kinase-dependent survival pathway.

Luparello, C., R. Sirchia, et al. (2003). "PTHrP [67-86] regulates the expression of stress proteins in breast cancer cells inducing modifications in urokinase-plasminogen activator and MMP-1 expression." *J. Cell Sci.* **116**(12): 2421-2430.

<http://jcs.biologists.org/cgi/content/abstract/116/12/2421>

It was previously reported that a midregion domain of parathyroid hormone-related protein (PTHrP), that is, [67-86]-amide, is able to restrain growth and promote matrigel penetration by the 8701-BC cell line, derived from a biopsy fragment of a primary ductal infiltrating carcinoma of the human breast, and that cell invasion in vitro is drastically impaired by inactivation of urokinase-plasminogen activator (uPa). In this study we started a more detailed investigation of the possible effects on gene expression arising from the interaction between PTHrP [67-86]-amide and 8701-BC breast cancer cells by a combination of conventional-, differential display-and semi-quantitative multiplex-polymerase chain reaction (PCR) assays. We present here the first evidence that the upregulation of some stress-related genes, most noticeably heat shock factor binding protein-1 (hsbp1) and heat shock protein 90 (hsp-90), is involved in the acquisition of an in vitro more invasive phenotype by cells treated with midregion PTHrP. This is conceivably accomplished by sequestering and inactivating heat shock factor-1 (hsf1) which is able to recognize Ets transcription-factor-binding sites present in some gene promoters, such as those of uPa and matrix metalloprotease-1 (MMP-1). In fact, our data show that incubation of PTHrP [67-86]-amide-treated cells with either antisense hsbp1-oligonucleotide or geldanamycin, an hsp90-inactivating antibiotic, results in downregulation of uPa and upregulation of MMP-1, and in a prominent inhibition of cell invasion in matrigel-containing Transwell chambers. Alternatively, incubation of untreated 8701-BC cells with quercetin, a flavonoid known to decrease the amount of free hsf1, is found to induce upregulation of uPa and downregulation of MMP-1, and an increase of matrigel invasion by cells, thus providing further supporting data of the involvement of hsf unavailability on the modulation of uPa and MMP-1 expression and on cell invasive behaviour. These studies confirm a previous postulate that over-secretion of uPa, rather than of other extracellular proteases, is a primary condition for the increase of invasive activity triggered by PTHrP [67-86]-amide in vitro, and support a role for midregion forms of PTHrP in potentially affecting pathological mammary growth and differentiation. They also identify two new key

protagonists in the complex scenario of breast tumor cell invasiveness in vitro, that is, hsbp1 and hsp90, which deserve further and more extensive studies as potential and attractive molecular targets for anti-breast cancer treatments.

Meissner, M., M. Reiss, et al. (2002). "A family of transmembrane microneme proteins of *Toxoplasma gondii* contain EGF-like domains and function as escorts." *J. Cell Sci.* **115**(3): 563-574.

<http://jcs.biologists.org/cgi/content/abstract/115/3/563>

TgMIC6, TgMIC7, TgMIC8 and TgMIC9 are members of a novel family of transmembrane proteins localized in the micronemes of the protozoan parasite *Toxoplasma gondii*. These proteins contain multiple epidermal growth factor-like domains, a putative transmembrane spanning domain and a short cytoplasmic tail. Sorting signals to the micronemes are encoded in this short tail. We established previously that TgMIC6 serves as an escorter for two soluble adhesins, TgMIC1 and TgMIC4. Here, we present the characterization of TgMIC6 and three additional members of this family, TgMIC7, -8 and -9. Consistent with having sorting signals localized in its C-terminal tail, TgMIC6 exhibits a classical type I membrane topology during its transport along the secretory pathway and during storage in the micronemes. TgMIC6 is processed at the N-terminus, probably in the trans-Golgi network, and the cleavage site has been precisely mapped. Additionally, like other members of the thrombospondin-related anonymous protein family, TgMIC2, TgMIC6 and TgMIC8 are proteolytically cleaved near their C-terminal domain upon discharge by micronemes. We also provide evidence that TgMIC8 escorts another recently described soluble adhesin, TgMIC3. This suggests that the existence of microneme protein complexes is not an exception but rather the rule. TgMIC6 and TgMIC8 are expressed in the rapidly dividing tachyzoites, while TgMIC7 and TgMIC9 genes are predominantly expressed in bradyzoites, where they presumably also serve as escorts.

Nakano, M., Y. Okamoto, et al. (2003). "Epigenetic assembly of centromeric chromatin at ectopic {alpha}-satellite sites on human chromosomes." *J. Cell Sci.* **116**(19): 4021-4034.

<http://jcs.biologists.org/cgi/content/abstract/116/19/4021>

To investigate the mechanism of chromatin assembly at human centromeres, we isolated cultured human cell lines in which a transfected alpha-satellite (alphoid) YAC was integrated ectopically into the terminal region of host chromosome 16, where it was stably maintained. Centromere activity of the alphoid YAC was suppressed at ectopic locations on the host chromosome, as indicated by the absent or reduced assembly of CENP-A and -C. However, long-term culture in selective medium, or short-term treatment with the histone deacetylase inhibitor Trichostatin A (TSA), promoted the re-assembly of CENPA, -B and -C at the YAC site and the release of minichromosomes containing the YAC integration site. Chromatin immunoprecipitation analyses of the re-formed minichromosome and the alphoid YAC-based stable human artificial chromosome both indicated that CENP-A and CENP-B assembled only on the inserted alphoid array but not on the YAC arms. On the YAC arms at the alphoid YAC integration sites, TSA treatment increased both the acetylation level of histone H3 and the transcriptional level of a marker gene. An increase in the level of transcription was also observed after long-term culture in selective medium. These activities, which are associated with changes in chromatin structure, might reverse the suppressed chromatin state of the YAC at ectopic loci, and thus might be involved in the epigenetic change of silent centromeres on ectopic alphoid loci.

O'Shaughnessy, P. J., H. Johnston, et al. (2002). "Failure of normal adult Leydig cell development in androgen-receptor-deficient mice." *J. Cell Sci.* **115**(17): 3491-3496.

<http://jcs.biologists.org/cgi/content/abstract/115/17/3491>

During testicular development, fetal and adult populations of Leydig cells arise sequentially. Previous studies have shown that androgen action is required for normal steroidogenic activity in the mouse testis. Therefore, to determine the role of androgens in regulating fetal and adult Leydig cell differentiation and function, Leydig development has been measured in mice lacking functional androgen receptors (AR-null). The Leydig cell number was normal on day 5 after birth in AR-null mice but failed to increase normally thereafter and was about 30% of the control level on day 20 and about 60% of control level in adult animals. Levels of 15 different mRNA species expressed specifically in Leydig cells were measured by real-time PCR in AR-null and control animals. Expression levels of all mRNA species were normal on day 5 when only fetal Leydig cells are present. In older animals, which contain predominantly adult Leydig cells, five of the mRNA species (3 β -hydroxysteroid dehydrogenase (3 β HSD) type 1, cytochrome P450_{scc}, renin, StAR protein and luteinising hormone receptor) were expressed at normal or increased levels in AR-null mice. All other mRNA species measured showed significantly reduced expression in older animals, and three of these mRNA species (17 β -hydroxysteroid dehydrogenase type III, prostaglandin D (PGD)-synthetase and 3 β HSD type VI), which are only expressed in the adult population of Leydig cells, were barely detectable in the adult AR-null mouse. The results show that in the absence of androgen receptors, fetal Leydig cell function is normal, but there is a developmental failure of adult Leydig cell maturation, with cells only acquiring partial characteristics of the adult population.

Paganoni, S. and A. Ferreira (2005). "Neurite extension in central neurons: a novel role for the receptor tyrosine kinases Ror1 and Ror2." *J. Cell Sci.* **118**(2): 433-446.

<http://jcs.biologists.org/cgi/content/abstract/118/2/433>

Neurite elongation and branching are key cellular events during brain development as they underlie the formation of a properly wired neuronal network. Here we report that the receptor tyrosine kinases Ror1 and Ror2 modulate the growth of neurites as well as their branching pattern in hippocampal neurons. Upon Ror1 or Ror2 suppression using antisense oligonucleotides or RNA interference (RNAi), neurons extended shorter and less branched minor processes when compared to those in control cells. In addition, Ror-depleted cells elongated longer, albeit less branched, axons than seen in control cells. Conversely, Ror overexpression both in non-neuronal cells and in hippocampal neurons resulted in the enhanced extension of short and highly branched processes. These phenotypes were accompanied by changes in the microtubule-associated proteins MAP1B and MAP2. Taken together, these results support a novel role for Ror receptors as modulators of neurite extension in central neurons.

Penttinen, C., J. Saharinen, et al. (2002). "Secretion of human latent TGF- β -binding protein-3 (LTBP-3) is dependent on co-expression of TGF- β ." *J. Cell Sci.* **115**(17): 3457-3468.

<http://jcs.biologists.org/cgi/content/abstract/115/17/3457>

Latent TGF- β -binding proteins (LTBPs) were initially identified through their binding to the growth factor. Three of the four known LTBPs are able to associate covalently with the small latent forms of TGF- β and mediate their efficient secretion. LTBPs have subsequently been found to associate with the extracellular matrix. We report here the cDNA cloning and

characterization of the human LTBP-3 protein, which is the smallest LTBP. The hLTBP-3 gene consists of 28 exons, including one alternatively spliced exon. The splice variant contains an additional epidermal-growth-factor-like repeat in the C-terminus. The gene is transcribed to produce a [~]4.6 kb mRNA, which is expressed at high levels in human heart, skeletal muscle, prostate and ovaries and in certain osteosarcoma and fibroblastic cell lines. Antibodies were generated against recombinant fragment of hLTBP-3 and used to detect the protein and its secretion from cultured COS-7 and osteosarcoma cells. Immunoblotting analysis indicated that efficient secretion of overexpressed hLTBP-3 from COS-7 cells required co-expression of TGF- β 1, which resulted in the secretion of high molecular weight complexes of [~]240 kDa. hLTBP-3 protein was secreted from cultured osteosarcoma cells as high molecular weight complexes rather than in the free form. Similar complexes were recognized with antibodies specific to β 1*LAP. These findings indicate that human LTBP-3 has an essential role in the secretion and targeting of TGF- β 1.

Santama, N., C. P. N. Er, et al. (2004). "Distribution and functions of kinectin isoforms." J. Cell Sci. **117**(19): 4537-4549.

<http://jcs.biologists.org/cgi/content/abstract/117/19/4537>

Kinectin is an integral transmembrane protein on the endoplasmic reticulum, binding to kinesin, interacting with Rho GTPase and anchoring the translation elongation factor-1 complex. There has been debate on the specific role(s) of kinectin in different species and cell types. Here we identified 15 novel kinectin isoforms in the mouse nervous system, constituting a family of alternatively spliced carboxyl-terminal variants. Isoform expression is subject to cell type- and developmental stage-specific regulation. We raised specific antibodies to the kinectin variants to characterise their differential intracellular localisation and discovered that certain kinectin isoforms are found in axons where kinectin was previously believed to be absent. We also demonstrated in vivo by overexpression and RNA interference assay that kinectin is selectively involved in the transport of specific types of organelles. A 160 kDa kinectin species is mainly concentrated in the endoplasmic reticulum, anchored via its transmembrane domain and is essential for endoplasmic reticulum membrane extension. A 120 kDa kinectin species is specifically associated with mitochondria, and its interaction with kinesin was found to influence mitochondrial dynamics. These findings contribute to a more unified view of kinectin function. They suggest that different cellular processes use specific kinectin isoforms to mediate intracellular motility and targeting by transient interaction with different motor proteins or other binding partners.

von Rotz, R. C., B. M. Kohli, et al. (2004). "The APP intracellular domain forms nuclear multiprotein complexes and regulates the transcription of its own precursor." J. Cell Sci. **117**(19): 4435-4448.

<http://jcs.biologists.org/cgi/content/abstract/117/19/4435>

The physiological functions of the beta-amyloid precursor protein (APP) may include nuclear signaling. To characterize the role of the APP adaptor proteins Fe65, Jip1b, X11 α (MINT1) and the chromatin-associated protein Tip60, we analyzed their interactions by confocal microscopy and co-immunoprecipitations. AICD corresponding to S3-cleaved APP bound to Fe65 that transported it to nuclei and docked it to Tip60. These proteins formed AICD-Fe65-Tip60 (AFT) complexes that were concentrated in spherical nuclear spots. γ -Secretase inhibitors prevented AFT-complex formation with AICD derived from full-length APP. The APP adaptor protein Jip1b also transported AICD to nuclei and docked it to Tip60, but AICD-Jip1b-Tip60 (AJT) complexes had different, speckle-like morphology. By contrast, X11 α trapped AICD in the cytosol. Induced AICD expression identified the APP-effector genes APP, BACE, Tip60, GSK3 β and KAI1, but not the Notch-effector gene Hes1 as transcriptional targets.

These data establish a role for APP in nuclear signaling, and they suggest that therapeutic strategies designed to modulate the cleavage of APP affect AICD-dependent signaling.

J. Clin. Endocrinol. Metab. (98)

Albrecht, E. D., G. W. Aberdeen, et al. (2003). "Acute Temporal Regulation of Vascular Endothelial Growth/Permeability Factor Expression and Endothelial Morphology in the Baboon Endometrium by Ovarian Steroids." J. Clin. Endocrinol. Metab. **88**(6): 2844-2852.

<http://jcem.endojournals.org/cgi/content/abstract/88/6/2844>

We recently showed that endometrial vascular endothelial growth/permeability factor (VEG/PF) mRNA expression was decreased by ovariectomy of baboons and restored by chronic administration of estrogen. However, it remains to be determined whether this effect of estrogen reflects genomic up-regulation of VEG/PF and leads to an increase in microvascular permeability, an early physiological event in angiogenesis. Therefore, we determined the temporal expression of VEG/PF mRNA in glandular epithelial and stromal cells isolated by laser capture microdissection from and width of microvascular paracellular clefts that regulate vessel permeability in the endometrium of ovariectomized baboons after acute estradiol and/or progesterone administration. Endometrial VEG/PF mRNA levels were increased in five of five animals within 2 h of estradiol administration and remained elevated at 4 and 6 h. The net increase in glandular epithelial (7.31 ± 2.72 attomol/fmol 18S ribosomal rRNA) and stromal (3.13 ± 0.36) cell VEG/PF mRNA levels after estradiol administration was over 8-fold ($P < 0.05$) and 2.6-fold ($P < 0.01$) greater, respectively, than after vehicle (0.90 ± 0.30 , glands and 1.20 ± 0.33 , stroma). In contrast, endometrial VEG/PF mRNA expression was unaltered by progesterone. After estradiol treatment, endometrial paracellular cleft width was increased ($P < 0.01$) from a mean ($\pm$ SE) of 71.6 ± 4.6 nm at 0 h to 101.1 ± 6.4 nm at 6 h, whereas vehicle or progesterone had no effect. We suggest that estrogen has a major role in regulating VEG/PF synthesis and early events in angiogenesis in the primate endometrium.

Andersen, K. L., S. M. Echwald, et al. (2005). "Variation of the McKusick-Kaufman Gene and Studies of Relationships with Common Forms of Obesity." J. Clin. Endocrinol. Metab. **90**(1): 225-230.

<http://jcem.endojournals.org/cgi/content/abstract/90/1/225>

Obesity is a prominent feature of the Bardet-Biedl syndrome (BBS), one subset of which, BBS6, is due to mutations in the chaperonin-like gene termed the McKusick-Kaufman syndrome (MKKS) gene. We tested whether variation in MKKS contributes to common and probably polygenic forms of obesity by performing mutation analysis of the coding region in 60 Danish white men with juvenile-onset obesity. Five variants were identified, including two synonymous mutations (Pro39Pro and Ile178Ile) and three nonsynonymous variants (Ala242Ser, Arg517Cys, and Gly532Val). Furthermore, the rare Ala242Ser was identified in two families and showed partial cosegregation with obesity. The Pro39Pro, Ile178Ile, and Arg517Cys variants are in complete linkage disequilibrium and defined a prevalent haplotype. In a case-control study, the Arg517Cys polymorphism allele prevalence was 11.4% [95% confidence interval (CI), 9.7-13.0] among 744 men with juvenile-onset obesity and 9.3% (CI, 7.9-10.7) among 867 control subjects ($P = 0.048$). However, among middle-aged men the allelic prevalence was 9.7% (CI, 7.9-11.4) among 523

obese men and 12.2% (CI, 10.8-13.6) among 1051 lean men ($P = 0.037$). In conclusion, it is unlikely that MKKS variants play a major role in the pathogenesis of nonsyndromic obesity, although in rare cases the A242S allele may contribute to obesity.

Arko, B., J. Prezelj, et al. (2002). "Sequence Variations in the Osteoprotegerin Gene Promoter in Patients with Postmenopausal Osteoporosis." *J. Clin. Endocrinol. Metab.* **87**(9): 4080-4084.

<http://jcem.endojournals.org/cgi/content/abstract/87/9/4080>

Osteoprotegerin (OPG) is a recently discovered member of the TNF receptor superfamily that acts as an important paracrine regulator of bone remodeling. OPG knockout mice develop severe osteoporosis, whereas administration of OPG can prevent ovariectomy-induced bone loss. These findings implicate a role for OPG in the development of osteoporosis. In the present study, we screened the OPG gene promoter for sequence variations and examined their association with bone mineral density (BMD) in 103 osteoporotic postmenopausal women. Single-strand conformation polymorphism analysis followed by DNA sequencing revealed a presence of four nucleotide substitutions: 209 G→A, 245 T→G, 889 C→T, and 950 T→C. The frequencies of genotypes were as follows: GG (89.3%), GA (10.7%) for 209 G→A polymorphism; TT (89.3%), TG (10.7%) for 245 T→G polymorphism; and TT (25.2%), TC (53.4%), CC (21.4%) for 950 T→C polymorphism. Substitution 889 C→T was found in only two patients. Statistically significant association of genotypes with BMD at the lumbar spine ($P = 0.005$) was observed for 209 G→A and 245 T→G polymorphisms. Haplotype GATG was associated with lower BMD as compared with GGTT haplotype. Our results suggest that 209 G→A and 245 T→G polymorphisms in the OPG gene promoter may contribute to the genetic regulation of BMD.

Ban, Y., E. S. Concepcion, et al. (2004). "Analysis of Immune Regulatory Genes in Familial and Sporadic Graves' Disease." *J. Clin. Endocrinol. Metab.* **89**(9): 4562-4568.

<http://jcem.endojournals.org/cgi/content/abstract/89/9/4562>

Graves' disease (GD) is seen in apparently sporadic and familial forms. At least two immune regulatory genes are associated with GD, human leukocyte antigen (HLA) and cytotoxic T lymphocyte antigen-4 (CTLA-4). The aim of our study was to examine the contributions of HLA and CTLA-4 to the familial clustering of GD by analyzing them for association with familial and sporadic GD. We analyzed 160 Caucasian GD patients (69 familial and 91 sporadic), and 150 matched controls. Analysis of all GD patients demonstrated significant associations between GD and HLA-DR3 [$P = 9.0 \times 10^{-7}$; relative risk (RR) = 3.8] and two CTLA-4 single nucleotide polymorphisms (SNPs), A/G49 SNP ($P = 0.03$; RR = 1.5), and CT60 SNP ($P = 0.03$; RR = 1.4). Moreover, there was evidence for joint susceptibility to risk between HLA-DR3 and CTLA-4, giving a combined RR of 5.9. Subset analysis demonstrated no significant difference between the frequencies of HLA-DR3 and the susceptibility alleles of CTLA-4 A/G49 and CT60 SNPs in the familial and sporadic GD subsets ($P > 0.05$). These results suggested that HLA-DR3 and CTLA-4 conferred a general increased risk for GD in both the sporadic and familial forms, and that the risk conferred by them was additive. However, HLA-DR3 and CTLA-4 did not have a stronger effect in the familial GD patients, suggesting that additional genes must contribute to the aggregation of GD within families.

Bujalska, I. J., E. A. Walker, et al. (2002). "A Switch in Dehydrogenase to Reductase Activity of 11 β -

Hydroxysteroid Dehydrogenase Type 1 upon Differentiation of Human Omental Adipose Stromal Cells." *J. Clin. Endocrinol. Metab.* **87**(3): 1205-1210.

<http://jcem.endojournals.org/cgi/content/abstract/87/3/1205>

As exemplified in patients with Cushing's syndrome, glucocorticoids play an important role in regulating adipose tissue distribution and function, but circulating cortisol concentrations are normal in most patients with obesity. However, human omental adipose stromal cells (ASCs) can generate glucocorticoid locally through the expression of the enzyme 11 β -hydroxysteroid dehydrogenase (11 β -HSD) type 1 (11 β -HSD1), which, in intact cells, has been considered to be an oxoreductase, converting inactive cortisone (E) to cortisol (F). Locally produced F can induce ASC differentiation, but the relationship between 11 β -HSD1 expression and adipocyte differentiation is unknown. Primary cultures of paired omental (om) and sc ASC and adipocytes were prepared from 17 patients undergoing elective abdominal surgery and cultured for up to 14 d. Expression and activity of 11 β -HSD isozymes were analyzed together with early (lipoprotein lipase) and terminal (glycerol 3 phosphate dehydrogenase) markers of adipocyte differentiation. On d 1 of culture, 11 β -HSD1 activity in intact om ASCs exceeded oxoreductase activity in every patient (78.9 $\pm$ 24.9 vs. 15.8 $\pm$ 3.7 [mean $\pm$ SE] pmol/mg per hour, $P < 0.001$), and in sc ASCs, relative activities were similar (40.6 $\pm$ 12.2 vs. 36.9 $\pm$ 8.8). Conversely, in freshly isolated om adipocytes, reductase activity exceeded dehydrogenase activity (23.6 $\pm$ 1.5 vs. 6.2 $\pm$ 0.8 pmol/mg per hour, $P < 0.01$). Following 14 d of culture in serum-free conditions with addition of 10 nM insulin (Ctr) or insulin with 100 nM F (+F), lipoprotein lipase/18S RNA levels increased in both the Ctr- and +F-treated ASCs, but glycerol 3 phosphate dehydrogenase increased only in the +F cultures. In both cases, however, 11 β -HSD1 oxoreductase activity exceeded dehydrogenase activity (Ctr: 53.3 $\pm$ 9.0 vs. 32.4 $\pm$ 10.5, $P < 0.05$; +F: 65.6 $\pm$ 15.6 vs. 37.1 $\pm$ 11.5 pmol/mg per hour, $P < 0.05$), despite no significant changes in 11 β -HSD1 mRNA levels. In sc ASCs, dehydrogenase activity was similar to reductase activity in both Ctr- and +F-treated cells. Type 2 11 β -HSD expression was undetectable in each case. These data show that in intact, undifferentiated om ASCs, 11 β -HSD1 acts primarily as a dehydrogenase, but in mature adipocytes oxoreductase activity predominates. Because glucocorticoids inhibit cell proliferation, we postulate that 11 β -HSD1 activity in uncommitted ASCs may facilitate proliferation rather than differentiation. Once early differentiation is initiated, a "switch" to 11 β -HSD1 oxoreductase activity generates F, thus promoting adipogenesis. Site-specific regulation of the set-point of 11 β -HSD1 activity may be an important mechanism underpinning visceral obesity.

Canto, P., D. Soderlund, et al. (2004). "Mutations in the Desert hedgehog (DHH) Gene in Patients with 46,XY Complete Pure Gonadal Dysgenesis." *J. Clin. Endocrinol. Metab.* **89**(9): 4480-4483.

<http://jcem.endojournals.org/cgi/content/abstract/89/9/4480>

Mutations of SRY are the cause of complete pure gonadal dysgenesis (PGD) in 10-15% of patients. In the remaining individuals, it has been suggested that mutations in other genes involved in the testis-determining pathway could be causative. We describe the first report in which three cases of 46,XY complete PGD are attributed to mutations of the Desert hedgehog (DHH) gene. DHH was sequenced using genomic DNA from paraffinembedded gonadal tissue from six patients with complete 46,XY PGD. Mutations were found in three patients: a homozygous mutation in exon 2, responsible for a L162P, and a homozygous 1086delG in exon 3. Mutated individuals displayed 46,XY complete PGD, differentiating from the only previously described patient with a homozygous DHH mutation, who exhibited a partial form of PGD with polyneuropathy, suggesting that localization of mutations influence phenotypic expression. This constitutes the first report where mutations of DHH are associated with the presence of 46,XY complete PGD, demonstrating that the genetic origin of this entity is heterogeneous and that disorders in other genes, different from SRY, involved in the testis-determining pathway are

implicated in abnormal testicular differentiation in humans. These data extend previous reports demonstrating DHH is a key gene in gonadal differentiation.

Caraccio, N., R. Giannini, et al. (2005). "Type I Interferons Modulate the Expression of Thyroid Peroxidase, Sodium/Iodide Symporter, and Thyroglobulin Genes in Primary Human Thyrocyte Cultures." J. Clin. Endocrinol. Metab. **90**(2): 1156-1162.

<http://jcem.endojournals.org/cgi/content/abstract/90/2/1156>

We evaluated in primary human thyrocyte cultures the effect of interferon (IFN)- α and - β on the expression of thyroid peroxidase (TPO), sodium/iodide symporter (NIS), and thyroglobulin (Tg) as well as T4 release. Human thyrocyte cultures were carried out with fresh normal thyroid tissue. Gene and protein expression of Tg, TPO, and NIS were assessed by RT-PCR and Western blot analysis after 24, 48, and 72 h of treatment with TSH alone (10 mIU/ml) and in combination with IFN α or - β (104 U/ml). IFN inhibited the TSH-stimulated gene expression of Tg, TPO, and NIS in a time-dependent manner without significant differences between IFN α and - β . Moreover, the addition of both type I IFNs clearly reduced the TSH-stimulated protein expression of Tg, TPO, and NIS after 72 h of exposure. Finally, this down-regulation was associated with a reduction of T4 release by almost 50%. In conclusion, our study shows that both IFN α and - β down-regulate the TSH-stimulated expression of Tg, TPO, and NIS as well as T4 release. Indeed, the development of hypothyroidism during type I IFN therapy may be related, at least in part, to an abnormal expression and function of key proteins involved in iodine uptake and organification.

Carpenter, T. O., B. K. Ellis, et al. (2005). "Fibroblast Growth Factor 7: An Inhibitor of Phosphate Transport Derived from Oncogenic Osteomalacia-Causing Tumors." J. Clin. Endocrinol. Metab. **90**(2): 1012-1020.

<http://jcem.endojournals.org/cgi/content/abstract/90/2/1012>

Oncogenic osteomalacia (OO), a tumor-associated phosphate-wasting syndrome, provides an opportunity to identify regulators of renal phosphate homeostasis. We established cultures from OO-associated tumors. Conditioned medium from these cultures inhibited phosphate uptake in renal tubular epithelial cells. We then compared RNA from tumor-derived cultures expressing inhibitory activity with RNA from tumor-derived cultures in which inhibitory activity was not evident and identified candidate mRNAs specifically expressed by cultures inhibiting renal phosphate transport. Testing of identified candidates revealed that one protein, fibroblast growth factor 7 (FGF7), was a potent and direct inhibitor of phosphate uptake in vitro. A neutralizing monoclonal antibody to FGF7 reversed FGF7-dependent phosphate transport inhibition and inhibitory activity in conditioned medium from tumor cell cultures. Immunoassay revealed abundant FGF7 in inhibitory conditioned medium and minimal amounts in nonconditioned medium or conditioned medium with no phosphate transport inhibitory activity. Furthermore, only small amounts of FGF23 were present in inhibitory conditioned medium, comparable to concentrations found in conditioned medium with no phosphate transport inhibitory activity. Thus, FGF7 was specifically identified when selecting for in vitro phosphate transport inhibitory activity of tumor-derived cultures and was confirmed as a potent inhibitor of phosphate transport. Finally, FGF7 message was confirmed in PCR products of mRNA extracted from fragments of each tumor. Members of the FGF family (other than FGF23) are expressed by OO-associated tumors and may play a role in mediating this syndrome.

Chan, E. C., S. Fraser, et al. (2002). "Human Myometrial Genes Are Differentially Expressed in Labor: A Suppression Subtractive Hybridization Study." *J. Clin. Endocrinol. Metab.* **87**(6): 2435-2441.

<http://jcem.endojournals.org/cgi/content/abstract/87/6/2435>

Human parturition is effected by a cascade of factors, of which many are unknown. We aim to identify the genes that are changed by labor in the human myometrium by suppression subtractive hybridization. We also seek to ascertain whether these genes are differentially expressed in the myometrium at the upper or fundal and lower segments of the uterus. Term myometrial tissues were obtained from laboring and nonlaboring women undergoing cesarean section after obtaining informed consent. Total RNA was used in suppression subtractive hybridization (CLONTECH PCR Select) to produce two subtracted cDNA libraries enriched for genes expressed during or before labor, labor and not-in-labor libraries, respectively. Dot blot screening of 400 positive clones, constituting 20% of the two subtracted libraries, revealed 30 differentially expressed clones, 14 of which were up-regulated by labor. Among the 10 known genes that were up-regulated in labor, 6 had apparent immune regulatory and inflammatory roles. Three are well-known inflammatory mediators and modulators that were previously linked with parturition: IL-8, manganese superoxide dismutase (MnSOD), and metalloproteinase-9. Three others, interferon-inducible 1-8d gene, elongation factor 1{alpha}, and nucleophosmin, have not been previously linked with labor. Constitutively expressed genes, including cyclophilin and {alpha}-actin, were found to be altered by labor. Quantitative real-time RT-PCR using Taqman probes further confirmed the up-regulation of some of these genes. The amounts of the specific genes assayed were standardized to 18S ribosomal RNA and are expressed as mean {+/-} SEM. Quantitative real-time RT-PCR showed that IL-8 mRNA rose from 0.003 {+/-} 0.002 in nonlaboring samples (n = 38) to 0.24 {+/-} 0.11 (n = 20) in gestational-age-matched spontaneously laboring women (P = 0.035). Similarly, MnSOD rose from 0.11 {+/-} 0.02 (n = 24) to 1.23 {+/-} 0.56 (n = 24) in gestational-age-matched women (P = 0.047). Additionally, cyclophilin, often used as a constitutive or housekeeping gene marker, increased from 0.0008 {+/-} 0.0002 (n = 6) to 0.002 {+/-} 0.0004 (n = 6; P = 0.008) during labor. Notably, MnSOD mRNA was differentially distributed between the upper (0.63 {+/-} 0.18) and lower (0.15 {+/-} 0.05; n = 15; P = 0.022) segments of the uterus, but IL-8 was not (n = 17; P = 0.97). Induced labor further showed significantly higher levels of IL-8 (0.63 {+/-} 0.21; n = 14) than spontaneous labor (0.22 {+/-} 0.11; n = 20; P = 0.046), but not MnSOD (P = 0.1). This work identifies novel as well as known genes that were not previously associated with parturition. It extends previous data indicating that there is differential expression of some, but not all genes within the gravid human uterus. Inflammatory genes constitute a major proportion of the known genes found to be up-regulated in labor, lending support to the hypothesis of an inflammatory mechanism for human parturition. This work further indicates that many factors associated with human labor and their complex interactions remain to be elucidated.

Chen, C.-P., Y.-C. Yang, et al. (2005). "Hypoxia and Transforming Growth Factor-{beta}1 Act Independently to Increase Extracellular Matrix Production by Placental Fibroblasts." *J. Clin. Endocrinol. Metab.* **90**(2): 1083-1090.

<http://jcem.endojournals.org/cgi/content/abstract/90/2/1083>

Villous fibrosis is associated with oxygen deprivation in placental pathology, but the signaling networks and growth factors involved in activating the relevant cellular repair mechanisms are largely unknown. TGF is a powerful enhancer of extracellular matrix (ECM) production and an important immune suppressor that has been linked with fibrosis in several tissues. Here, cell culture methods were used to investigate possible links between hypoxia, elevated TGF{beta}1, and altered ECM production in placenta. Term placental fibroblasts were isolated and cultured under hypoxia (3% O₂) or in the presence of TGF{beta}1, and the expression of fibronectin, collagen I, and collagen IV was examined using immunohistochemistry, ELISA of cell monolayers

with associated ECM, and real-time RT-PCR. The effect of hypoxia on endogenous production of TGF{beta}1-3 was also examined. Both TGF{beta}1 and hypoxia increased fibronectin, collagen I, and collagen IV protein and mRNA in placental fibroblasts. However, TGF{beta}1-3 production was not increased by culturing the cells under hypoxic conditions for 5 d. Thus, increased ECM expression under hypoxia was not mediated directly by increased TGF{beta}. We conclude that ECM production can be stimulated independently by hypoxia and TGF{beta}1.

Christensen, J. H., C. Siggaard, et al. (2004). "Differential Cellular Handling of Defective Arginine Vasopressin (AVP) Prohormones in Cells Expressing Mutations of the AVP Gene Associated with Autosomal Dominant and Recessive Familial Neurohypophyseal Diabetes Insipidus." J. Clin. Endocrinol. Metab. **89**(9): 4521-4531.

<http://jcem.endojournals.org/cgi/content/abstract/89/9/4521>

An unusual mutation in the arginine vasopressin (AVP) gene, predicting a P26L amino acid substitution of the AVP prohormone, is associated with autosomal recessive familial neurohypophyseal diabetes insipidus (FNDI). To investigate whether the cellular handling of the P26L prohormone differed from that of the Y21H prohormone associated with autosomal dominant inheritance of FNDI, the mutations were examined by heterologous expression in cell lines. Immunoprecipitation demonstrated retarded processing and secretion of the Y21H prohormone, whereas the secretion of the P26L prohormone seemed to be unaffected. Confocal laser scanning microscopy showed accumulation of the Y21H prohormone in the endoplasmic reticulum, whereas the P26L prohormone and/or processed products were localized in secretory granules in the cellular processes. RIA analysis showed reduced amounts of immunoreactive Y21H-AVP and P26L-AVP in the cell culture medium. Thus, the recessive mutation does not seem to affect the intracellular trafficking but rather the final processing of the prohormone. Our results provide an important negative control in support of the hypothesis that autosomal dominant inheritance of FNDI is caused by mutations in the AVP gene that alter amino acid residues important for folding and/or dimerization of the neurophysin II moiety of the AVP prohormone and subsequent transport from the endoplasmic reticulum.

Chu, J., R. Zhang, et al. (2002). "Male Fertility Is Compatible with an Arg840Cys Substitution in the AR in a Large Chinese Family Affected with Divergent Phenotypes of AR Insensitivity Syndrome." J. Clin. Endocrinol. Metab. **87**(1): 347-351.

<http://jcem.endojournals.org/cgi/content/abstract/87/1/347>

Androgen insensitivity syndrome (AIS) is a disorder of male sexual development caused by an absent or dysfunctional AR. Fertile cases with mild AIS and slightly impaired AR activity had been reported in literature, and their external genitalia were documented to be usually normal or subnormal. We reported here an Arg840Cys substitution in the AR gene in a large Chinese pedigree affected with AIS. The mutant gene may result in infertility for some affected males with or without hypospadias. However, it was also observed that the mutation did not affect the fertility of the other patients. The gonadotropin levels for one of these patients were within the normal range. Thus, whether normal levels of the gonadotropins are necessary for the preserved fertility of patients affected with this genetic disorder remains to be elucidated.

Collins, J. E., J. M. Heward, et al. (2003). "Association of a Rare Thyroglobulin Gene Microsatellite Variant with Autoimmune Thyroid Disease." J. Clin. Endocrinol. Metab. **88**(10): 5039-5042.

<http://jcem.endojournals.org/cgi/content/abstract/88/10/5039>

Genetic and environmental factors contribute to the development of Graves' disease and Hashimoto's thyroiditis. These diseases, although clinically distinct, share many immunological and histological features. Susceptibility genes for autoimmune thyroid disease (AITD) have been investigated, although only the human leukocyte antigen and cytotoxic T lymphocyte-associated antigen-4 gene regions have been consistently associated with disease. Recent data, however, have shown linkage and association of chromosome 8q24 (containing the thyroglobulin gene) to AITD. Therefore, we performed a case-control association study on patients with AITD and controls using previously associated markers (D8S284 and Tgms2). No differences in allele frequencies were observed between AITD cases and controls for D8S284. Compared with the three common alleles (frequencies >10%), the rare alleles of Tgms2 were increased ($\chi^2=10.6$; $P=0.001$) at Tgms2. This group included the 336-bp allele (increased in cases vs. controls: $\chi^2=24.97$; $P<0.001$), which has previously been reported to be associated with AITD. The rarity of this allele in the United Kingdom, however, precluded analysis in our family dataset. Although these findings may represent a random chance event, in view of previous reports of linkage and association of this gene region to AITD, this may be an example of a rare causal variant of a complex disease.

Dalrymple, A., D. M. Slater, et al. (2004). "Physiological Induction of Transient Receptor Potential Canonical Proteins, Calcium Entry Channels, in Human Myometrium: Influence of Pregnancy, Labor, and Interleukin-1 β ." *J. Clin. Endocrinol. Metab.* **89**(3): 1291-1300.

<http://jcem.endojournals.org/cgi/content/abstract/89/3/1291>

This study investigated gestational regulation of transient receptor potential canonical (TrpC) proteins, putative calcium entry channels in human myometrium, and the potential modulation of TrpC expression by IL-1 β , a cytokine implicated in labor. Total RNA and proteins were isolated from myometrial biopsies obtained from NP women, pregnant women at term not in labor (TNL), or term active labor (TAL) and from primary cultured human myometrial smooth muscle cells incubated with IL-1 β or IL-1 β with or without nimesulide. Semiquantitative RT-PCR demonstrated significant up-regulation of TrpC1 in TAL and TNL ($P\leq 0.01$) and TrpC6 ($P\leq 0.01$) and TrpC7 ($P\leq 0.05$) in TAL samples. TrpC3 and TrpC4 mRNA expression was unaffected. Western blot demonstrated significant up-regulation of TrpC1 in TAL and TNL ($P\leq 0.05$) and TrpC3 ($P\leq 0.01$), TrpC4 ($P\leq 0.05$), and TrpC6 ($P\leq 0.01$) in TAL samples. IL-1 β did not alter TrpC1, 3, 4, 6, or 7 mRNA expression; but IL-1 β exclusively up-regulated TrpC3 protein expression ($P\leq 0.05$). TrpC3 up-regulation was unaffected by cyclooxygenase blockade. These data demonstrate physiological regulation of TrpC mRNA and protein and suggest an important role for TrpC proteins in human myometrium during labor.

de Sanctis, L., A. Corrias, et al. (2004). "Familial PAX8 Small Deletion (c.989_992delACCC) Associated with Extreme Phenotype Variability." *J. Clin. Endocrinol. Metab.* **89**(11): 5669-5674.

<http://jcem.endojournals.org/cgi/content/abstract/89/11/5669>

The PAX8 gene, mapped on 2q12-q14, encodes for a transcription factor involved in thyroid cell proliferation and differentiation. Five mutations in PAX8 have been so far described in both sporadic and rare familial forms of thyroid dysgenesis with proposed autosomal dominant inheritance, all associated with thyroid hypoplasia and/or dysfunction. Fifty-four subjects with congenital hypothyroidism detected during neonatal screening and associated with an ultrasound or scintiscan picture of thyroid dysgenesis were investigated for PAX8 mutations. The entire

PAX8 coding region with exon-intron boundaries was amplified from genomic DNA, and a mutational screening was performed by denaturing HPLC followed by direct sequencing when denaturing HPLC elution abnormalities appeared. A new heterozygous deletion (c.989_992delACCC) in exon 7 causing a frameshift with premature stop codon after codon 277 was identified in a subject with thyroid hypoplasia. This mutation is the only one so far identified that lies outside the paired domain. The predicted mutant protein completely lacks the C-terminal region but contains the paired box, octapeptide, and homeodomain. It retains the ability to bind a paired-domain sequence in vitro but is transcriptionally inactive. These results provide evidence that the C-terminal region is essential for transcriptional activity. The new mutation has been inherited from the completely euthyroid mother. It was also present in a brother with slightly elevated TSH only. Thus, it is associated with thyroid dysgenesis in the proband and both euthyroidism and compensated hypothyroidism in her family. This suggests that other factors/genes may modulate phenotypic expression.

Fukuyama, S., M. Hiramatsu, et al. (2004). "Novel Mutations of the Chloride Channel Kb Gene in Two Japanese Patients Clinically Diagnosed as Bartter Syndrome with Hypocalciuria." J. Clin. Endocrinol. Metab. **89**(11): 5847-5850.

<http://jcem.endojournals.org/cgi/content/abstract/89/11/5847>

Hypokalemic metabolic tubulopathy, such as in Bartter syndrome and Gitelman syndrome, is caused by the dysfunction of renal electrolyte transporters. Despite advances in molecular genetics with regard to hypokalemic metabolic tubulopathy, recent reports have suggested that the phenotype-genotype correlation is still confusing, especially in classic Bartter and Gitelman syndromes. We report here two Japanese patients who suffered from clinically diagnosed classic Bartter syndrome but who presented hypocalciuria. Hypocalciuria is generally believed to be a pathognomonic finding of NCCT malfunction. To better understand the genotype-phenotype correlation in these two cases, we screened four renal electrolyte transporter genes [Na-K-2Cl cotransporter (NKCC2), renal outer medullary K channel (ROMK), Cl channel Kb (ClC-Kb), and Na-Cl cotransporter (NCCT)] by the PCR direct sequencing method. We identified three ClC-Kb allelic variants, including two new mutations (L27R and W610X in patient 1 and a G to C substitution of a 3' splice site of intron 2 and W610X in patient 2). We did not find any mutations in the other three genes. Our present data suggest that some ClC-Kb mutations may affect calcium handling in renal tubular cells.

Georgopoulos, A., O. Aras, et al. (2002). "Unlike Type 2 Diabetes, Type 1 Does Not Interact with the Codon 54 Polymorphism of the Fatty Acid Binding Protein 2 Gene." J. Clin. Endocrinol. Metab. **87**(8): 3735-3739.

<http://jcem.endojournals.org/cgi/content/abstract/87/8/3735>

In type 2 diabetes, the threonine (Thr) for alanine (Ala) codon 54 polymorphism of the fatty acid binding protein 2 gene is associated with elevated fasting and postprandial triglycerides and dyslipidemia when compared with the wild type (Ala-54/Ala-54). To assess whether this is the case in patients with type 1 diabetes, who usually do not manifest the metabolic syndrome, we screened 181 patients with similar glycemic control as the type 2 patients. Thirty percent were heterozygous, and 9% were homozygous for the polymorphism. Mean ($\pm$ SEM) fasting plasma triglyceride levels in patients with the wild type (n = 84), those heterozygous for Ala-54/Thr-54 (n = 44), and those homozygous for the Thr-54 (n = 13) were 1.0 ± 0.07 , 1.1 ± 0.17 , and 1.2 ± 0.23 mmol/liter, respectively. In addition, there were no differences in total, low-density lipoprotein, high-density lipoprotein, and non-high density lipoprotein cholesterol among the three groups. After a fat load, the postprandial area under the curve of triglyceride in plasma,

chylomicrons, and very low-density lipoprotein were similar between the wild type (n = 18) and the Thr-54 homozygotes (n = 12). In conclusion, in contrast to type 2, type 1 diabetes does not interact with the codon 54 polymorphism of the fatty acid binding protein 2 gene to cause hypertriglyceridemia/dyslipidemia. Insulin resistance could account possibly for this difference.

Gertow, K., M. Bellanda, et al. (2004). "Genetic and Structural Evaluation of Fatty Acid Transport Protein-4 in Relation to Markers of the Insulin Resistance Syndrome." J. Clin. Endocrinol. Metab. **89**(1): 392-399.

<http://jcem.endojournals.org/cgi/content/abstract/89/1/392>

Disturbances in fatty acid metabolism are involved in the etiology of insulin resistance and the related dyslipidemia, hypertension, and procoagulant state. The fatty acid transport proteins (FATPs) are implicated in facilitated cellular uptake of nonesterified fatty acids (NEFAs), thus potentially regulating NEFA concentrations and metabolism. The aim of this study was to investigate polymorphic loci in the FATP4 gene with respect to associations with fasting and postprandial lipid and lipoprotein variables and markers of insulin resistance in 608 healthy, middle-aged Swedish men and to evaluate possible mechanisms behind any associations observed. Heterozygotes for a Gly209Ser polymorphism (Ser allele frequency 0.05) had significantly lower body mass index and, correcting for body mass index, significantly lower triglyceride concentrations, systolic blood pressure, insulin concentrations, and homeostasis model assessment index compared with common homozygotes. A three-dimensional model of the FATP4 protein based on structural and functional similarity with adenylate-forming enzymes revealed that the variable residue 209 is exposed in a region potentially involved in protein-protein interactions. Furthermore, the model indicated functional regions with respect to NEFA transport and acyl-coenzyme A synthase activity and membrane association. These findings propose FATP4 as a candidate gene for the insulin resistance syndrome and provide a structural basis for understanding FATP function in NEFA transport and metabolism.

Goswami, R., T. Mohapatra, et al. (2004). "Parathyroid Hormone Gene Polymorphism and Sporadic Idiopathic Hypoparathyroidism." J. Clin. Endocrinol. Metab. **89**(10): 4840-4845.

<http://jcem.endojournals.org/cgi/content/abstract/89/10/4840>

The pathogenetic mechanisms involved in the development of sporadic idiopathic hypoparathyroidism are currently under investigation. Although autoantibodies against the calcium-sensing receptor (CaSR) have been implicated to play a role, these could be demonstrated in only 49% of a group of 51 patients with sporadic idiopathic hypoparathyroidism that we previously studied. Therefore, we investigated 49 of these patients further, regardless of their antibody status, and looked for mutations in the section of the PTH gene sequence that coded for prepro-PTH as well as the 3'-untranslated region (3'-UTR) of the gene, which is believed to be involved in the stability of its mRNA. We also examined the relationship between the clinical manifestations of the disease and the occurrences of two commonly observed single nucleotide polymorphisms (SNPs) in the PTH gene. In 49 of the patients with idiopathic hypoparathyroidism and in 55 healthy controls, the SNPs were characterized by restriction analysis using DralI and BstBI enzymes. In a subset of these patients, exons 2 and 3 of the PTH gene (n = 37) and its 3'-UTR region (n = 40) were also sequenced. No mutations were observed in the segment of the PTH gene coding for the signal peptide, prohormone, or the 3'-UTR region. However, three well described SNPs were observed: 1) an A->G substitution in intron 1 in 35.1% of the patients; 2) a G->A substitution in intron 2, characterized by BstBI, in one or both alleles in 27%; and 3) a C->A substitution at codon 52 (CGA) of exon 3, characterized by DralI, in one or both alleles in 59.7% of the patients. There was no significant difference in the

frequency of occurrence of these SNPs between the patient and the control groups. Furthermore, the mean age at onset of symptoms, body mass index, frequency of cataract, tetany, convulsion, basal ganglia calcification, serum calcium, inorganic phosphorus, and intact PTH were not significantly different between patients with and without the above-described SNPs. Thus, the data from this report demonstrate that in patients with sporadic idiopathic hypoparathyroidism, neither the clinical manifestations nor the biochemical indexes of the disease are related to the occurrence of mutations or SNPs in the PTH gene. Because neither patient nor control samples exhibited any variations in the sequence of their 3'-UTR regions, it is unlikely that mRNA instability is a factor in the pathogenesis of the disease. Additional studies are required to investigate the role of other genes and autoantigens that may be involved in the genesis of idiopathic hypoparathyroidism.

Grundberg, E., T. Carling, et al. (2004). "A Deletion Polymorphism in the RIZ Gene, a Female Sex Steroid Hormone Receptor Coactivator, Exhibits Decreased Response to Estrogen in Vitro and Associates with Low Bone Mineral Density in Young Swedish Women." J. Clin. Endocrinol. Metab. **89**(12): 6173-6178.

<http://jcem.endojournals.org/cgi/content/abstract/89/12/6173>

Low bone mineral density (BMD) is a major risk factor for osteoporotic fracture, and the trait is under genetic control by a large number of genes. It is recognized that estrogen plays an important role in the maintenance of bone mass by binding to estrogen receptor {alpha} (ER{alpha}). RIZ1 has previously been shown to be a specific ER{alpha} coactivator and strongly enhances its function both in vivo and in vitro. We performed in vitro studies comparing the abilities of RIZ1 P704 polymorphic variants (homozygous presence, P704+; absence, P704-; heterozygosity P704+/- of a proline at position 704) to coactivate the ER{alpha} and also examined the polymorphism associated to BMD of 343 Swedish women, aged 20-39 yr. The expression vector containing P704- RIZ1 showed an impaired response in coactivating ER{alpha} in a ligand- and dose-dependent manner compared with P704+ RIZ (P < 0.0001). The genotype frequencies were 19% (P704+), 32% (P704-), and 49% (P704+/-) and were in Hardy-Weinberg equilibrium. BMD at the heel was higher in the P704+ genotype group than in the P704+/- group (P = 0.02), which was evident also after corrections for fat and lean mass (P = 0.03). We conclude that RIZ1 may be a new candidate gene for involvement in the variation seen in BMD.

Guettier, J.-M., A. Georgopoulos, et al. (2005). "Polymorphisms in the Fatty Acid-Binding Protein 2 and Apolipoprotein C-III Genes Are Associated with the Metabolic Syndrome and Dyslipidemia in a South Indian Population." J. Clin. Endocrinol. Metab. **90**(3): 1705-1711.

<http://jcem.endojournals.org/cgi/content/abstract/90/3/1705>

The Chennai Urban Population Study investigates a South Indian population with a high prevalence of cardiovascular disease associated with the metabolic syndrome (MS). The Ala54Thr polymorphism in the fatty acid-binding protein 2 (FABP2) gene as well as the T-455C and C-482T polymorphisms in the apolipoprotein C-III (APOC3) gene promoter have been associated with features of the MS in specific populations. This study evaluates in Asian-Indians the association between these polymorphisms with MS and dyslipidemia, defined according to National Cholesterol Education Program Adult Treatment Panel III. Allelic frequencies in 70 controls and 110 patients with diabetes from the Chennai Urban Population Study were 52.9% for FABP2 Thr54, 73.0% for APOC3 -482T, and 80.2% for APOC3 -455C. The polymorphisms were in agreement with Hardy-Weinberg equilibrium. Controls carrying FABP2 Thr54 were more likely to have MS than noncarriers (Fisher's exact test P = 0.031; odds ratio = 6.9 with a 95% confidence interval of 1.1, 43.9). Those carrying at least one polymorphic allele in both genes had

a higher likelihood of having MS than wild type (Fisher's exact test $P = 0.003$; odds ratio = 12.1 with a 95% confidence interval of 1.88, 77.6). Dyslipidemia was associated with the polymorphism as well. The polymorphisms were not associated with MS in patients with diabetes. The association of the polymorphisms with MS and dyslipidemia could contribute to the high cardiovascular disease prevalence in this population.

Gylling, M., E. Kaariainen, et al. (2003). "The Hypoparathyroidism of Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy Protective Effect of Male Sex." J. Clin. Endocrinol. Metab. **88**(10): 4602-4608.

<http://jcem.endojournals.org/cgi/content/abstract/88/10/4602>

In autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, hypoparathyroidism (HP) is the most common endocrine component. It occurs in most (but not all) patients. Determinants of its occurrence are unknown, and there is no proof for its autoimmune nature. Recently, the Ca^{2+} -sensing receptor (CaSR) was reported to be an autoantigen in HP. With our group of 90 patients, we aimed at identifying the determinants and pathomechanism of HP. For the determinants, we evaluated gender and the HLA class II. For the pathomechanism, we searched for parathyroid autoantibodies, including antibodies against CaSR and PTH. Also, we studied whether AIRE is expressed in the human parathyroid, because its absence could be a pathogenetic factor. We found a clear gender linkage with lower and later incidence in males. Of the 14 patients who had escaped HP, 13 were males. This was associated with adrenal failure, which was the first or only endocrinopathy in 47% of males vs. 7% of females. In contrast, we found no linkage to the HLA class II. By immunofluorescence, 19% of the patients had antibodies to parathyroid epithelia. By immunoblotting, these recognized several parathyroid proteins. No antibodies were observed against the CaSR or PTH. By RT-PCR, AIRE mRNA was not found in the parathyroid.

Halmos, G., A. V. Schally, et al. (2002). "Expression of Growth Hormone-Releasing Hormone and Its Receptor Splice Variants in Human Prostate Cancer." J. Clin. Endocrinol. Metab. **87**(10): 4707-4714.

<http://jcem.endojournals.org/cgi/content/abstract/87/10/4707>

Antagonists of GHRH inhibit the growth of various human tumors, including prostate cancer, but the tumoral receptors mediating the antiproliferative effect of GHRH antagonists have not been clearly identified. Recently, we demonstrated that human cancer cell lines express splice variants (SVs) of receptors for GHRH, of which SV1 exhibits the greatest similarity to the pituitary GHRH receptors. In this study we investigated the expression of GHRH and SVs of GHRH receptor and the binding characteristics of the GHRH receptor isoform in 20 surgical specimens of organ-confined and locally advanced human prostatic adenocarcinomas. The mRNA expression of GHRH and SVs of GHRH receptor was investigated by RT-PCR. The affinity and density of receptors for GHRH were determined by ligand competition assays based on binding of ^{125}I -labeled GHRH antagonist JV-1-42 to tumor membranes. Twelve of 20 tumors (60%) exhibited specific, high affinity binding for JV-1-42, with a mean dissociation constant (Kd) of 0.81 nmol/liter and a mean maximal binding capacity of 185.2 fmol/mg membrane protein. The mRNA of SV1 was detected in 13 of 20 (65%) prostate cancer specimens and was consistent with the presence of GHRH binding. RT-PCR analyses also revealed the expression of mRNA for GHRH in 13 of 15 (86%) prostatic carcinoma specimens examined. The presence of GHRH and its tumoral receptor SVs in prostate cancers suggests the possible existence of an autocrine mitogenic loop. The antitumor effects of GHRH antagonists in prostate cancer could be exerted in part by interference with this local GHRH system.

Hansen, S. K., E.-M. D. Nielsen, et al. (2005). "Analysis of separate and combined effects of common variation in KCNJ11 and PPARG on risk of type 2 diabetes." *J. Clin. Endocrinol. Metab.*: jc.2004-1942.

<http://jcem.endojournals.org/cgi/content/abstract/jc.2004-1942v1>

The separate and combined effects of the PPARG Pro12Ala and the KCNJ11 Glu23Lys polymorphisms on risk of type 2 diabetes were investigated in relatively large-scale case-control studies. Separate effects of the variants were examined among 1187/1461 type 2 diabetic patients and 4791/4986 middle-aged glucose-tolerant subjects. The combined analysis involved 1164 type 2 diabetic patients and 4733 middle-aged glucose-tolerant subjects. In the separate analyses, the K-allele of the KCNJ11 Glu23Lys associated with type 2 diabetes (OR: 1.19, $P = 0.0002$), whereas the PPARG Pro12Ala showed no significant association with type 2 diabetes. The combined analysis indicated that the two polymorphisms acted in an additive manner to increase the risk of type 2 diabetes and we found no evidence for a synergistic interaction between them. Analysis of a model with equal additive effects of the two variants showed that the OR for type 2 diabetes increased with 1.14 per risk allele ($P = 0.003$). Together, the two polymorphisms conferred a population attributable risk for type 2 diabetes of 28%. In conclusion, our results showed no evidence of a synergistic interaction between the KCNJ11 Glu23Lys and PPARG Pro12Ala polymorphisms but indicated that they may act in an additive manner to increase the risk of type 2 diabetes.

Haugen, B. R., L. L. Larson, et al. (2004). "Retinoic Acid and Retinoid X Receptors Are Differentially Expressed in Thyroid Cancer and Thyroid Carcinoma Cell Lines and Predict Response to Treatment with Retinoids." *J. Clin. Endocrinol. Metab.* **89**(1): 272-280.

<http://jcem.endojournals.org/cgi/content/abstract/89/1/272>

Therapy for patients with advanced thyroid carcinoma is limited. Clinical and in vitro studies suggest that some patients with advanced thyroid cancer may respond to therapy with retinoic acid. mRNA expression of the six retinoic acid (RAR) and retinoid X receptor (RXR) isoforms (RAR $\{\alpha\}$, - $\{\beta\}$, - $\{\gamma\}$ and RXR $\{\alpha\}$, - $\{\beta\}$, - $\{\gamma\}$) was measured in four human thyroid cell lines, and protein expression was subsequently measured in 10 thyroid cancer cell lines. Two isoforms, RAR $\{\beta\}$ and RXR $\{\gamma\}$, were differentially expressed in the four cell lines. Comparison of 10 thyroid tumors and matched normal thyroid tissue confirmed differential tumor expression of RAR $\{\beta\}$ and RXR $\{\gamma\}$ and lack of the RXR $\{\gamma\}$ isoform in normal thyroid tissue. Cell lines expressing both RAR $\{\beta\}$ and RXR $\{\gamma\}$ demonstrated significant growth suppression when treated with retinoids, whereas cell lines lacking these isoforms were unaffected. Expression of RAR $\{\beta\}$, the isoform associated with suppression of tumor growth in other cancer types, was not affected by treatment with retinoids in the thyroid cancer cell lines. LG346 increased apoptosis and decreased cells in the S-phase in an anaplastic carcinoma cell line, suggesting that this retinoid causes growth suppression of these cells by multiple mechanisms. In summary, we identified the RAR $\{\beta\}$ and RXR $\{\gamma\}$ isoform to be differentially expressed in thyroid cancer cell lines and tumor tissue. These isoforms seem to predict response to retinoid therapy in thyroid cancer cell lines.

Helliwell, R. J. A., L. W. Chamley, et al. (2004). "Characterization of the Endocannabinoid System in Early Human Pregnancy." *J. Clin. Endocrinol. Metab.* **89**(10): 5168-5174.

<http://jcem.endojournals.org/cgi/content/abstract/89/10/5168>

In recent years, it has been demonstrated that high circulating levels of the endogenous cannabinoid anandamide, resulting from low expression of its metabolizing enzyme fatty acid amide hydrolase (FAAH), may contribute to spontaneous miscarriage and poor outcome in women undergoing in vitro fertilization. The site of action of this compound, however, has not been determined. In this study, we examined the distribution of the cannabinoid receptors, CB1 and CB2, and the endocannabinoid-metabolizing enzyme FAAH in first trimester human placenta. Here, we show that FAAH is expressed throughout the human first trimester placenta, in extravillous trophoblast columns, villous cytotrophoblasts, syncytiotrophoblasts, and macrophages. Furthermore, FAAH mRNA levels appear to be regulated during gestation, with levels peaking at 11 wk before declining again. The immune system-associated cannabinoid CB2 receptors were localized only to placental macrophages. Interestingly, the cannabinoid receptor CB1 was not identified in first trimester placenta despite having previously been shown to be present in placental tissues at term. These findings suggest that the placenta may form a barrier preventing maternal-fetal transfer of anandamide and/or modulate local levels of anandamide by regulation of FAAH expression with gestation.

Hiromatsu, Y., T. Fukutani, et al. (2005). "Interleukin-13 Gene Polymorphisms Confer the Susceptibility of Japanese Populations to Graves' Disease." J. Clin. Endocrinol. Metab. **90**(1): 296-301.

<http://jcem.endojournals.org/cgi/content/abstract/90/1/296>

Graves' disease (GD) is an autoimmune disorder with genetic predisposition. IL-13 is an important mediator of antiinflammatory immune responses and is expressed in the thyroid and orbit. The aim of the present study was to investigate whether IL-13 gene polymorphisms are associated with the development of GD. IL-13 gene polymorphisms were studied in Japanese GD patients (n = 310) and healthy control subjects without antithyroid autoantibodies or a family history of autoimmune disorders (n = 244). A C/T polymorphism at position -1112 of the promoter region was measured using the direct sequencing method, and an Arg130Gln (G2044A) polymorphism in exon 4 was examined using the PCR-restriction fragment length polymorphism method. There was a significant decrease in -1112T allele frequency in GD patients compared with controls (16% vs. 23%; P = 0.0019). The frequency of the 2044A allele on exon 4 also appeared lower in GD patients compared with controls. Haplotype analysis showed a significant decrease in the -1112T/2044A haplotype in GD patients. There was no association between IL-13 gene polymorphisms and ophthalmopathy, severity, or serum IgE levels. In conclusion, IL-13 gene polymorphisms are associated with GD susceptibility in Japan.

Huang, S. C., J. Torres-Cruz, et al. (2003). "Amplification and Overexpression of Mutant RET in Multiple Endocrine Neoplasia Type 2-Associated Medullary Thyroid Carcinoma." J. Clin. Endocrinol. Metab. **88**(1): 459-463.

<http://jcem.endojournals.org/cgi/content/abstract/88/1/459>

We have previously identified two second hit mechanisms involved in the development of multiple endocrine neoplasia type 2 (MEN 2)-associated tumors: trisomy 10 with duplication of the mutant RET allele and loss of the wild-type RET allele. However, some of the MEN 2-associated tumors investigated did not demonstrate either mechanism. Here, we studied the TT cell line derived from MEN 2-associated medullary thyroid carcinoma with a RET germline mutation in codon 634, for alternative mechanisms of tumorigenesis. Although we observed a 2:1 ratio between mutant and wild-type RET at the genomic DNA level in this cell line, fluorescence in situ hybridization

analysis revealed neither trisomy 10 nor loss of the normal chromosome 10. Instead, a tandem duplication event was responsible for amplification of mutant RET. In further studies we could for the first time demonstrate that the genomic chromosome 10 abnormalities in this cell line cause an increased production of mutant RET mRNA. These findings provide evidence for a third second hit mechanism resulting in overrepresentation and overexpression of mutant RET in MEN 2-associated tumors.

Hung, C. J., D. G. Ginzinger, et al. (2003). "Expression of Vascular Endothelial Growth Factor-C in Benign and Malignant Thyroid Tumors." J. Clin. Endocrinol. Metab. **88**(8): 3694-3699.

<http://jcem.endojournals.org/cgi/content/abstract/88/8/3694>

In contrast to vascular endothelial growth factor (VEGF), which stimulates angiogenesis, VEGF-C is thought to stimulate lymphangiogenesis. The role of VEGF-C in thyroid cancer pathogenesis has not been clarified. One might expect a different pattern of VEGF-C expression in the various types of thyroid cancer because of their different means of metastases. In this investigation, we determined whether the differential expression of VEGF-C might explain the different propensity to lymph node metastasis in thyroid cancers. One hundred eleven normal and neoplastic thyroid tissues were analyzed by real-time quantitative PCR. Papillary thyroid cancers had a higher VEGF-C expression than other thyroid malignancies ($P < 0.0005$ ANOVA). Among the normal thyroid tissues from patients with malignant or benign thyroid diseases, there was no significant difference in VEGF-C expression. Paired comparison of VEGF-C expression between thyroid cancers and normal thyroid tissues from the same patients showed a significant increase of VEGF-C expression in papillary thyroid cancer (1.10 ± 0.41 vs. 0.70 ± 0.13 ; $P = 0.001$) and a significant decrease of VEGF-C expression in medullary thyroid cancer (0.11 ± 0.13 vs. 0.78 ± 0.29 ; $P = 0.001$). In contrast, there was no significant difference of VEGF-C expression between cancer and normal tissues in other types of thyroid cancer. In summary, VEGF-C expression is increased in papillary thyroid cancer, compared with paired normal thyroid tissues, but not in other thyroid cancers that are also prone to lymph node metastasis. The lymphangiogenic role of VEGF-C in thyroid cancers therefore appears to be complex and other factors are likely to be also involved.

Hwang, J. H., J. H. Hwang, et al. (2003). "CXC Chemokine Receptor 4 Expression and Function in Human Anaplastic Thyroid Cancer Cells." J. Clin. Endocrinol. Metab. **88**(1): 408-416.

<http://jcem.endojournals.org/cgi/content/abstract/88/1/408>

Anaplastic thyroid carcinomas (ATCs) are highly aggressive, extremely lethal human cancers with poor therapeutic response. Chemokines are a superfamily of small cytokine-like proteins that induce, through their interaction with G protein-coupled receptors, cytoskeletal rearrangement, firm adhesion to endothelial cells, and directional migration. In this study, we characterized the expression of CXC chemokine receptor 4 (CXCR4) and analyzed its functions in ARO cells, a human ATC cell. The normal primary cultured thyroid cells and ATC cell lines expressed CXCR4 and stromal cell-derived factor (SDF)-1 α transcripts, detected by RT-PCR. Fluorescence activated cell sorting analysis of CXCR4 expression in normal and ATC cells showed that ARO cells expressed significant levels of CXCR4. FRO, NPA, and normal thyroid cells did not express membrane CXCR4, as determined by fluorescence activated cell sorting analysis. To identify the functional role of CXCR4 in ARO cells, we treated ARO cells with SDF-1 α and analyzed the signaling pathways, cellular migration, and proliferation. SDF-1 α enhanced the migration but did not affect the proliferation of ARO cells or activate the Janus kinase/signal transducer and activator of transcription signaling pathways. However, SDF-1 α /CXCR4 activation resulted in phosphorylation of the p70S6 kinase and its target protein, ribosomal S6 protein, and also

activation of the ERK1/ERK2 signaling pathways. Furthermore, SDF-1 α /CXCR4-mediated activation of the p70S6 kinase and phosphorylation of the S6 protein were inhibited by treatment with an mTOR/FRAP inhibitor. The specificity of the CXCR4-mediated migration of ARO cells was demonstrated by the dose-dependent inhibition of migration by neutralizing anti-CXCR4. The ATC cells, FRO and NPA, which do not express CXCR4, did not demonstrate significant SDF-1 α -mediated migration in vitro. In addition, the CXCR4-mediated migration of ARO cells was inhibited by treatment with pertussis toxin (a Gi-protein inhibitor) and PD 98059 (a mitogen-activated ERK kinase inhibitor) but not by LY294002 and wortmanin, phosphatidylinositol 3-kinase inhibitors. These findings suggest that a subset of ATC cells expresses functional CXCR4, which may be important in tumor cell migration and local tumor invasion.

Jin, Y., W. Teng, et al. (2003). "Genome-Wide Scan of Graves' Disease: Evidence for Linkage on Chromosome 5q31 in Chinese Han Pedigrees." J. Clin. Endocrinol. Metab. **88**(4): 1798-1803.

<http://jcem.endojournals.org/cgi/content/abstract/88/4/1798>

Graves' disease (GD), which is a common organ-specific autoimmune disorder, is multifactorial and develops in genetically susceptible individuals. Despite many studies of candidate genes, only associations with human leukocyte antigen and cytotoxic T lymphocyte antigen 4 have been generally detected, and the number of susceptibility genes remains unknown. To identify chromosomal regions contributing to GD, we conducted a genome-wide scan on 322 individuals from 54 Chinese Han multiplex GD pedigrees. Parametric linkage analysis revealed the strongest evidence for linkage at D5S436 on chromosome 5q31, with a maximum two-point LOD score of 2.8 and a maximum multipoint LOD score of 2.3. To further assess the significance of this suggestive finding, we typed four additional markers around D5S436 in this chromosome region, and a maximum two-point LOD score of 4.31 and a maximum multipoint LOD score of 4.12 were obtained for marker D5S2090 (with heterogeneity, $[IMG]f1.gif" BORDER="0"> = 0.38$). Nonparametric multipoint analysis also showed significant excess allele sharing, with a P value as low as 0.001, at the same locus. Our findings provide evidence for a susceptibility locus for GD on chromosome 5q31 and support the existence of genetic heterogeneity in GD.

Johannsen, T. H., D. Mallet, et al. (2005). "Delayed Diagnosis of Congenital Adrenal Hyperplasia with Salt Wasting Due to Type II 3 β -Hydroxysteroid Dehydrogenase Deficiency." J. Clin. Endocrinol. Metab. **90**(4): 2076-2080.

<http://jcem.endojournals.org/cgi/content/abstract/90/4/2076>

Classical 3 β -hydroxysteroid dehydrogenase (3 β -HSD) deficiency is a rare cause of congenital adrenal hyperplasia. We report two sisters presenting with delayed diagnoses of classical 3 β -HSD, despite salt wasting (SW) episodes in infancy. Sibling 1 was referred for premature pubarche, slight growth acceleration, and advanced bone age, whereas sibling 2 had no signs of virilization. At referral, increased 17 α -hydroxyprogesterone associated with premature pubarche at first suggested a nonclassical 21-hydroxylase deficiency. Sequencing of the CYP21 gene showed both girls only heterozygotes (V281L mutation). This result, combined with SW in infancy, suggested a 3 β -HSD deficiency because of increased dehydroepiandrosterone sulfate levels. Further hormonal studies showed markedly elevated Δ^5 -steroids, in particular 17 α -hydroxypregnenolone greater than 100 nmol/liter (the clue to the diagnosis) and elevated Δ^5 -/ Δ^4 -steroid ratios. Sequencing of the type II 3 β -HSD gene documented that both girls were compound heterozygotes for T181I and 1105delA mutations. Retrospectively, elevated levels of 17 α -hydroxyprogesterone were found on blood spots from Guthrie's test. There is no previous report of the combination of SW and premature pubarche due to mutations in the type II 3 β -HSD gene. Because neonatal

diagnosis could have prevented life-threatening crises in these girls, this report further supports the benefits for neonatal screening for congenital adrenal hyperplasia whatever the etiology.

Kannisto, K., K. H. Pietilainen, et al. (2004). "Overexpression of 11 β -Hydroxysteroid Dehydrogenase-1 in Adipose Tissue Is Associated with Acquired Obesity and Features of Insulin Resistance: Studies in Young Adult Monozygotic Twins." *J. Clin. Endocrinol. Metab.* **89**(9): 4414-4421.

<http://jcem.endojournals.org/cgi/content/abstract/89/9/4414>

11 β -Hydroxysteroid dehydrogenase type 1 (11 β -HSD-1) catalyzes the interconversion of inactive cortisone to active cortisol. Overexpression of 11 β -HSD-1 in murine adipose tissue results in glucocorticoid receptor (GR) α overexpression, central obesity, and insulin resistance. It is controversial whether 11 β -HSD-1 or GR α expression are increased in human adipose tissue in obesity. We studied effects of acquired obesity on 11 β -HSD-1 gene (real-time PCR) and protein (Western blotting) expression in sc adipose tissue in 17 monozygotic twin pairs aged 24-27 yr with a mean intrapair difference in body mass index (BMI) of 3.8 kg/m² (range 0.4-10.1 kg/m²). Intrapair correlations were calculated to study effects of acquired obesity on 11 β -HSD-1 expression. Western blot analysis of adipose tissue homogenates identified approximately 50- and approximately 68-kDa proteins specific for 11 β -HSD-1. Both structural forms correlated positively with 11 β -HSD-1 mRNA concentrations. Intrapair differences in 11 β -HSD-1 mRNA, and the 50- and 68-kDa proteins in sc adipose tissue correlated positively with those in BMI (kilograms per square meter) ($r = 0.78$ for 11 β -HSD-1 mRNA, $P = 0.0002$; $r = 0.87$ for the 11 β -HSD-1 50-kDa protein, $P = 0.0003$; and $r = 0.62$ for the 11 β -HSD-1 68-kDa protein, $P = 0.033$), total body fat (percent) ($r = 0.65$, $P = 0.005$; $r = 0.83$, $P = 0.001$; and $r = 0.69$, $P = 0.013$, respectively) and sc fat (cubed centimeters) ($r = 0.66$, $P = 0.004$; $r = 0.94$, $P = 0.0001$; and $r = 0.71$, $P = 0.009$, respectively). Furthermore, 11 β -HSD-1 mRNA and 50-kDa protein expression, but not 68-kDa protein expression, correlated positively with intrapair differences in intraabdominal fat mass (cubed centimeters) ($r = 0.62$, $P = 0.008$; $r = 0.69$, $P = 0.013$; $r = 0.48$, $P = 0.112$) and serum fasting insulin concentration (milliunits per liter) ($r = 0.76$, $P = 0.0004$; $r = 0.60$, $P = 0.037$; and $r = 0.43$, $P = 0.160$, respectively). Intrapair differences in GR α expression were significantly inversely correlated with those in BMI and total and sc fat mass. In conclusion, expression of 11 β -HSD-1 in sc adipose tissue is increased in human acquired obesity and is closely related to accumulation of sc and intraabdominal fat and features of insulin resistance.

Kebebew, E., M. Peng, et al. (2004). "Id1 Gene Expression Is Up-Regulated in Hyperplastic and Neoplastic Thyroid Tissue and Regulates Growth and Differentiation in Thyroid Cancer Cells." *J. Clin. Endocrinol. Metab.* **89**(12): 6105-6111.

<http://jcem.endojournals.org/cgi/content/abstract/89/12/6105>

The Id (inhibitor of DNA binding) proteins are a family of helix-loop-helix (HLH) proteins (Id1, Id2, Id3, and Id4) that lack the basic domain necessary for DNA binding. The Id1 protein enhances cell proliferation and inhibits cellular differentiation in a variety of cell types. We have previously demonstrated that the Id1 gene is up-regulated in papillary and medullary thyroid cancers. In this study we characterized the expression and distribution of the Id1 protein in normal, hyperplastic, and neoplastic human thyroid tissue. We also evaluated the effect of the Id1 gene on thyroid cancer cell growth and markers of thyroid cell differentiation. We used semiquantitative immunohistochemistry to characterize Id1 protein expression in normal, hyperplastic (multinodular goiter and Graves' disease), and neoplastic thyroid tissue from 103 patients. Normal thyroid tissue had the lowest level of Id1 protein expression ($P < 0.0001$). Anaplastic thyroid cancer had the highest level (vs. benign and malignant thyroid tissues, $P < 0.01$). Id1

protein expression was higher in malignant thyroid tissue than in hyperplastic thyroid tissue ($P < 0.02$). We found no significant association between the level of Id1 protein expression and patient age, sex, tumor-node-metastasis stage, tumor size, primary tumor vs. lymph node metastasis, primary tumor vs. recurrent tumors, and extent of tumor differentiation. Inhibiting Id1 mRNA expression in thyroid cancer cell lines using Id1 antisense oligonucleotides resulted in growth inhibition ($P < 0.03$) and decreased thyroglobulin and sodium-iodine symporter mRNA expression ($P < 0.02$). In conclusion, Id1 is overexpressed in hyperplastic and neoplastic thyroid tissue and directly regulates the growth of thyroid cancer cells of follicular cell origin, but is not a marker of aggressive phenotype in differentiated thyroid cancer.

Kitanaka, S., Y. Miki, et al. (2004). "Promoter-Specific Repression of Hepatocyte Nuclear Factor (HNF)-1{beta} and HNF-1{alpha} Transcriptional Activity by an HNF-1{beta} Missense Mutant Associated with Type 5 Maturity-Onset Diabetes of the Young with Hepatic and Biliary Manifestations." *J. Clin. Endocrinol. Metab.* **89**(3): 1369-1378.

<http://jcem.endojournals.org/cgi/content/abstract/89/3/1369>

Mutations in the hepatocyte nuclear factor (HNF)-1{beta} lead to type 5 maturity-onset diabetes of the young (MODY5). HNF-1{beta} forms a homodimer or a heterodimer with HNF-1{alpha} and regulates various target genes. HNF-1{beta} mutations are rare, and no functional analysis has been performed in conjunction with HNF-1{alpha}. HNF-1{beta} is expressed in the liver and biliary system and controls liver-specific and bile acid-related genes. Moreover, liver-specific Hnf-1{beta} knockout mice present with severe jaundice. However, no patients with HNF-1{beta} mutations have biliary manifestations. In this report, we found a novel missense mutation in the HNF-1{beta} gene in a patient with neonatal cholestasis and liver dysfunction together with the common features of MODY5. Functional analysis revealed that the mutant HNF-1{beta} had diminished transcriptional activity by loss of the DNA binding activity. The mutant had a promoter-specific dominant-negative transcriptional effect on wild-type HNF-1{beta} and inhibited its DNA binding. Moreover, the mutant had a promoter- and cell-specific transcriptional repressive effect on HNF-1{alpha} and a promoter-specific inhibitory effect on HNF-1{alpha} DNA binding. From these results, we considered that the different phenotype of patients with HNF-1{beta} mutations might be caused by the different HNF-1{beta} activity in conjunction with the different repression of HNF-1{alpha} activity in selected promoters and tissues.

Kumar, S. and R. S. Bahn (2003). "Relative Overexpression of Macrophage-Derived Cytokines in Orbital Adipose Tissue from Patients with Graves' Ophthalmopathy." *J. Clin. Endocrinol. Metab.* **88**(9): 4246-4250.

<http://jcem.endojournals.org/cgi/content/abstract/88/9/4246>

Graves' ophthalmopathy (GO) is an autoimmune disorder involving the adipose and connective tissues of the orbit. The study of cytokines present in these tissues may reveal the nature of the cells and immune responses involved in GO pathogenesis. In the current study, we performed relative quantification of the expression of cytokine genes in orbital adipose tissue from patients with GO ($n = 6$) and normal individuals ($n = 2$). Real-time RT-PCR was performed using fluorescent probes and primers for cytokines including IL-1{beta}, IL-2, IL-4, IL-5, IL-8, IL-10, IFN-{\gamma}, and TNF-{\alpha}. Results showed IL-1{beta} to be the gene having the greatest fold expression increase over normal in four of six patients. TNF-{\alpha} was increased in all six GO patients. In addition, IL-8, IL-10, and IFN-{\gamma} were increased in five of six GO patients. We found no evidence of either IL-4 or IL-5 expression in any of the GO or normal samples. The increased expression of the macrophage-derived cytokines IL-1{beta}, TNF-{\alpha}, and IL-10 suggests the presence of macrophage activation and ongoing antigen presentation within the

orbit in GO. In addition, the overexpression of IFN- γ , without evidence of IL-4 or IL-5 expression, supports the concept that cell-mediated, rather than humoral, immunity plays the predominant role in pathogenesis of this disorder.

Kumar, S., M. J. Coenen, et al. (2004). "Evidence for Enhanced Adipogenesis in the Orbits of Patients with Graves' Ophthalmopathy." J. Clin. Endocrinol. Metab. **89**(2): 930-935.

<http://jcem.endojournals.org/cgi/content/abstract/89/2/930>

The signs and symptoms of Graves' ophthalmopathy (GO) result from increased volume of the orbital contents, including adipose, connective, and extraocular muscle tissues. We wanted to determine whether the expanded adipose tissue volume might be in part attributable to de novo adipogenesis. We measured levels of mRNA encoding leptin, adiponectin, peroxisome proliferator-activated receptor γ (PPAR γ), preadipocyte factor-1, and TSH receptor (TSHr) genes in orbital adipose tissues from GO patients (n = 22) and normal individuals (n = 18) and in orbital preadipocyte cultures derived from GO patients (n = 6) and normal subjects (n = 3) using quantitative real-time RT PCR. We found increased leptin, adiponectin, PPAR γ , and TSHr expression in GO compared with normal orbital tissue samples, with positive correlations in the GO tissues between TSHr and leptin, adiponectin and PPAR γ . In vitro differentiation of GO and normal preadipocytes resulted in enhanced adiponectin, leptin, and TSHr expression, with greater expression of the latter two genes in the GO cultures. These results suggest that de novo adipogenesis within orbital tissues with parallel enhanced expression of TSHr may be important in the pathogenesis of GO, and that potential therapies for GO might include inhibition of the adipogenic pathway.

Kung, A. W. C., K. S. Lau, et al. (2004). "Association of Novel Single Nucleotide Polymorphisms in the Calcium Channel α_1 Subunit Gene (Cav1.1) and Thyrotoxic Periodic Paralysis." J. Clin. Endocrinol. Metab. **89**(3): 1340-1345.

<http://jcem.endojournals.org/cgi/content/abstract/89/3/1340>

Thyrotoxic (hypokalemic) periodic paralysis (TPP) is a frequent complication of thyrotoxicosis among Chinese men. To determine the genetic association of TPP, we studied 97 male TPP patients, 77 Graves' disease patients without TPP, and 100 normal male subjects. Mutations of the voltage-dependent calcium channel (Cav1.1), sodium channel (Nav1.4), and potassium channel (Kv3.4), and association of the microsatellite markers on chromosome 1 in the region of the Na/K-ATPase subunits α_1 , α_2 , and β_1 were studied. None of the TPP patients carried the known mutations in Cav1.1, Nav1.4, and Kv3.4 genes. There was no association of TPP with the microsatellite markers that mapped to 1p13, 1q21-23, and 1q22-25. We detected 12 single nucleotide polymorphisms (SNPs) in Cav1.1 in our population, of which three were novel. Significant differences in the SNP genotype distribution between TPP compared with Graves' disease controls and normal controls were seen at the 5' flanking region nucleotide (nt) -476 (P = 0.02), intron 2 nt 57 (P < 0.01), and intron 26 nt 67 (P < 0.001). Because these SNPs lie at or near the thyroid hormone responsive element, it is possible that they may affect the binding affinity of the thyroid hormone responsive element and modulate the stimulation of thyroid hormone on the Cav1.1 gene.

Larsen, L. H., S. M. Echwald, et al. (2005). "Prevalence of Mutations and Functional Analyses of Melanocortin 4 Receptor Variants Identified among 750 Men with Juvenile-Onset Obesity." J.

Clin. Endocrinol. Metab. **90**(1): 219-224.

<http://jcem.endojournals.org/cgi/content/abstract/90/1/219>

Mutations in the gene encoding the melanocortin 4 receptor (MC4R) are associated with the most common monogenic form of obesity. We examined 750 Danish men with juvenile-onset obesity (body mass index 33.3 ± 2.4 kg/m²) and 706 control subjects (body mass index 21.4 ± 2.1 kg/m²) for mutations in MC4R. A total of 14 different mutations were identified of which two, Ala219Val and Leu325Phe, were novel variants. The variant receptor, Leu325Phe, was unable to bind [Nle⁴,D-Phe⁷]- α -MSH, whereas the Ala219Val variant showed a significantly impaired melanotan II induction of cAMP, compared with the wild-type receptor. The remaining 11 mutations have previously been reported, but selected MC4R variants were further characterized in vitro in the present study. A previously identified nonsense mutation, Tyr35stop, had a relatively high allele frequency (0.6%), suggesting a possible founder effect in the Danish population. This study shows a carrier frequency of 2.5% of pathogenic mutations in the MC4R gene in a population-based study of obese men. Thus, variation in this gene is the most common known specific genetic cause of obesity among Scandinavian men.

Liu, W., S. G. Thomas, et al. (2003). "Myostatin Is a Skeletal Muscle Target of Growth Hormone Anabolic Action." J. Clin. Endocrinol. Metab. **88**(11): 5490-5496.

<http://jcem.endojournals.org/cgi/content/abstract/88/11/5490>

Myostatin is a cytokine that has recently been shown to selectively and potently inhibit myogenesis. To investigate the mechanisms of anabolic actions of GH on skeletal muscle growth, we examined the in vitro and in vivo effects of GH on myostatin regulation. Twelve GH-deficient hypopituitary adult subjects were treated with recombinant GH (5 μ g/kg $\cdot$ d) in a double-blind, placebo-controlled fashion. Body composition and physical function were assessed and skeletal muscle biopsies from the vastus lateralis performed at 6-monthly intervals during 18 months of treatment. Myostatin mRNA expression was significantly inhibited to $31 \pm 9\%$ ($P < 0.001$) of control by GH but not by placebo administration ($79 \pm 11\%$) as determined by quantitative real-time PCR normalized for the housekeeping glyceraldehyde-3-phosphate dehydrogenase gene. The inhibitory effect of GH on myostatin was sustained after 12 and 18 months of GH treatment. These effects were associated with increases in lean body mass and translated into enhanced aerobic performance as determined by maximal oxygen uptake and ventilation threshold. Parallel in vitro studies of skeletal muscle cells demonstrated significant reduction of myostatin expression by myotubes in response to GH, compared with vehicle treatment. Conversely, GH receptor antagonism resulted in up-regulation of myostatin in myoblasts. Given the potent catabolic actions of myostatin, our data suggest that myostatin represents a potential key target for GH-induced anabolism.

Lockwood, C. J., P. Kumar, et al. (2004). "Effects of Thrombin, Hypoxia, and Steroids on Interleukin-8 Expression in Decidualized Human Endometrial Stromal Cells: Implications for Long-Term Progestin-Only Contraceptive-Induced Bleeding." J. Clin. Endocrinol. Metab. **89**(3): 1467-1475.

<http://jcem.endojournals.org/cgi/content/abstract/89/3/1467>

Abnormal uterine bleeding is the major reason for discontinuing long-term progesterone-only contraceptives (LTPOCs). Prior studies demonstrated that endometria exposed to the LTPOC, Norplant, display aberrant angiogenesis, leukocyte infiltration, and hypoxia-associated impaired blood flow. Paradoxically, human endometrial stromal cells (HESCs) of these specimens exhibit

elevated expression of tissue factor (TF), the primary initiator of hemostasis via thrombin generation. The current study demonstrates that TF levels are also elevated in HESCs that are decidualized after insertion of Mirena, an intrauterine system that releases levonorgestrel directly into the endometrial canal and produces elevated perivascular levels of the proinflammatory and angiogenic cytokine IL-8. Because bleeding, inflammation, and ischemia-associated increased vascular permeability enhance access of plasma factor VII to HESC-expressed TF to generate thrombin, we evaluated the effects of steroids, thrombin, and hypoxia on HESC expression of IL-8. Confluent HESCs were incubated in a serum-containing medium for 7 d with vehicle control or estradiol (E2) plus medroxyprogesterone acetate (MPA). The medium was then exchanged for corresponding defined medium with and without thrombin, and the cultures were incubated in parallel for up to 48 h in a standard incubator (normoxia) or a sealed chamber at 0-1% O₂ (hypoxia). Under normoxia, immunoreactive IL-8 levels in the conditioned medium were reduced to one-third of control levels during decidualization with E2+MPA ($P < 0.05$; $n = 5$). In E2+MPA-treated cultures, thrombin (0.1 U/ml to 2.5 U/ml) elicited a dose-dependent reversal of this inhibition, elevating IL-8 up to 60-fold ($P < 0.05$; $n = 5$) for more than 24 h and steady-state IL-8 mRNA levels by 3-fold for 3 h. The specific inactivator, hirudin, blocked most of the effects of thrombin, whereas TRAP-14, an agonist of the protease-activated receptor for thrombin, enhanced IL-8 output. In the absence of thrombin, hypoxia elevated IL-8 output 5-fold in E2+MPA-treated HESCs ($P < 0.02$, $n = 4$), with thrombin exerting additive effects. In contrast to its effects in progestin-treated HESCs, hypoxia did not elevate IL-8 output in control cultures. This study suggests that inhibition of IL-8 expression in decidualized HESCs contributes to the antiinflammatory milieu of the luteal phase. However, LTPOC-induced hypoxia and excess thrombin generation enhance IL-8 expression in decidualized HESCs, thereby eliciting aberrant angiogenesis and inflammation that promote the onset of abnormal uterine bleeding.

Mallya, S. M., J. J. Gallagher, et al. (2003). "Analysis of Microsatellite Instability in Sporadic Parathyroid Adenomas." *J. Clin. Endocrinol. Metab.* **88**(3): 1248-1251.

<http://jcem.endojournals.org/cgi/content/abstract/88/3/1248>

Microsatellite instability (MSI) is the form of genomic instability associated with defective DNA mismatch repair (MMR) in human tumorigenesis. Recent reports have suggested a role for MSI in the pathogenesis of sporadic parathyroid adenomas. However, because of their small sample sizes and/or lack of systematic analysis of genome-wide MSI, these studies have not provided conclusive evidence that MMR defects are a common occurrence in parathyroid neoplasia. To further investigate whether MSI plays an important role in parathyroid tumorigenesis, we analyzed 49 sporadic parathyroid adenomas for MSI using a panel of 5 microsatellite DNA markers that has been recommended for sensitive detection of MSI by the NCI Workshop and validated in other tumor types. These microsatellite loci were amplified by PCR using fluorescent-labeled primers from the 49 samples of template tumor DNA and matching normal DNA isolated from the same patients' peripheral blood leukocytes. None of the 49 tumors showed evidence of MSI at any of the analyzed loci of the NCI marker panel. These observations strongly suggest that defective DNA MMR plays a minor role, if any, in the pathogenesis of sporadic parathyroid adenomas.

Mantovani, G., S. Bondioni, et al. (2004). "Parental Origin of Gs{alpha} Mutations in the McCune-Albright Syndrome and in Isolated Endocrine Tumors." *J. Clin. Endocrinol. Metab.* **89**(6): 3007-3009.

<http://jcem.endojournals.org/cgi/content/abstract/89/6/3007>

Activating mutations of the Gs{alpha} gene are detected in different endocrine tumors, such as GH-secreting adenomas and toxic thyroid adenomas, and in hyperfunctioning glands from

patients with McCune-Albright syndrome (MAS). There is increasing evidence that the Gs{alpha} gene is subjected to imprinting control and that Gs{alpha} imprinting plays a key role in the pathogenesis of different human diseases. The aim of this study was to investigate the presence of a parent specificity of Gs{alpha} mutations in 10 patients affected with MAS and 12 isolated tumors (10 GH-secreting adenomas, one toxic thyroid adenoma, and one hyperfunctioning adrenal adenoma). The parental origin of Gs{alpha} mutations was assessed by evaluating NESP55 and exon 1A transcripts, which are monoallelically expressed from the maternal and paternal alleles, respectively. By this approach, we demonstrated that in isolated GH-secreting adenomas, as well as in MAS patients with acromegaly, Gs{alpha} mutations were on the maternal allele. By contrast, the involvement of other endocrine organs in MAS patients was not associated with a particular parent specificity, as precocious puberty and hyperthyroidism were present in patients with mutations on either the maternal or the paternal allele. Moreover, isolated hyperfunctioning thyroid and adrenal adenomas displayed the mutation on the maternal and paternal alleles, respectively. These data confirm the importance of Gs{alpha} imprinting in the pituitary gland and point out the high degree of tissue specificity of this phenomenon.

Mantovani, G., M. Maghnie, et al. (2003). "Growth Hormone-Releasing Hormone Resistance in Pseudohypoparathyroidism Type Ia: New Evidence for Imprinting of the Gs{alpha} Gene." J. Clin. Endocrinol. Metab. **88**(9): 4070-4074.

<http://jcem.endojournals.org/cgi/content/abstract/88/9/4070>

Heterozygous inactivating mutations in the Gs{alpha} gene cause Albright's hereditary osteodystrophy. Consistent with the observation that only maternally inherited mutations lead to resistance to hormone action [pseudohypoparathyroidism type Ia (PHP Ia)], recent studies provided evidence for a predominant maternal origin of Gs{alpha} transcripts in endocrine organs, such as thyroid, gonad, and pituitary. The aim of this study was to investigate the presence of pituitary resistance to hypothalamic hormones acting via Gs{alpha}-coupled receptors in patients with PHP Ia. Six of nine patients showed an impaired GH responsiveness to GHRH plus arginine, consistent with a complete GH deficiency (GH peak from 2.6-8.6 {micro}g/liter, normal > 16.5), and partial (GH peak 13.9 and 13.6 {micro}g/liter) and normal responses were found in two and one patient, respectively. Accordingly, IGF-I levels were below and in the low-normal range in seven and two patients. All patients had a normal cortisol response to 1 {micro}g ACTH test, suggesting a normal corticotroph function that was confirmed by a normal ACTH and cortisol response to CRH test in three patients. In conclusion, we report that in addition to PTH and TSH resistance, patients with PHP Ia display variable degrees of GHRH resistance, consistent with Gs{alpha} imprinting in human pituitary.

Mazzocchi, G., L. K. Malendowicz, et al. (2004). "Cholecystokinin (CCK) Stimulates Aldosterone Secretion from Human Adrenocortical Cells via CCK2 Receptors Coupled to the Adenylate Cyclase/Protein Kinase A Signaling Cascade." J. Clin. Endocrinol. Metab. **89**(3): 1277-1284.

<http://jcem.endojournals.org/cgi/content/abstract/89/3/1277>

Cholecystokinin (CCK) IS a regulatory peptide that acts via two receptor subtypes, CCK1-R and CCK2-R. RT-PCR demonstrated the expression of both CCK1-R and CCK2-R in the zona glomerulosa (ZG), but not zona fasciculata-reticularis cells of the human adrenal cortex. CCK and the CCK2-R agonist pentagastrin enhanced basal aldosterone secretion from ZG cells without affecting cortisol production from zona fasciculata-reticularis cells. The aldosterone response to CCK and pentagastrin was suppressed by a CCK2-R antagonist, but not by a CCK1-R antagonist. Pentagastrin evoked a sizeable cAMP, but not inositol triphosphate, response from ZG cells, whereas CCK plus CCK2-R antagonist was ineffective. The cAMP response to

pentagastrin was abrogated by CCK2-R antagonist or the adenylate cyclase inhibitor SQ-22536, and the aldosterone response was abolished by both SQ-22536 and the protein kinase A inhibitor H-89. Both CCK and pentagastrin increased steroidogenic acute regulatory protein mRNA expression in ZG cells; the effect was abrogated by CCK2-R antagonist. We conclude that CCK exerts secretagogue action on human ZG cells, acting through CCK2-Rs coupled to the adenylate cyclase/protein kinase A signaling cascade, which, in turn, stimulates the expression of steroidogenic acute regulatory protein, the rate-limiting step of steroidogenesis.

Mazzocchi, G., L. K. Malendowicz, et al. (2002). "Expression and Function of Vasoactive Intestinal Peptide, Pituitary Adenylate Cyclase-Activating Polypeptide, and Their Receptors in the Human Adrenal Gland." J. Clin. Endocrinol. Metab. **87**(6): 2575-2580.

<http://jcem.endojournals.org/cgi/content/abstract/87/6/2575>

VIP and pituitary adenylate cyclase-activating polypeptide (PACAP) are two regulatory peptides that possess remarkable amino acid sequence homology and act through common receptors, named PAC1, VPAC1, and VPAC2. PAC1 receptor is selective for PACAP, whereas VPAC1 and VPAC2 receptors bind both VIP and PACAP. We have investigated the expression and function of VIP, PACAP, and their receptors in the zona glomerulosa (ZG), zonae fasciculata and reticularis, and adrenal medulla (AM) of the human adrenal cortex. RT-PCR and RIA detected VIP and PACAP expression exclusively in AM cells. RT-PCR demonstrated the presence of PAC1 mRNA only in AM and of VPAC1 and VPAC2 mRNAs in both ZG and AM cells. VIP and PACAP concentration-dependently increased aldosterone and catecholamine secretion from cultured ZG and AM cells. The catecholamine response to both peptides was higher than the aldosterone response, and the secretagogue action of PACAP was more intense than that of VIP. The aldosterone response of cultured ZG cells to VIP or PACAP was unaffected by the PAC1 receptor antagonist PACAP-(6-38) (PAC1-A), but was significantly decreased by the VPAC1 receptor antagonist [Ac-His1,D-Phe2,Lys15,Arg16]VIP-(3-7),GH-releasing factor-(8-27)-NH₂ (VPAC1-A). The catecholamine response of cultured AM cells to VIP was lowered by VPAC1-A and unaffected by PAC1-A; conversely, the catecholamine response to PACAP was reduced by both PAC1-A and VPAC1-A. Simultaneous exposure to both antagonists did not abolish the catecholamine response to PACAP. Collectively, our findings allow us to conclude that in human adrenals 1) VIP and PACAP biosynthesis exclusively occurs in AM cells; 2) ZG cells are provided with functional VPAC1 and VPAC2 receptors, whose activation by VIP or PACAP elicits a moderate aldosterone response; 3) AM cells possess PAC1, VPAC1, and VPAC2 receptors, whose activation evokes a marked catecholamine response; and 4) the catecholamine response to PACAP is more intense than that to VIP, because it is mediated by all subtypes of VIP/PACAP receptors.

McCabe, C. J., K. Boelaert, et al. (2002). "Vascular Endothelial Growth Factor, Its Receptor KDR/Flk-1, and Pituitary Tumor Transforming Gene in Pituitary Tumors." J. Clin. Endocrinol. Metab. **87**(9): 4238-4244.

<http://jcem.endojournals.org/cgi/content/abstract/87/9/4238>

Pituitary tumorigenesis is a poorly understood process involving dysregulation of the cell cycle, proliferation, and angiogenesis. The novel securin pituitary tumor transforming gene (PTTG) disrupts cell division and stimulates fibroblast growth factor (FGF)-2-mediated angiogenesis. We investigated expression of the angiogenic vascular endothelial growth factor (VEGF) and its receptor KDR/Flk-1 in 103 human pituitary tumors, and we assessed functional relationships between these genes in vitro. Nonfunctioning tumors (n = 81) demonstrated markedly raised VEGF mRNA (3.2-fold, P < 0.05) and protein concentrations, compared with normal pituitaries (n

= 10). KDR was also highly induced in nonfunctioning tumors (14-fold, $P < 0.001$, $n = 78$) as well as in the whole cohort of pituitary tumors, compared with normal pituitary samples (14-fold, $P < 0.0001$, $n = 100$). In vitro, PTTG induced VEGF, but not KDR, expression in fetal neuronal NT2 cells (2.7-fold, $P < 0.001$, $n = 8$), MCF-7 breast carcinoma cells (1.9-fold, $P = 0.03$, $n = 10$), and choriocarcinoma JEG-3 cells ($P = 0.0002$, $n = 8$). A mutated PTTG construct that cannot be phosphorylated showed identical VEGF up-regulation (2.9-fold, $P < 0.001$, $n = 8$) in NT2 cells, compared with wild-type PTTG, but a further mutated construct with abrogation of the key protein:protein interaction domain of PTTG resulted in a significant reduction in VEGF stimulation, compared with wild-type (0.37-fold reduction, $P < 0.001$, $n = 8$). FGF-2 findings mirrored those of VEGF, although antibody depletion of secreted FGF-2 in the cell medium failed to influence VEGF up-regulation by PTTG. Overall, our findings implicate altered VEGF and KDR signaling in pituitary tumorigenesis, and we propose that PTTG stimulation of FGF-2 and VEGF expression in the presence of up-regulated growth factor receptors may account for angiogenic growth and progression of human pituitary tumors.

McCann, J. A., Y. Q. Xu, et al. (2004). "The Insulin-Like Growth Factor-II Receptor Gene Is Associated with Type 1 Diabetes: Evidence of a Maternal Effect." *J. Clin. Endocrinol. Metab.* **89**(11): 5700-5706.

<http://jcem.endojournals.org/cgi/content/abstract/89/11/5700>

Susceptibility to type 1 diabetes (T1D) is a complex trait, involving several loci. One of these putative loci, insulin-dependent diabetes mellitus-8 (IDDM8) at 6q, has been found to be subject to parental effects, suggesting the involvement of an imprinted gene. IGF-II receptor (IGF2R), the best-studied imprinted gene in the IDDM8 region, encodes the IGF-2 receptor, a protein involved in many biological processes, including immune function and β -cell regeneration. Mice express only the maternal allele. In humans, the molecular IGF2R imprint (maternal-specific methylation) is present, but it affects expression in only a small subset of individuals. To examine whether IGF2R might contribute to the IDDM8 effect, we examined transmission distortion at several single nucleotide polymorphisms (SNPs) in 404 parent-offspring trios. After correcting for multiple testing, significant distortion was found at only one silent SNP on exon 16 ($P = 0.002$). SNPs upstream and downstream showed weak linkage disequilibrium and no transmission distortion, localizing the association to a 53-kb block within IGF2R. Interestingly, the exon 16 SNP association was limited to maternally inherited alleles. SLC22A2 and SLC22A3, two genes downstream of IGF2R that are imprinted in the mouse, showed no T1D association. Thus, we present evidence that maternal alleles at an IGF2R polymorphism are associated with T1D. It is thus possible that at some tissue or developmental stage not yet examined, IGF2R is universally imprinted.

McTernan, P. G., F. M. Fisher, et al. (2003). "Resistin and Type 2 Diabetes: Regulation of Resistin Expression by Insulin and Rosiglitazone and the Effects of Recombinant Resistin on Lipid and Glucose Metabolism in Human Differentiated Adipocytes." *J. Clin. Endocrinol. Metab.* **88**(12): 6098-6106.

<http://jcem.endojournals.org/cgi/content/abstract/88/12/6098>

Resistin, an adipocyte secreted factor, has been suggested to link obesity with type 2 diabetes in rodent models, but its relevance to human diabetes remains uncertain. Although previous studies have suggested a role for this adipocytokine as a pathogenic factor, its functional effects, regulation by insulin, and alteration of serum resistin concentration by diabetes status remain to be elucidated. Therefore, the aims of this study were to analyze serum resistin concentrations in type 2 diabetic subjects; to determine the in vitro effects of insulin and rosiglitazone (RSG) on the

regulation of resistin, and to examine the functional effects of recombinant human resistin on glucose and lipid metabolism in vitro. Serum concentrations of resistin were analyzed in 45 type 2 diabetic subjects and 34 nondiabetic subjects. Subcutaneous human adipocytes were incubated in vitro with insulin, RSG, and insulin in combination with RSG to examine effects on resistin secretion. Serum resistin was increased by approximately 20% in type 2 diabetic subjects compared with nondiabetic subjects ($P = 0.004$) correlating with C-reactive protein. No other parameters, including adiposity and fasting insulin levels, correlated with serum resistin in this cohort. However, in vitro, insulin stimulated resistin protein secretion in a concentration-dependent manner in adipocytes [control, 1215 $\{+/-\}$ 87 pg/ml (mean $\{+/-\}$ SEM); 1 nM insulin, 1414.0 $\{+/-\}$ 89 pg/ml; 1 $\{\mu\text{M}\}$ insulin, 1797 $\{+/-\}$ 107 pg/ml ($P < 0.001$)]. RSG (10 nM) reduced the insulin-mediated rise in resistin protein secretion (1 nM insulin plus RSG, 971 $\{+/-\}$ 35 pg/ml; insulin, 1 $\{\mu\text{M}\}$ insulin plus RSG, 1019 $\{+/-\}$ 28 pg/ml; $P < 0.01$ vs. insulin alone). Glucose uptake was reduced after treatment with 10 ng/ml recombinant resistin and higher concentrations ($P < 0.05$). Our in vitro studies demonstrated a small, but significant, reduction in glucose uptake with human recombinant resistin in differentiated preadipocytes. In human abdominal sc adipocytes, RSG blocks the insulin-mediated release of resistin secretion in vitro. In conclusion, elevated serum resistin in human diabetes reflects the subclinical inflammation prevalent in type 2 diabetes. Our in vitro studies suggest a modest effect of resistin in reducing glucose uptake, and suppression of resistin expression may contribute to the insulin-sensitizing and glucose-lowering actions of the thiazolidinediones.

Meeus, L., B. Gilbert, et al. (2004). "Characterization of a Novel Loss of Function Mutation of PAX8 in a Familial Case of Congenital Hypothyroidism with In-Place, Normal-Sized Thyroid." J. Clin. Endocrinol. Metab. **89**(9): 4285-4291.

<http://jcem.endojournals.org/cgi/content/abstract/89/9/4285>

Thyroid dysgenesis is the most common cause of congenital hypothyroidism, a relatively frequent disease affecting 1 in 3000-4000 newborns. Whereas most cases are sporadic, mutations in transcription factors implicated in thyroid development have been shown to cause a minority of cases transmitted as monogenic Mendelian diseases. PAX8 is one of these transcription factors, and so far, five mutations have been identified in its paired domain in patients with thyroid dysgenesis. We have identified a novel mutation of PAX8, in the heterozygous state, in a father and his two children both presenting with congenital hypothyroidism associated with an in-place thyroid of normal size at birth. In addition, one of the affected siblings displayed unilateral kidney agenesis. The mutation substitutes a highly conserved serine in position 54 of the DNA-binding domain of the protein (S54G mutation) by a glycine. Functional analyses of the mutant protein (PAX8-S54G) demonstrated that it is unable to bind a specific cis-element of the thyroperoxidase gene promoter in EMSAs and that it has almost completely lost the ability to act in synergy with Ttf1 to transactivate transcription from the thyroglobulin promoter/enhancer. These results indicate that loss of function mutations of the PAX8 gene may cause congenital hypothyroidism in the absence of thyroid hypoplasia.

Meloni, A., R. Perniola, et al. (2002). "Delineation of the Molecular Defects in the AIRE Gene in Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy Patients from Southern Italy." J. Clin. Endocrinol. Metab. **87**(2): 841-846.

<http://jcem.endojournals.org/cgi/content/abstract/87/2/841>

In this study, we have carried out molecular analysis of the AIRE (autoimmune regulator) gene in 11 patients (from 8 families) affected by autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, originating from a restricted area of Southern Italy (the Salento peninsula in Puglia). Of

the 16 mutant AIRE alleles from the 8 probands studied, 12 carried a missense mutation (W78R in 9, P539L in 2, and P252L in 1), 2 carried the Q358X nonsense mutation, and 2 carried the 1058delT frameshift mutation. All these mutations except the 1058delT are novel. Each of the detected mutations either predicts a premature termination of the protein or results in a nonconservative amino acid change, most likely adversely affecting the function of the protein. The W78R missense mutation is relatively common in these patients, having been detected (in homozygosity or compound heterozygosity) in 6 of the 8 probands tested, indicating the presence of a founder effect. The results of this study contribute to the delineation of the molecular pathology of the AIRE gene and enhance our ability to perform a molecular diagnosis in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients from Southern Italy.

Menko, F. H., R. B. van der Lijst, et al. (2002). "Atypical MEN Type 2B Associated with Two Germline RET Mutations on the Same Allele Not Involving Codon 918." J. Clin. Endocrinol. Metab. **87**(1): 393-397.

<http://jcem.endojournals.org/cgi/content/abstract/87/1/393>

A kindred was diagnosed with atypical MEN type 2B characterized by medullary thyroid cancer and mucosal neurilemmomas in multiple family members. Mutation analysis revealed a double RET germline mutation, Val804Met and Ser904Cys, in affected individuals. The clinical phenotype, the functional effect of the mutations, and the clinical implications of our findings are discussed.

Messina, M., D. M. T. Yu, et al. (2003). "Calcitonin-Specific Transcription and Splicing Targets Gene-Directed Enzyme Prodrug Therapy to Medullary Thyroid Carcinoma Cells." J. Clin. Endocrinol. Metab. **88**(3): 1310-1318.

<http://jcem.endojournals.org/cgi/content/abstract/88/3/1310>

Recurrent and metastatic medullary thyroid carcinoma (MTC) remains difficult to treat due to its limited responsiveness to chemotherapy, radiotherapy, and imaging. To investigate an alternative therapeutic approach, we examined the feasibility of targeting gene-directed enzyme/prodrug therapy delivered by adenoviral vectors to MTC. We previously described a modified human calcitonin (CT)/CT gene-related peptide promoter that produced increased expression while maintaining specificity for MTC cells. In this study, we introduced an additional level of specificity by using cell-specific splicing and examined whether the selectivity of the gene-directed enzyme/prodrug therapy for MTC was enhanced when both the promoter and splicing features were combined in a single transcription unit. Two replication-defective adenoviruses were constructed that expressed the Escherichia coli purine nucleoside phosphorylase (PNP) gene under the transcriptional control of a modified T2 promoter (Ad.T2-PNP) or the T2 promoter in combination with a CT minigene cassette in which the PNP gene was imbedded within the CT gene exon 4 (Ad.T2-CT/PNP). The specificity of PNP expression by Ad.T2-PNP, Ad.T2-CT/PNP, and control viruses in the MTC cell line, TT, and in a panel of non-MTC cell lines was evaluated. The highest level of PNP gene expression and the most effective cell killing in the presence of prodrug occurred in TT cells infected with Ad.T2-PNP, followed by Ad.T2-CT/PNP. Infection of most non-MTC cell lines, even with high multiplicities of Ad.T2-PNP, produced only low-level PNP expression that resulted in minimal cell killing in the presence of prodrug. High-level expression of PNP and effective cell killing was observed with both adenoviral gene constructs. The highest level of cell specificity was achieved with the combined use of promoter and splicing regulation in the Ad.T2-CT/PNP virus.

Meysing, A. U., H. Kanasaki, et al. (2004). "GNRHR Mutations in a Woman with Idiopathic Hypogonadotropic Hypogonadism Highlight the Differential Sensitivity of Luteinizing Hormone and Follicle-Stimulating Hormone to Gonadotropin-Releasing Hormone." J. Clin. Endocrinol. Metab. **89**(7): 3189-3198.

<http://jcem.endojournals.org/cgi/content/abstract/89/7/3189>

Mutations in the GnRH receptor gene (GNRHR) are a cause of idiopathic hypogonadotropic hypogonadism. We describe a normosmic female subject with congenital idiopathic hypogonadotropic hypogonadism in whom treatment with pulsatile GnRH resulted in an unusual response. The subject not only required an increased dose of pulsatile GnRH for ovarian follicular development, but LH secretion did not increase appropriately, estradiol levels remained low, and she did not ovulate spontaneously. Sequencing of the GNRHR coding sequence revealed compound heterozygous mutations leading to amino acid substitutions [N10K+Q11K] and P320L. The introduction of the P320L mutation into the GnRH receptor led to failure of detectable ligand binding and failure of stimulation of inositol phosphate production and gonadotropin subunit gene promoter activity in response to GnRH in transiently transfected cells. The [N10K+Q11K] mutation resulted in reduced binding of a GnRH agonist to 25% of the wild-type receptor. In addition, the EC₅₀ value for GnRH stimulation of inositol phosphate production was significantly increased, and the dose-response curves for stimulation of α gonadotropin subunit, LH β , and FSH β gene transcription by GnRH were similarly shifted to the right. Stimulation of FSH β gene transcription was more sensitive to GnRH than LH β for both wild-type and [N10K+Q11K] GnRH receptors, resulting in a greater loss of LH β stimulation than FSH β by the [N10K+Q11K] mutant at any given submaximal GnRH concentration. We propose that the mutations in the GnRH receptor result in a rightward shift of the dose-response curves of gonadotropin responses to pulsatile GnRH in the subject and unmask the differential sensitivities of LH and FSH to GnRH, resulting in low LH and estradiol levels despite appropriate FSH secretion and follicular growth.

Miki, Y., T. Nakata, et al. (2002). "Systemic Distribution of Steroid Sulfatase and Estrogen Sulfotransferase in Human Adult and Fetal Tissues." J. Clin. Endocrinol. Metab. **87**(12): 5760-5768.

<http://jcem.endojournals.org/cgi/content/abstract/87/12/5760>

Estrogens play a key role in various target tissues. Enzymes involved in the biosynthesis and metabolism of these sex steroids also regulate estrogenic actions in these tissues. Estrone sulfate (E1S) is a major circulating plasma estrogen that is converted into the biologically active estrogen, estrone (E1), by steroid sulfatase (STS). E1 is also sulfated and reverted into E1S by estrogen sulfotransferase (EST). These two enzymes have recently been shown to play important roles in the in situ estrogen actions of various sex steroid-dependent human tumors. However, the distribution of STS and EST in normal adult and fetal human tissues remains largely unknown. Therefore, in this study, in addition to examining the tissue distribution of both STS and EST mRNA in human adult and fetal tissues using RT followed by quantitative PCR, we studied the activity of these enzymes using 3H-labeled E1/E1S as substrates in the homogenates of various human adult tissues. We also examined the localization of STS and EST protein in human adult and fetal tissues using immunohistochemistry, and that of EST mRNA in the adult kidney using laser dissection microscopy and PCR. STS mRNA, enzyme activity, and immunoreactivity were either absent or detected at very low levels in all adult and fetal tissues examined in this study. EST mRNA expression, however, was detected in all of the tissues examined, except for adult spleen and pancreas. EST enzyme activities were consistent with those of mRNA expression in the great majority of the tissues examined. Marked EST

immunoreactivity was detected in hepatocytes, adrenal gland (adult, zona fasciculata to the reticularis; fetus, fetal zone), and epithelial cells of the gastrointestinal tract, smooth muscle cells of the tunica media in aorta, Leydig cells of the testis, and syncytiotrophoblast of the placenta. Patterns of EST immunolocalization were similar between adult and fetal human tissues, but EST immunoreactivity was detected in the urinary tubules of adult kidney, whereas in the fetal kidney, it was localized in the interstitial cells surrounding the urinary tubules. In the adult kidney, the presence of EST mRNA was also confirmed in the cells of urinary tubules using laser dissection microscopy and RT-PCR. Although the number of human tissues available for examination in this study was limited, our results suggest that between the enzymes involved in estrogen activation or inactivation, EST and not STS is the more widely expressed enzyme in various peripheral tissues in humans. We speculate that EST may play an important role in protecting peripheral tissues from possible excessive estrogenic effects.

Milne, S. A. and H. N. Jabbour (2003). "Prostaglandin (PG) F₂{alpha} Receptor Expression and Signaling in Human Endometrium: Role of PGF₂{alpha} in Epithelial Cell Proliferation." J. Clin. Endocrinol. Metab. **88**(4): 1825-1832.

<http://jcem.endojournals.org/cgi/content/abstract/88/4/1825>

Prostaglandin (PG) F₂{alpha}, a member of the prostanoid bioactive lipid family, is secreted by human endometrium throughout the menstrual cycle and is present in both menstrual fluid and medium of endometrial explants in culture. PGF₂{alpha} mediates its effects through a seven-transmembrane G-protein-coupled receptor (FP). The aim of this study was to examine the temporal expression, signaling, and role of FP receptor in the human endometrium. Quantitative RT-PCR analysis demonstrated highest expression of FP receptor in the mid- to late-proliferative phase, compared with early-proliferative and secretory phase endometrium. In situ hybridization studies localized FP receptor mRNA expression to the epithelial cell compartment during the mid- to late-proliferative phase. Moreover, treatment of endometrial tissue with 1-100 nM PGF₂{alpha} induced a concentration-dependent increase in inositol phosphate mobilization, indicating functional FP receptor expression. The Ishikawa human endometrial epithelial cell line was used to investigate further the signaling and role of PGF₂{alpha} in endometrial epithelial cells. Ishikawa cells endogenously express the FP receptor, and treatment with 1-100 nM PGF₂{alpha} elicits a concentration-dependent increase in inositol phosphate release. Moreover, treatment of Ishikawa cells with 100 nM PGF₂{alpha} induced phosphorylation of ERK1/2 that was abolished when cells were cotreated with 50 {micro}M PD98059 (MAPK kinase inhibitor) or 10 {micro}M U73122 [phospholipase C (PLC) inhibitor]. Treatment of Ishikawa cells with PGF₂{alpha} for 24 h induced a significant concentration-dependent increase in Ishikawa cell proliferation. Coincubation of the cells with 50 {micro}M PD98059 or 2 {micro}M U73122 demonstrated that PLC inhibition significantly reduced PGF₂{alpha}-induced proliferation, whereas MAPK kinase inhibition had no effect. In summary, these studies demonstrate increased FP receptor expression in endometrial epithelial cells during the proliferative phase of the menstrual cycle and identify a role for PGF₂{alpha} in epithelial cell proliferation via a PLC-dependent pathway.

Mitchell, S. M. S., A. T. Hattersley, et al. (2004). "Lack of Support for a Role of the Insulin Gene Variable Number of Tandem Repeats Minisatellite (INS-VNTR) Locus in Fetal Growth or Type 2 Diabetes-Related Intermediate Traits in United Kingdom Populations." J. Clin. Endocrinol. Metab. **89**(1): 310-317.

<http://jcem.endojournals.org/cgi/content/abstract/89/1/310>

The insulin gene variable number of tandem repeats minisatellite (INS-VNTR) class III allele is associated with altered fetal growth, type 2 diabetes risk (especially when paternally inherited),

and insulin and IGF2 gene expression. Further studies are needed to establish the role of the INS-VNTR in fetal growth and assess whether its effects depend on the parent of origin. We analyzed the INS-VNTR-linked -23 Hph1 polymorphism in 2283 subjects, comprising 1184 children and 1099 parents. There were no differences ($P < 0.05$) in birth weight between offspring of the three genotypes: III/III ($n = 108$) vs. I/I ($n = 558$), effect size, -8 g ($P = 0.87$); and I/III ($n = 464$) vs. I/I, effect size, -19 g ($P = 0.54$). We observed no differences in head circumference [III/III ($n = 95$) vs. I/I ($n = 470$), effect size, -0.14 cm; $P = 0.31$] or birth length. No differences were observed when stratifying by postnatal growth realignments [nonchangers III/III ($n = 37$) vs. I/I ($n = 170$), effect size, -43 g; $P = 1.00$] or by parent of origin of the class III allele (presence of paternal III allele effect size, -15 g; $P = 0.74$). INS-VNTR was nominally associated ($P < 0.05$) with body mass index and insulin resistance, but not with β -cell function, in young adults. In the largest study to date, we found a lack of support for a role for INS-VNTR in fetal growth and nominal association with type 2 diabetes-related intermediate traits.

Moffett, S. P., J. M. Zmuda, et al. (2005). "Tumor Necrosis Factor Alpha Polymorphism, Bone Strength Phenotypes, and the Risk of Fracture in Older Women." *J. Clin. Endocrinol. Metab.*: jc.2004-2235.

<http://jcem.endojournals.org/cgi/content/abstract/jc.2004-2235v1>

Tumor necrosis factor- α (TNF α) is a proinflammatory cytokine that promotes osteoclastic bone resorption. We evaluated the association between a G-308A polymorphism (rs1800629) at the TNFA locus and osteoporosis phenotypes in 4306 older women participating in the Study of Osteoporotic Fractures (SOF). Femoral neck BMD and structural geometry were measured using dual-energy x-ray absorptiometry and hip structural analysis. Incident fractures were confirmed by physician adjudication of radiology reports. Despite similar femoral neck BMD, women with the A/A genotype had greater subperiosteal width ($P = 0.01$) and endocortical diameter ($P = 0.03$) than those with the G/G genotype. The net result of these structural differences was that there was a greater distribution of bone mass away from the neutral axis of the femoral neck in women with the A/A genotype, resulting in greater indices of bone bending strength (cross-sectional moment of inertia: $P = 0.004$; section modulus: $P = 0.003$). Among 376 incident hip fractures during 12.1 yr of follow-up, a 22% decrease in the risk of hip fracture was seen per copy of the A allele (RR: 0.78; 95% C.I.: 0.63, 0.96) which was not influenced by adjustments for potential confounding factors, BMD or bone strength indices. The G-308A polymorphism was not associated with a reduced risk of other fractures. These results suggest a potential role of genetic variation in TNF α in the etiology of osteoporosis.

Mulatero, P., T. A. Williams, et al. (2002). "Blood Pressure in Patients with Primary Aldosteronism Is Influenced by Bradykinin B2 Receptor and α -Adducin Gene Polymorphisms." *J. Clin. Endocrinol. Metab.* **87**(7): 3337-3343.

<http://jcem.endojournals.org/cgi/content/abstract/87/7/3337>

Primary aldosteronism (PA) is the most common cause of endocrine hypertension. PA is most frequently presented as moderate to severe hypertension, but the clinical and biochemical features vary widely. The aim of our study was to identify genetic variants that influence the phenotype of patients with PA. We hypothesized that genetic variants potentially affecting aldosterone production (aldosterone synthase, CYP11B2), renal proximal tubule reabsorption (α -adducin), or the mechanisms of counterbalance leading to vasodilatation and sodium excretion (bradykinin B2-receptor, B2R) could influence the clinical and biochemical characteristics of patients with PA. We studied three polymorphisms of these genes (C-344T of CYP11B2, G460W of α -adducin, and C-58T of B2R) in 167 primary aldosteronism patients

(56 with aldosterone-producing adenoma and 111 with idiopathic hyperaldosteronism). B2R and {alpha}-adducin genotypes were strong independent predictors of both systolic and diastolic blood pressure levels; plasma renin activity and aldosterone also play a marginal role on BP levels. Body mass index, age, sex, and CYP11B2 genotype displayed no significant effect on the clinical parameters of our population. In particular, {alpha}-adducin and B2R polymorphisms accounted for 13.2% and 11.0% of the systolic and diastolic blood pressure variance, respectively. These data suggest that genetic variants of {alpha}-adducin and the bradykinin B2-R influence the blood pressure levels in patients with primary aldosteronism.

Nystrom, A. M., M. L. Bondeson, et al. (2004). "A Novel Nonsense Mutation of the Mineralocorticoid Receptor Gene in a Swedish Family with Pseudohypoaldosteronism Type I (PHA1)." J. Clin. Endocrinol. Metab. **89**(1): 227-231.

<http://jcem.endojournals.org/cgi/content/abstract/89/1/227>

Pseudohypoaldosteronism type I (PHA1) is a condition associated with salt wasting leading to dehydration, hypotension, hyperkalemia, and metabolic acidosis. Sporadic cases and two familial forms, one autosomal dominant and one autosomal recessive form, have been described. The autosomal dominant or sporadic form manifests milder salt wasting that remits with age. Mutations in the gene encoding the mineralocorticoid receptor (MR) have been identified in patients with the autosomal dominant inheritance. However, recent studies suggest that the autosomal dominant and sporadic forms are genetically heterogeneous and that additional genes might be involved. We report on the study of 15 members of a Swedish five-generation family with the autosomal dominant form of PHA1. Interestingly, neuropathy was found in two of five affected individuals. A novel heterozygous nonsense mutation C436X in exon 2 was identified in the index patient by linkage analysis, PCR, and direct sequencing of the MR gene. Analysis of the family demonstrated that the mutation segregated with PHA1 in the family. It is unclear whether the neuropathy is associated with the mutation found. Our results together with previously published data suggest that loss-of-function mutations of the MR gene located at 4q31.1, commonly are associated with the autosomal dominant form of PHA1.

Osgood, D., D. Corella, et al. (2003). "Genetic Variation at the Scavenger Receptor Class B Type I Gene Locus Determines Plasma Lipoprotein Concentrations and Particle Size and Interacts with Type 2 Diabetes: The Framingham Study." J. Clin. Endocrinol. Metab. **88**(6): 2869-2879.

<http://jcem.endojournals.org/cgi/content/abstract/88/6/2869>

The scavenger receptor class B type I (SR-BI) is a key component in the reverse cholesterol transport pathway. We have previously reported three common polymorphisms associated with plasma lipids and body mass index. We hypothesized that diabetic status may interact with these polymorphisms in determining plasma lipid concentrations and particle size. We evaluated this hypothesis in 2463 nondiabetic (49% men) and 187 diabetic (64% men) participants in the Framingham Study. SR-BI and APOE genotypes, anthropometric, clinical, biochemical, and lifestyle variables were determined. After multivariate adjustment, we found a consistent association between the exon 8 polymorphism and high-density lipoprotein cholesterol concentration and particle size. Interaction effects were not significant for exon 8 and intron 5 polymorphisms. However, we found statistically significant interactions between SR-BI exon 1 genotypes and type 2 diabetes, indicating that diabetic subjects with the less common allele (allele A) have lower lipid concentrations. For low-density lipoprotein cholesterol, the adjusted means ($\pm$ SE) were 3.31 $\pm$ 0.03 and 3.29 $\pm$ 0.04 mmol/liter for G/G and G/A or A/A in nondiabetics, respectively, compared with 3.19 $\pm$ 0.10 and 2.75 $\pm$ 0.01 mmol/liter for G/G and G/A or A/A in diabetics ($P = 0.03$ for interaction). Similar results were obtained for HDL2-C. In

conclusion, SR-BI gene variation modulates the lipid profile, particularly in type 2 diabetes, contributing to the metabolic abnormalities in these subjects.

Pagnin, E., G. Fadini, et al. (2005). "Diabetes Induces p66shc Gene Expression in Human Peripheral Blood Mononuclear Cells: Relationship to Oxidative Stress." J. Clin. Endocrinol. Metab. **90**(2): 1130-1136.

<http://jcem.endojournals.org/cgi/content/abstract/90/2/1130>

Oxidative stress plays a role in cardiovascular dysfunction. This is of interest in diabetes, a clinical condition characterized by oxidative stress and increased prevalence of cardiovascular disease. The role of p66shc in oxidative stress-related response has been demonstrated by resistance to and reduction of oxidative stress and prolonged lifespan in p66shc^{-/-} mice. In this study we assess p66shc gene expression in peripheral blood mononuclear cells (PBM) from type 2 diabetic patients and healthy subjects. The p66shc mRNA level was assessed using RT-PCR with two sets of primers mapping for different p66shc regions. p66shc is expressed in both monocytes and lymphocytes. The level of p66shc mRNA was significantly higher in type 2 diabetic patients compared with controls (0.38 {+/-} 0.07 densitometric units vs. 0.13 {+/-} 0.08; $P < 0.0001$). In addition, total plasma 8-isoprostane levels, a marker of oxidative stress, were higher in type 2 diabetics (0.72 {+/-} 0.04 ng/ml) than in normal subjects (0.43 {+/-} 0.04, $P < 0.001$) and were significantly correlated to the p66shc mRNA level in PBM from type 2 diabetics ($r^2 = 0.47$; $P = 0.0284$). In conclusion, diabetes induces p66shc gene expression in circulating PBM; this up-regulation in expression is significantly associated with markers of oxidative stress. p66shc gene expression in PBM may represent a useful tool to investigate the oxidative stress involved in the pathogenesis of long-term diabetic complications.

Perez-Martinez, P., F. Perez-Jimenez, et al. (2005). "A Polymorphism Exon 1 Variant at the Locus of the Scavenger Receptor Class B Type I (SCARB1) Gene Is Associated with Differences in Insulin Sensitivity in Healthy People during the Consumption of an Olive Oil-Rich Diet." J. Clin. Endocrinol. Metab. **90**(4): 2297-2300.

<http://jcem.endojournals.org/cgi/content/abstract/90/4/2297>

Scavenger receptor class B type I (SCARB1) was described as the first high-density lipoprotein receptor. Increasing evidence indicates that SCARB1 plays additional roles particularly in type 2 diabetes mellitus. Our aim was to determine whether the presence of an exon 1 (G[->A] polymorphism at the SCARB1 gene modifies the insulin sensitivity to dietary fat. Methods: We studied 59 healthy volunteers (30 men and 29 women, 42 G/G homozygous and 17 G/A heterozygous). Subjects consumed three diets for 4 wk each: a saturated fatty acid (SFA)-rich diet (38% fat, 20% SFA), followed by a carbohydrate (CHO)-rich diet (30% fat, 55% CHO) or a monounsaturated fatty acid (MUFA)-rich diet (38% fat, 22% MUFA) after a randomized crossover design. For each diet, we investigated peripheral insulin sensitivity with the insulin suppression test. Results: Steady-state plasma glucose after the MUFA diet was lower in G/A compared with G/G subjects ($P = 0.030$). This effect was not observed after CHO and SFA diets ($P = 0.177$ and 0.957 , respectively). Plasma nonesterified free fatty acid values were lower in subjects carrying the A allele for all the diet periods. Conclusions: Our findings show that carriers of the G/A genotype have significant increases in insulin sensitivity after a MUFA-rich diet compared with G/G individuals.

Pitteloud, N., J. S. Acierno, Jr., et al. (2005). "Reversible Kallmann Syndrome, Delayed Puberty, and Isolated Anosmia Occurring in a Single Family with a Mutation in the Fibroblast Growth Factor Receptor 1 Gene." *J. Clin. Endocrinol. Metab.* **90**(3): 1317-1322.

<http://jcem.endojournals.org/cgi/content/abstract/90/3/1317>

Kallmann syndrome (KS) is a clinically and genetically heterogeneous disorder. Recently, loss-of-function mutations in the fibroblast growth factor receptor 1 (FGFR1) gene have been shown to cause autosomal dominant KS. To date, the detailed reproductive phenotype of KS associated with mutations in the FGFR1 has yet to be described. We report a kindred comprising a male proband with KS and spontaneous reversibility, whose mother had delayed puberty and whose maternal grandfather isolated anosmia. The proband presented at age 18 yr with KS and was subsequently treated with testosterone (T) therapy. Upon discontinuation of T therapy, he recovered from his hypogonadotropic hypogonadism, as evidenced by a normal LH secretion pattern, sustained normal serum T levels, and active spermatogenesis. The three members of this single family harbor the same FGFR1 mutation (Arg622X) in the tyrosine kinase domain. This report demonstrates 1) the first genetic cause of the rare variant of reversible KS, 2) the reversal of hypogonadotropic hypogonadism in a proband carrying an FGFR1 mutation suggests a role of FGFR1 beyond embryonic GnRH neuron migration, and 3) a loss of function mutation in the FGFR1 gene causing delayed puberty.

Powell, B. L., L. Haddad, et al. (2005). "Analysis of Multiple Data Sets Reveals No Association between the Insulin Gene Variable Number Tandem Repeat Element and Polycystic Ovary Syndrome or Related Traits." *J. Clin. Endocrinol. Metab.* **90**(5): 2988-2993.

<http://jcem.endojournals.org/cgi/content/abstract/90/5/2988>

Context: Variation at the insulin gene VNTR (variable number tandem repeat) minisatellite has been reported to be associated with polycystic ovary syndrome (PCOS), but findings have been inconsistent and all studies have featured small sample sizes. Objective: To gain a robust understanding of the role of the INS-VNTR in PCOS susceptibility. Design: Case-control, family-based association and quantitative trait analyses. Setting and Participants: A UK population comprising 255 parent-offspring trios, 185 additional cases, and 1062 control subjects (cases and controls all British/Irish) as well as 1599 women from a northern Finland population-based birth cohort characterized for PCO symptomatology and testosterone levels. VNTR class was inferred from genotyping of the -23HphI variant. Intervention(s): None. Main Outcome Measure(s): INS-VNTR genotype frequencies between subject groups, body mass index, and testosterone levels by genotype. Results: Case-control analyses in both UK and Finnish samples failed to confirm previously reported class III allele associations with PCOS (UK, $P = 0.43$, Finnish, $P = 0.31$; Kruskal-Wallis $\{\chi^2\}$). Transmission analysis in trios showed no excess transmission of either allele ($P = 0.62$), regardless of parent of origin (maternal: $P = 0.73$; paternal: $P = 0.66$). No association between genotype and testosterone levels was seen in any sample (UK PCOS subjects, $P = 0.95$; Finnish symptomatic cases, $P = 0.38$; Finnish control women, $P = 0.58$). Conclusions: Despite the strong biological candidacy and supportive data from previous studies, we conclude that variation at the INS-VNTR has no major role in the development of PCOS.

Reed, B. Y., W. L. Gitomer, et al. (2002). "Identification and Characterization of a Gene with Base Substitutions Associated with the Absorptive Hypercalciuria Phenotype and Low Spinal Bone Density." *J. Clin. Endocrinol. Metab.* **87**(4): 1476-1485.

<http://jcem.endojournals.org/cgi/content/abstract/87/4/1476>

Absorptive hypercalciuria (AH) is a kidney stone-forming condition frequently complicated by bone loss. Previously, we mapped the locus for an inherited form of AH to chromosome 1q23.3-q24. We have sequenced a putative gene (subsequently shown by others to be homologous with the rat soluble adenylate cyclase gene) in this region in 12 unrelated Caucasian AH patients. Eighteen base substitutions were identified in the soluble adenylate cyclase human homolog gene. All sequence variations were further evaluated in 3-68 additional unrelated AH patients and 19-132 normal subjects, and 1 additional base substitution was identified. Six of the identified sequence variations occurred with increased frequency in the AH population and tracked with the AH phenotype in AH families. Calculated odds ratios showed that the occurrence of any 4 of these individual base substitutions was associated with a 2.2- to 3.5-fold increase in estimated risk for AH ($P < 0.02$). In addition, 1 or more base changes was associated with a lower L2-L4 vertebral bone density. Sequence analysis of 3 other genes within the AH linkage interval showed no difference in the distribution of sequence variations between AH and normal populations. This is the first description of a specific gene defect associated with AH.

Rossi, G. P., P. G. Andreis, et al. (2002). "Endothelin-1[1-31]: A Novel Autocrine-Paracrine Regulator of Human Adrenal Cortex Secretion and Growth." *J. Clin. Endocrinol. Metab.* **87**(1): 322-328.

<http://jcem.endojournals.org/cgi/content/abstract/87/1/322>

Endothelin (ET)-1[1-21] stimulates steroid secretion and zona glomerulosa growth and is expressed in the human and rat adrenal cortex together with its receptor subtypes A and B (ETA and ETB). Although ET-1[1-21] is generated from bigET-1 by an ET-converting enzyme (ECE-1), there is evidence of an alternative chymase-mediated biosynthetic pathway leading to the production of an ET-1[1-31] peptide, the role of which in adrenal pathophysiology is largely unknown. Gene expression and immunohistochemical studies allowed localization of chymase in the normal human adrenal cortex. Sizable amounts, not only of ET-1[1-21] but also of ET-1[1-31], were found in the adrenal vein plasma of three patients. ET-1[1-21] and ET-1[1-31] elicited a clear-cut secretory response by dispersed human adrenocortical cells, ET-1[1-31] being significantly less potent than ET-1[1-21]. The secretagogue effect of ET-1[1-31] was abolished by the ETA receptor antagonist BQ-123 and was unaffected by the ETB receptor antagonist BQ-788. Because, in humans, the secretagogue effect of ET-1[1-21] involves both ETA and ETB receptors, the weaker action of ET-1[1-31] could be attributable to a selective ETA receptor activation. Two lines of evidence support this contention: 1) ET-1[1-31] was more effective than ET-1[1-21] in stimulating ETA-mediated cell proliferation of human adrenocortical cells cultured in vitro; and 2) autoradiography showed that a) ET-1[1-31] displaced in vitro [^{125} I]ET-1[1-21] binding to the ETA, but not ETB receptors, in human internal thoracic artery rings; and b) BQ-123, but not BQ-788, eliminated [^{125} I]ET-1[1-31] binding in the rat adrenal cortex.

Sales, K. J., S. Battersby, et al. (2004). "Prostaglandin E2 Mediates Phosphorylation and Down-Regulation of the Tuberous Sclerosis-2 Tumor Suppressor (Tuberin) in Human Endometrial Adenocarcinoma Cells via the Akt Signaling Pathway." *J. Clin. Endocrinol. Metab.* **89**(12): 6112-6118.

<http://jcem.endojournals.org/cgi/content/abstract/89/12/6112>

Prostaglandin (PG) E2 promotes tumor growth via interaction with its G protein-coupled receptors and activation of intracellular signaling. Tuberous sclerosis 2 (tuberin) is a tumor suppressor, which negatively regulates cell growth. Its phosphorylation results in its inactivation and targeted down-regulation, thus lifting the growth inhibition effects. This study investigated the expression and localization of tuberin in neoplastic and normal endometrium and the effect of PGE2 on phosphorylation of tuberin via the Akt pathway. Quantitative RT-PCR and Western blot analysis

demonstrated reduced expression of tuberin in neoplastic tissue, compared with normal endometrial tissue. Tuberin expression was localized by immunohistochemistry to the glandular epithelial compartment in neoplastic and normal endometrium. We investigated the effect of PGE2 on phosphorylation of tuberin via the Akt pathway. Treatment of neoplastic and normal endometrium with 100 nM PGE2 enhanced phosphorylated tuberin immunoreactivity in the glandular epithelium. PGE2 also phosphorylated Akt and tuberin in Ishikawa endometrial adenocarcinoma cells, leading to a reduction in expression of total tuberin protein. Cotreatment of cells with wortmannin or LY294002 inhibited the PGE2-induced phosphorylation of Akt and tuberin. These data suggest that PGE2 signaling may promote endometrial tumorigenesis by inactivation of tuberin after its phosphorylation via the Akt signaling pathway.

Sales, K. J., S. A. Milne, et al. (2004). "Expression, Localization, and Signaling of Prostaglandin F2{alpha} Receptor in Human Endometrial Adenocarcinoma: Regulation of Proliferation by Activation of the Epidermal Growth Factor Receptor and Mitogen-Activated Protein Kinase Signaling Pathways." J. Clin. Endocrinol. Metab. **89**(2): 986-993.

<http://jcem.endojournals.org/cgi/content/abstract/89/2/986>

Prostaglandin F2{alpha}(PGF2{alpha}) is a bioactive lipid biosynthesized by cyclooxygenase (COX) enzymes and mediates its biological activity via the heptahelical Gq-coupled PGF2{alpha}receptor (FP receptor). This study investigated the expression and molecular signaling of the FP receptor in human endometrial adenocarcinomas. Real-time RT-PCR and Western blot analysis confirmed FP receptor expression in endometrial adenocarcinoma of all grades and differentiation. The expression of FP receptor was up-regulated in all endometrial adenocarcinomas compared with normal endometrium. The site of FP receptor expression was localized by in situ hybridization and immunohistochemistry to the neoplastic epithelial cells in all adenocarcinomas. Treatment of endometrial adenocarcinoma explants with PGF2{alpha} resulted in mobilization of inositol phosphate signaling, indicating functional FP receptor expression. We investigated whether PGF2{alpha} could trans-activate the epidermal growth factor receptor (EGFR) and trigger the MAPK signaling pathway. Treatment of adenocarcinoma explants and endometrial adenocarcinoma cells (Ishikawa) with PGF2{alpha}-phosphorylated EGFR, triggered MAPK signaling and enhanced the proliferation of Ishikawa cells. Inactivation of phospholipase C, EGFR kinase, and MAPK kinase with specific inhibitors abolished PGF2{alpha}-induced trans-activation of EGFR, MAPK signaling, and Ishikawa cell proliferation. These data suggest that PGF2{alpha}-FP receptor promote endometrial tumorigenesis via a phospholipase C-mediated phosphorylation of the EGFR and MAPK signaling pathways.

Salvatore, G., R. Giannini, et al. (2004). "Analysis of BRAF Point Mutation and RET/PTC Rearrangement Refines the Fine-Needle Aspiration Diagnosis of Papillary Thyroid Carcinoma." J. Clin. Endocrinol. Metab. **89**(10): 5175-5180.

<http://jcem.endojournals.org/cgi/content/abstract/89/10/5175>

Point mutations in BRAF are genetic hallmarks of papillary thyroid carcinoma (PTC). In this retrospective study, we examined thyroid aspirates and corresponding paraffin-embedded surgical samples for the presence of BRAF mutations. Altogether, we examined 96 cases, including 69 PTCs, 19 follicular adenomas, and eight nontoxic nodular goiters for BRAF; 60 of these samples were also examined for RET/PTC rearrangements. The results were correlated with the cytological diagnosis and the final histopathology. The BRAF mutation (V599E) was detected in 38% of the samples that were PTC on histopathology; RET/PTC was found in 18% of the PTC cases. In all the cases, the presence of the genetic alteration was confirmed in the surgically resected tumor. The identification of BRAF mutation and RET/PTC refined the

diagnosis of PTC in five of 15 samples that were considered either indeterminate or insufficient at cytology. No mutation was found in aspirates of follicular adenomas and nontoxic nodular goiters. These results indicate that BRAF mutation and RET/PTC rearrangements are molecular markers of PTC that can be applied to FNA in adjunct to traditional cytology.

Segersten, U., P. Correa, et al. (2002). "25-Hydroxyvitamin D3-1{alpha}-Hydroxylase Expression in Normal and Pathological Parathyroid Glands." J. Clin. Endocrinol. Metab. **87**(6): 2967-2972.

<http://jcem.endojournals.org/cgi/content/abstract/87/6/2967>

Active vitamin D, 1,25-dihydroxyvitamin D3 [1,25(OH)2D3], plays a pivotal role in calcium homeostasis and bone metabolism. Circulating levels of 1,25(OH)2D3 are thought to be dependent mainly on the activity of the renal cytochrome P450 enzyme 25-hydroxyvitamin D3-1{alpha}-hydroxylase (1{alpha}-hydroxylase), which is potently induced by PTH. However, 1{alpha}-hydroxylase activity or expression has also been reported at several extrarenal sites, at which local synthesis of 1,25(OH)2D3 appears to fulfill autocrine or paracrine functions. This includes tissues such as placenta and brain that also express LRP-2/megalin, an endocytic receptor for multiple ligands, which is involved in the renal uptake of the substrate for 1{alpha}-hydroxylase, 25-hydroxyvitamin D3. We have previously demonstrated LRP-2/megalin in parathyroid cells, and here we present results from RT-PCR and immunohistochemical analyses showing coincident expression of 1{alpha}-hydroxylase in normal and pathological parathyroid tissue. With real-time quantitative RT-PCR analysis, the expression of 1{alpha}-hydroxylase mRNA was higher in the majority of parathyroid adenomas and secondary hyperplastic glands but lower in parathyroid carcinomas, compared with normal parathyroid tissue. The findings imply that in addition to feedback control by circulating 1,25(OH)2D3 levels, parathyroid cells may also be influenced by local 1{alpha}-hydroxylase activity with possible growth regulatory and differentiating effects.

Shahid, M., V. S. Dhillon, et al. (2005). "Three New Novel Point Mutations Localized within and Downstream of High-Mobility Group-box Region in SRY Gene in Three Indian Females with Turner Syndrome." J. Clin. Endocrinol. Metab. **90**(4): 2429-2435.

<http://jcem.endojournals.org/cgi/content/abstract/90/4/2429>

Point mutations and deletions in the SRY gene result in XY sex reversal in pure gonadal dysgenesis. To date, a majority of these affect the high-mobility group (HMG) domain of SRY, which plays a key role in its DNA binding activity. We carried out molecular genetics studies in three Turner syndrome patients all presenting with 45,X/46,XY mosaic karyotype. Case 1 demonstrated an insertion of T (thymine) within helix I of HMG box leading to frame shift mutation (N82X). In case 2, insertion of A (adenine) downstream of HMG box resulted in a nonsense frame shift mutation (L159fsX167). These mutations resulted in truncated and altered proteins. In case 3, G>C missense mutation is found at codon 74 within helix I of HMG box (Q74H). No other mutations were found in the SRY gene of these patients. An allele-specific oligonucleotide study further confirmed that these variants are not common polymorphisms. To our knowledge, this is the first time these mutations are described at these codons resulting in mutated SRY proteins. Lack of a second sex chromosome in a majority of cells [mosaic karyotype and mutation(s) in the SRY gene] in these patients may have triggered the short stature.

Shattuck, T. M., J. Costa, et al. (2002). "Mutational Analysis of Smad3, a Candidate Tumor Suppressor

Implicated in TGF- β and Menin Pathways, in Parathyroid Adenomas and Enteropancreatic Endocrine Tumors." J. Clin. Endocrinol. Metab. **87**(8): 3911-3914.

<http://jcem.endojournals.org/cgi/content/abstract/87/8/3911>

Based upon molecular allelotyping and comparative genomic hybridization studies, chromosome 15q is the likely location of a tumor suppressor gene important in the pathogenesis of sporadic enteropancreatic endocrine tumors and parathyroid adenomas. Interest has focused on Smad3 as a candidate endocrine tumor suppressor gene because 1) it is localized to 15q and 2) it encodes a TGF β signaling molecule that has been identified as a binding partner of the multiple endocrine neoplasm type 1 gene product menin, itself involved in enteropancreatic and parathyroid neoplasia. To determine whether Smad3 plays a primary role in development of these tumors, 20 enteropancreatic tumors and 67 parathyroid adenomas were investigated for loss of heterozygosity at DNA markers surrounding Smad3. Twenty percent of enteropancreatic tumors and 24% of parathyroid adenomas showed loss. All 9 coding exons and intron-exon boundaries of the Smad3 gene were then sequenced in genomic DNA from all 20 enteropancreatic and 25 parathyroid tumors, including every case with loss of heterozygosity. No acquired clonal mutations, insertions, or microdeletions in Smad3 were detected in any tumors. Because inactivating somatic mutation is the hallmark of an authentic tumor suppressor, Smad3 is unlikely to function as a classical tumor suppressor gene in the pathogenesis of sporadic parathyroid or enteropancreatic endocrine tumors.

Slawik, M., N. Reisch, et al. (2004). "Characterization of an Adrenocorticotropin (ACTH) Receptor Promoter Polymorphism Leading to Decreased Adrenal Responsiveness to ACTH." J. Clin. Endocrinol. Metab. **89**(7): 3131-3137.

<http://jcem.endojournals.org/cgi/content/abstract/89/7/3131>

The ACTH receptor has a pivotal role in the regulation of adrenal cortisol secretion. Here, we describe a polymorphism within the transcription initiation site of the ACTH receptor promoter altering the consensus sequence from CTC to CCC. The prevalence of the polymorphism in 1266 unrelated healthy men was 80.2% for CTC/CTC, 19.0% for CTC/CCC, and 0.8% for CCC/CCC, respectively. In vitro studies using luciferase assays demonstrated a lower basal (CCC, 73 $\pm$ 4%; CTC, 100 $\pm$ 5%; $P = 0.02$) and forskolin-stimulated (CCC, 143 $\pm$ 13%; CTC, 194 $\pm$ 15%; $P = 0.0008$) promoter activity in the CCC construct compared with CTC. The clinical significance of the in vitro findings was investigated by a 6-h ACTH stimulation test with increasing ACTH1-24 doses in normal subjects, demonstrating a blunted cortisol response in CCC/CCC subjects compared with CTC/CTC individuals (area under the curve, 12176 $\pm$ 966; 16334 $\pm$ 1051 nmol/liter $\cdot$ min; $P < 0.03$). Accordingly, after CRH stimulation, subjects with CCC/CCC showed a higher ACTH/cortisol ratio ($P < 0.05$) suggesting a decreased adrenal responsiveness to endogenous ACTH. In conclusion, we describe an ACTH receptor promoter polymorphism that results in a lower promoter activity in vitro and is associated with a lower cortisol secretion to prolonged ACTH stimulation in vivo. This polymorphism might influence cortisol homeostasis under stress conditions.

Sooranna, S. R., N. Engineer, et al. (2005). "The MAPK dependent expression of prostaglandin H synthase-2 and interleukin 8 mRNA by myometrial cells: the differential effect of stretch and interleukin-1 β ." J. Clin. Endocrinol. Metab.: jc.2004-1390.

<http://jcem.endojournals.org/cgi/content/abstract/jc.2004-1390v1>

Infection and uterine stretch are the common causes of preterm labor. Interleukin (IL)-1 β plays a key role in infection-induced preterm labor and increases prostaglandin H synthase 2 (PGHS-2) and IL-8 expression. We have shown that mechanical stretch of uterine myocytes in vitro up-regulates the expression of PGHS-2 and IL-8. In this study, we have tested the hypotheses that both IL-1 β and mechanical stretch increase the myometrial expression of PGHS-2 and IL-8 via MAPK activation and that their effects are synergistic. MAPK activation was assessed in myocytes obtained from pregnant women undergoing caesarean section before the onset of labor after exposure to IL-1 β and stretch either alone or in combination. Specific inhibitors of ERK, p38 and JNK were used to define the role of each in the increased expression of PGHS-2 and IL-8 mRNA. We found that both IL-1 β and stretch activated all 3 MAPK subtypes but that they had no synergistic effect. The inhibitor studies showed that stretch-induced increases in both PGHS-2 and IL-8 mRNA expression were ERK1/2 and p38-dependent and that IL-1 β -induced increases of PGHS-2 mRNA expression were also ERK1/2 and p38-dependent, but those of IL-8 were only dependent on ERK1/2 activation. These data show that exposure of human uterine myocytes to both stretch and IL-1 β activates the MAPK system which is responsible for the increase in PGHS-2 and IL-8 mRNA expression. We found no evidence of a synergistic effect of IL-1 β and stretch on myometrial expression of PGHS-2 and IL-8 mRNA.

Specht, M. C., O. N. Tucker, et al. (2002). "Cyclooxygenase-2 Expression in Thyroid Nodules." J. Clin. Endocrinol. Metab. **87**(1): 358-363.

<http://jcem.endojournals.org/cgi/content/abstract/87/1/358>

Factors contributing to the development of thyroid neoplasia remain poorly understood. Recent evidence indicates that overexpression of the inducible cyclooxygenase, COX-2, is important in the pathogenesis of epithelial carcinomas. These studies were undertaken to evaluate whether COX-2 is up-regulated in human thyroid neoplasia. Benign (n = 14), and malignant (n = 14) thyroid nodules were analyzed for expression of COX-2 mRNA by quantitative RT-PCR. Immunoblotting and immunohistochemistry were performed on representative samples. Three human thyroid cancer cell lines were similarly analyzed for COX-2 expression. Levels of COX-2 mRNA were significantly increased in thyroid nodule samples compared with adjacent thyroid tissue in the malignant specimens but not in the benign specimens. Additionally, COX-2 mRNA levels were significantly increased in malignant nodule samples compared with benign nodule samples. COX-2 protein expression was higher in 8 of 10 thyroid nodules compared with the adjacent tissue. Immunohistochemical analysis localized expression of COX-2 to the malignant epithelial cells. Immunofluorescence demonstrated COX-2 protein expression in all three thyroid cell lines. Finally, COX-2 expression could be detected by RT-PCR in fine needle aspiration specimens of thyroid nodules. These data indicate that COX-2 is up-regulated in human thyroid cancer, but not in benign thyroid nodules, and suggest that COX-2 expression may serve as a marker of malignancy in thyroid nodules.

Steinberg, G. R., A. C. Smith, et al. (2004). "AMP-Activated Protein Kinase Is Not Down-Regulated in Human Skeletal Muscle of Obese Females." J. Clin. Endocrinol. Metab. **89**(9): 4575-4580.

<http://jcem.endojournals.org/cgi/content/abstract/89/9/4575>

Obesity in humans is associated with lipid accumulation in skeletal muscle, insulin and leptin resistance, and type 2 diabetes. AMP-activated protein kinase (AMPK) is an important regulator of fatty acid (FA) metabolism in skeletal muscle. To address the hypothesis that lipid accumulation in skeletal muscle of obese subjects may be due to down-regulation of AMPK, we measured mRNA and protein levels of AMPK isoforms, AMPK α 1 and - α 2 activity,

AMPK kinase activity, acetyl-coenzyme A carboxylase (ACC{beta}) expression and phosphorylation, and FA metabolism in biopsies of rectus abdominus muscle from lean and obese women. We also examined the effect of 5-aminoimidazole-4-carboxamide riboside (AICAR) on AMPK activity and the effects of AICAR and leptin on FA metabolism. Skeletal muscle of obese subjects had increased total FA uptake and triglyceride esterification, and leptin failed to stimulate FA oxidation. However, AMPK mRNA and protein expression, AMPK{alpha}1 and -{alpha}2 activities, AMPK kinase activity, ACC{beta} phosphorylation, and FA oxidation were similar in lean and obese subjects. Moreover, AICAR increased AMPK{alpha}2 activity, ACC{beta} phosphorylation, and palmitate oxidation to a similar degree in muscle from lean and obese subjects. We conclude that the abnormal lipid metabolism and leptin resistance of skeletal muscle of obese subjects is not due to down-regulation of AMPK. In addition, the similar stimulation by AICAR of AMPK in skeletal muscle of lean and obese subjects suggests that direct pharmacological activation of AMPK may be a therapeutic approach for stimulating FA oxidation in the treatment of human obesity.

Syed, A. A., J. A. E. Irving, et al. (2004). "Low Prevalence of the N363S Polymorphism of the Glucocorticoid Receptor in South Asians Living in the United Kingdom." J. Clin. Endocrinol. Metab. **89**(1): 232-235.

<http://jcem.endojournals.org/cgi/content/abstract/89/1/232>

Similarities between clinical states of glucocorticoid excess and obesity have raised suspicion of a link between the two conditions. An Asn363Ser (N363S) polymorphism in exon 2 of the glucocorticoid receptor has been associated with glucocorticoid sensitivity and excess adiposity in people of European origin. Compared with Europid populations, South Asians have a higher prevalence of cardiovascular risk factors, including type 2 diabetes and central obesity. The aim of this study was to determine the prevalence of the 363S allele in people of South Asian origin living in northeast England in relation to obesity and other cardiovascular risk factors. DNA from 142 males and 153 females was characterized for 363S allele status. Two N363S heterozygotes were identified; both subjects had raised body mass index and central obesity. Despite a higher prevalence of overweight (body mass index [IMG]=" BORDER="0"> 25 kg/m²) people in the South Asian group compared with the Europid population in the same geographical area (66 vs. 56%, respectively), the 363S allele frequency was significantly lower in the South Asian group (0.3 vs. 3%, respectively). Therefore, the N363S polymorphism is unlikely to be an important factor in obesity and/or dysmetabolic traits in people of South Asian origin living in the United Kingdom.

Taillandier, A., S. L. Sallinen, et al. (2005). "Childhood Hypophosphatasia Due to a de Novo Missense Mutation in the Tissue-Nonspecific Alkaline Phosphatase Gene." J. Clin. Endocrinol. Metab. **90**(4): 2436-2439.

<http://jcem.endojournals.org/cgi/content/abstract/90/4/2436>

Hypophosphatasia is an inherited disorder due to mutations in the bone alkaline phosphatase (ALPL) gene. We report here a patient with childhood hypophosphatasia diagnosed at 1.4 yr because of pectus excavatum, large anterior fontanel, rachitic skeletal changes, and low serum alkaline phosphatase. Sequencing of the ALPL gene produced evidence of two distinct missense mutations, E174K (c.571G>A), of maternal origin, and a de novo mutation, M45I (c.186G>C). The study of various microsatellite polymorphisms ruled out false paternity and therefore confirmed that M45I occurred de novo in the paternal germline or in the early development of the patient. Site-directed mutagenesis showed that M45I results in the absence of in vitro alkaline phosphatase activity, suggesting that the mutation is a severe allele. In conclusion, childhood

hypophosphatasia in this patient is the result of compound heterozygosity for the moderate mutation E174K and a novel severe de novo mutation M45I.

Tan, Y. M., J. Cardinal, et al. (2003). "Autosomal Dominant Hypocalcemia: A Novel Activating Mutation (E604K) in the Cysteine-Rich Domain of the Calcium-Sensing Receptor." J. Clin. Endocrinol. Metab. **88**(2): 605-610.

<http://jcem.endojournals.org/cgi/content/abstract/88/2/605>

We report a novel activating mutation (E604K) of the calcium-sensing receptor in a family with autosomal dominant hypocalcemia. Whereas all affected individuals exhibited marked hypocalcemia, some cases with untreated hypocalcemia exhibited seizures in infancy, whereas others were largely asymptomatic from birth into adulthood. The missense mutation E604K (G2182A; GenBank accession no. U20759), which affects an amino acid residue in the C terminus of the cysteine-rich domain of the extracellular head, cosegregated with hypocalcemia in all seven individuals for whom DNA was available. Two unaffected, normocalcemic members of the family did not exhibit the mutation. The molecular impact of the mutation on two key components of the signaling response was assessed in HEK-293 cells transiently transfected with cDNA corresponding to either the wild-type calcium-sensing receptor or the E604K mutation derived by site-directed mutagenesis. There was a significant leftward shift in the concentration response curves for the effects of extracellular Ca²⁺ on both intracellular Ca²⁺ mobilization (determined by aequorin luminescence) and MAPK activity (determined by luciferase expression). The C terminus of the cysteine-rich domain of the extracellular head may normally act to suppress receptor activity in the presence of low extracellular Ca²⁺ concentrations.

Tanaka, K., H. Minoura, et al. (2003). "Ghrelin Is Involved in the Decidualization of Human Endometrial Stromal Cells." J. Clin. Endocrinol. Metab. **88**(5): 2335-2340.

<http://jcem.endojournals.org/cgi/content/abstract/88/5/2335>

Successful implantation involves a complex interaction between the endometrium and the embryo. It is well known that several neuropeptides are expressed in the endometrium and placenta during embryonal implantation, suggesting an important role as chemical mediators of the fetomaternal relationship. Ghrelin has recently been identified as the endogenous ligand for the GH secretagogue receptor. Ghrelin is a peptide hormone with many physiological functions, and its expression in the human placenta has been reported. To investigate the involvement of ghrelin in embryonal implantation, we assessed the spatio-temporal expression pattern of ghrelin and its receptor in the human endometrium and placenta through the normal menstrual cycle and in early pregnancy. We also examined the effect of ghrelin on the decidualization of endometrial stromal cells (ESC). Weak expression of ghrelin mRNA was detected in the nonpregnant endometrium, and it was dramatically increased in the decidualized endometrium. A GH secretagogue receptor mRNA was detected in the endometrium throughout the normal menstrual cycle and in early pregnancy, but not in the first trimester placenta. Immunohistochemical analysis using an antighrelin antibody revealed strong signals in decidual cells and extravillous trophoblast cells. Coculture with first trimester placenta up-regulated ghrelin mRNA expression by primary cultured ESC, although sex steroids and 8-bromo-cAMP had no effect. In addition, ghrelin enhanced the decidualization of ESC induced by 8-bromo-cAMP (8-Br-cAMP) in vitro. Thus, ghrelin is a novel paracrine/autocrine factor that is involved in cross-talk between the endometrium and embryo during embryonal implantation.

Tomlinson, J. W., J. S. Moore, et al. (2004). "Weight Loss Increases 11 β -Hydroxysteroid Dehydrogenase Type 1 Expression in Human Adipose Tissue." *J. Clin. Endocrinol. Metab.* **89**(6): 2711-2716.

<http://jcem.endojournals.org/cgi/content/abstract/89/6/2711>

The global epidemic of obesity has heightened the need to understand the mechanisms that underpin its pathogenesis. Clinical observations in patients with Cushing's syndrome have highlighted the link between cortisol and central obesity. However, although circulating cortisol levels are normal or reduced in obesity, local regeneration of cortisol, from inactive cortisone, by 11 β -hydroxysteroid dehydrogenase type 1 (11 β HSD1) has been postulated as a pathogenic mechanism. Although levels of expression of 11 β HSD1 in adipose tissue in human obesity are debated in the literature, global inhibition of 11 β HSD1 improves insulin sensitivity. We have determined the effects of significant weight loss on cortisol metabolism and adipose tissue 11 β HSD1 expression after 10-wk ingestion of a very low calorie diet in 12 obese patients (six men and six women; body mass index, 35.9 $\pm$ 0.9 kg/m²; mean $\pm$ SE). All patients achieved significant weight loss (14.1 $\pm$ 1.3% of initial body weight). Total fat mass fell from 41.8 $\pm$ 1.9 to 32.0 $\pm$ 1.7 kg (P < 0.0001). In addition, fat-free mass decreased (64.4 $\pm$ 3.4 to 58.9 $\pm$ 2.9 kg; P < 0.0001) and systolic blood pressure and total cholesterol also fell [systolic blood pressure, 135 $\pm$ 5 to 121 $\pm$ 5 mm Hg (P < 0.01); total cholesterol, 5.4 $\pm$ 0.2 to 4.8 $\pm$ 0.2 mmol/liter (P < 0.05)]. The serum cortisol/cortisone ratio increased after weight loss (P < 0.01). 11 β HSD1 mRNA expression in isolated adipocytes increased 3.4-fold (P < 0.05). Decreased 11 β HSD1 activity and expression in obesity may act as a compensatory mechanism to enhance insulin sensitivity through a reduction in tissue-specific cortisol concentrations. Inhibition of 11 β HSD1 may therefore be a novel, therapeutic strategy for insulin sensitization.

Tomlinson, J. W., B. Sinha, et al. (2002). "Expression of 11 β -Hydroxysteroid Dehydrogenase Type 1 in Adipose Tissue Is Not Increased in Human Obesity." *J. Clin. Endocrinol. Metab.* **87**(12): 5630-5635.

<http://jcem.endojournals.org/cgi/content/abstract/87/12/5630>

Central obesity is associated with increased morbidity and mortality. Preadipocyte proliferation and differentiation contribute to increases in adipose tissue mass, yet the mechanisms that underlie these processes remain unclear. Patients with glucocorticoid excess develop a reversible form of central obesity, but circulating cortisol levels in idiopathic obesity are invariably normal. We have hypothesized that the enzyme 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1), by converting inactive cortisone to active cortisol in adipose tissue, might be an important autocrine regulator of fat mass. Paired omental and sc fat biopsies were obtained from 32 women (median age, 43 yr; range, 28-65; median body mass index, 27.5 kg/m²; range, 19.7-39.2) undergoing elective abdominal surgery. 11 β -HSD1 activity and mRNA levels were assessed in whole tissue and in isolated preadipocytes and adipocytes using specific enzyme assays and real-time PCR. Preadipocyte proliferation was measured using tritiated thymidine incorporation. Whole adipose tissue 11 β -HSD1 mRNA levels did not differ between omental and sc samples (P = 0.73). In addition, mRNA levels did not correlate with body mass index (omental: r = 0.1; P = 0.6; sc: r = 0.15; P = 0.4). In keeping with earlier studies, 11 β -HSD1 mRNA levels were higher in omental compared with sc preadipocytes. However, in cultured omental preadipocytes, 11 β -HSD1 activity inversely correlated with body mass index (r = -0.47; P = 0.03). In omental preadipocytes, both cortisol and cortisone decreased proliferation (P < 0.05). Inhibition of 11 β -HSD1 with glycyrrhetic acid partially reversed the cortisone-induced decrease in preadipocyte proliferation (P < 0.05). Enhanced preadipocyte proliferation within omental adipose tissue as a consequence of decreased 11 β -HSD1 mRNA levels and activity may contribute to increases in visceral adipose tissue mass in obese patients.

Tsatsaris, V., F. Goffin, et al. (2003). "Overexpression of the Soluble Vascular Endothelial Growth Factor Receptor in Preeclamptic Patients: Pathophysiological Consequences." J. Clin. Endocrinol. Metab. **88**(11): 5555-5563.

<http://jcem.endojournals.org/cgi/content/abstract/88/11/5555>

Several growth factors such as vascular endothelial growth factor (VEGF)-A and placental growth factor (PIGF) are involved in the placental vascular development. We investigated whether dysregulation in the VEGF family may explain the defective uteroplacental vascularization characterizing preeclampsia. We compared pregnancies complicated by early onset severe preeclampsia or intrauterine growth retardation to normal pregnancies. Maternal plasma, placentas, and placental bed biopsies were collected. The mRNA levels of VEGF-A, PIGF, and their receptors were quantified in placentas and placental beds. Levels of VEGF-A, PIGF, and soluble VEGF receptor (VEGFR) were assessed in maternal plasma. In compromised pregnancies, elevated levels of VEGF-A and VEGFR-1 mRNAs may reflect the hypoxic status of the placenta. On contrast, the membrane-bound VEGFR-1 was decreased in the placental bed of preeclamptic patients. Preeclampsia was associated with low levels of circulating PIGF and increased levels of total VEGF-A and soluble VEGFR-1. Free VEGF-A was undetectable in maternal blood. Immunohistochemical studies revealed that VEGF-A and PIGF were localized in trophoblastic cells. Altogether, our results suggest two different pathophysiological mechanisms associated with preeclampsia. The first one is related to an overproduction of competitive soluble VEGFR-1 that may lead to suppression of VEGF-A and PIGF effects. The second one is the down-regulation of its membrane bound form (VEGFR-1) in the placental bed, which may result in the defective uteroplacental development.

Tschugguel, W., W. Dietrich, et al. (2003). "Differential Regulation of Proteasome-Dependent Estrogen Receptor {alpha} and {beta} Turnover in Cultured Human Uterine Artery Endothelial Cells." J. Clin. Endocrinol. Metab. **88**(5): 2281-2287.

<http://jcem.endojournals.org/cgi/content/abstract/88/5/2281>

Estrogen-induced loss of estrogen receptor (ER) {alpha} expression limits estrogen responsiveness in many target cells. However, whether such a mechanism contributes to changes in vascular endothelial ER{alpha} and/or ER{beta} levels is unclear. Using RT-PCR assays, we did not find any regulation of ER{alpha} or ER{beta} mRNA expression in human uterine artery endothelial cell (HUAEC) nuclear extracts on stimulation with 17{beta}-estradiol for 1 or 2 h. By contrast, Western analysis on HUAEC extracts revealed that 17{beta}-estradiol was capable of down-regulating both ER{alpha} and ER{beta} protein starting 1 h after treatment, an effect that can be blocked by pretreatment with tamoxifen as well as with the proteasome inhibitor lactacystin. The proteolysis inhibitors insulin, cycloheximide, and puromycin impede ER{alpha}, but not ER{beta}, turnover. Ubiquitin, but not its competitive inhibitor methyl-ubiquitin, induces rapid turnover of both ERs in a cell-free system of MCF-7 and HUAEC extracts. We, thus, propose the existence of estrogen-induced ER degradation that serves to control physiological responses in an estrogen target tissue, i.e. human vascular endothelium, by down-regulating ER{alpha} as well as ER{beta} through different proteasomal uptake mechanisms.

van Tilburg, J. H. O., L. A. Sandkuijl, et al. (2003). "A Genome-Wide Scan in Type 2 Diabetes Mellitus Provides Independent Replication of a Susceptibility Locus on 18p11 and Suggests the Existence of Novel Loci on 2q12 and 19q13." J. Clin. Endocrinol. Metab. **88**(5): 2223-2230.

<http://jcem.endojournals.org/cgi/content/abstract/88/5/2223>

A genome-wide scan was performed, using nonparametric linkage analyses, to find susceptibility loci for type 2 diabetes mellitus in the Dutch population. We studied 178 families from The Netherlands, who constituted 312 affected sibling pairs. The first stage of the genome scan consisted of 270 DNA markers, with an average intermarker spacing of 13 cM. Because obesity and type 2 diabetes mellitus are interrelated, the data set was stratified for the subphenotype body mass index, corrected for age and gender. This resulted in a suggestive maximum multipoint LOD score of 2.3 (single-point P value, 9.7×10^{-4} ; genome-wide P value, 0.028) for the most obese 20% pedigrees of the data set, between marker loci D18S471 and D18S843. In the lowest 80% obese pedigrees, two interesting loci on chromosome 2 and 19 were found, with LOD scores of 1.5 and 1.3. We provide independent evidence that the chromosome 18p11 locus, reported earlier from a Finnish/Swedish population, is of definite interest for type 2 diabetes mellitus in connection with obesity. Subsequently, our results indicate that two novel loci may reside on chromosomes 2 and 19, with minor effects involved in the development of type 2 diabetes mellitus in the Dutch population.

Vanttinen, T., T. Kuulasmaa, et al. (2002). "Expression of Activin/Inhibin Receptor and Binding Protein Genes and Regulation of Activin/Inhibin Peptide Secretion in Human Adrenocortical Cells." J. Clin. Endocrinol. Metab. **87**(9): 4257-4263.

<http://jcem.endojournals.org/cgi/content/abstract/87/9/4257>

Activins and inhibins are glycoprotein hormones produced mainly in gonads but also in other organs. They are believed to be important para/autocrine regulators of various cell functions. We investigated activin/inhibin receptor and binding protein gene expression and the regulation of activin/inhibin secretion in human adrenal cells. RT-PCR revealed inhibin/activin {alpha}-, {beta}A/B-subunit, follistatin, activin type I/II receptor, and inhibin receptor (betaglycan and inhibin-binding protein) mRNA expression in fetal and adult adrenals and cultured adrenocortical cells. Cultured cells secreted activin A and inhibin A/B as determined by specific ELISAs. ACTH stimulated inhibin A/B secretion in fetal (1.8- and 1.8-fold of control, respectively) and in adult cells (3.4- and 1.7-fold of control, respectively) without significant effect on activin A. 8-bromoadenosine cAMP (protein kinase A activator) increased activin A and inhibin A/B secretion in the human adrenocortical NCI-H295R cell line (32-, 17-, and 3-fold of control, respectively). 12-O-tetradecanoyl phorbol-13-acetate (protein kinase C activator) stimulated both activin A and inhibin A secretion (764- and 32-fold of control, respectively), and activin treatment increased inhibin B secretion in these cells (25-fold of control). In conclusion, human adrenocortical cells produce dimeric activins and inhibins. ACTH stimulates inhibin secretion and decreases activin/inhibin secretion ratio, probably via the protein kinase A signal transduction pathway. This, together with the adrenocortical activin/ inhibin receptor and binding protein expression, suggests a physiological role for activins and inhibins in the human adrenal gland.

Vantyghem, M. C., P. Pigny, et al. (2004). "Patients with Familial Partial Lipodystrophy of the Dunnigan Type Due to a LMNA R482W Mutation Show Muscular and Cardiac Abnormalities." J. Clin. Endocrinol. Metab. **89**(11): 5337-5346.

<http://jcem.endojournals.org/cgi/content/abstract/89/11/5337>

Diseases due to mutations in the lamin A/C gene (LMNA) are highly heterogeneous, including neuromuscular and cardiac dystrophies, lipodystrophies, and premature ageing syndromes. In this study we characterized the neuromuscular and cardiac phenotypes of patients bearing the

heterozygous LMNA R482W mutation, which is the most frequent genotype associated with the familial partial lipodystrophy of the Dunnigan type (FPLD). Fourteen patients from two unrelated families, including 10 affected subjects, were studied. The two probands had been referred for lipoatrophy and/or diabetes. Lipodystrophy, exclusively observed in LMNA-mutated patients, was of variable severity and limited to postpubertal subjects. Lipodystrophy and metabolic disturbances were more severe in women, even if an enlarged neck was a constant finding. The severity of hypertriglyceridemia and hirsutism in females was related to that of insulin resistance. Clinical muscular alterations were only present in LMNA-mutated patients. Clinical and histological examination showed an invalidating, progressive limb-girdle muscular dystrophy in a 42-yr-old woman that had been present since childhood, associated with a typical postpubertal FPLD phenotype. Six of eight adults presented the association of calf hypertrophy, perihumeral muscular atrophy, and a rolling gait due to proximal lower limb weakness. Muscular histology was compatible with muscular dystrophy in one of them and/or showed a nonspecific excess of lipid droplets (in three cases). Immunostaining of lamin A/C was normal in the six muscular biopsies. Surprisingly, calpain 3 expression was undetectable in the patient with the severe limb-girdle muscular dystrophy, although the gene did not reveal any molecular alterations. At the cardiac level, cardiac septal hypertrophy and atherosclerosis were frequent in FPLD patients. In addition, a 24-yr-old FPLD patient had a symptomatic second degree atrioventricular block. In conclusion, we showed that most lipodystrophic patients affected by the FPLD-linked LMNA R482W mutation show muscular and cardiac abnormalities. The occurrence and severity of the myopathic and lipoatrophic phenotypes varied and were not related. The muscular phenotype was evocative of limb girdle muscular dystrophy. Cardiac hypertrophy and advanced atherosclerosis were frequent. FPLD patients should receive careful neuromuscular and cardiac examination whatever the underlying LMNA mutation.

Veldhuis, J. D., G. Zhang, et al. (2002). "Troglitazone, an Insulin-Sensitizing Thiazolidinedione, Represses Combined Stimulation by LH and Insulin of de Novo Androgen Biosynthesis by Thecal Cells in Vitro." *J. Clin. Endocrinol. Metab.* **87**(3): 1129-1133.

<http://jcem.endojournals.org/cgi/content/abstract/87/3/1129>

Polycystic ovarian syndrome (anovulatory hyperandrogenism) is marked by adolescent onset of systemic hyperinsulinism, oligoovulation, hirsutism, excessive LH and androgen secretion, and variable reduction in fertility. Insulin and LH are believed to act in concert to promote ovarian androgen hypersecretion in this disorder. Administration of troglitazone, an insulin-sensitizing agent and putative PPAR{gamma} agonist, can decrease hyperinsulinism, suppress T production, and ameliorate oligoovulation in some women with this endocrinopathy. The present study tests the hypothesis that troglitazone directly inhibits de novo androgen biosynthesis stimulated jointly by LH and insulin in primary cultures of (porcine) thecal cells. We show that troglitazone dose-dependently antagonizes LH/insulin's combined stimulation of androstenedione and T production by thecal cells in vitro. Consistent steroidogenic inhibition of 80-95% was achieved at drug concentrations of 3-6.8 {micro}M ($P < 0.001$). Exposure of thecal cells to the thiazolidinedione derivative also blocked bihormonally stimulated accumulation of CYP17 (cytochrome P450 17 {alpha}-hydroxylase/C17-20 lyase) gene expression, as reflected by decreased accumulation of cognate heterogeneous nuclear RNA and mRNA (by 30-65%; $P < 0.05$). Moreover, troglitazone suppressed LH/insulin-induced phosphorylation of the 52-kDa immunoprecipitated CYP17 enzyme by 88% ($P < 0.001$). A putative natural agonist of PPAR{gamma} nuclear transcription, 15-deoxy-{delta}-12,14-prostaglandin J2, also inhibited LH/insulin-driven androstenedione biosynthesis and CYP17 gene expression in thecal cells. In conclusion, a synthetic thiazolidinedione (troglitazone) and a natural ligand of PPAR{gamma} (15-deoxy-{delta}-12,14-prostaglandin J2) effectively impede the concerted stimulation by LH and insulin of in vitro thecal cell androgen production, CYP17 gene expression, and CYP17 protein phosphorylation. This ensemble of inhibitory actions on LH/insulin-stimulated steroidogenesis offers a plausible mechanistic basis for at least part of the observed clinical efficacy of troglitazone in mitigating

androgen excess in women with polycystic ovarian syndrome.

Vella, V., G. Pandini, et al. (2002). "A Novel Autocrine Loop Involving IGF-II and the Insulin Receptor Isoform-A Stimulates Growth of Thyroid Cancer." *J. Clin. Endocrinol. Metab.* **87**(1): 245-254.

<http://jcem.endojournals.org/cgi/content/abstract/87/1/245>

The insulin receptor (IR) occurs in two isoforms (IR-A and IR-B) resulting from alternative splicing of exon 11 of the gene. The IR-A isoform is predominantly expressed in fetal tissues and malignant cells and binds IGF-II with high affinity. We previously observed that IRs are overexpressed in thyroid cancer cells; now we evaluated whether these cells preferentially express IR-A and produce IGF-II, which would activate a growth-promoting autocrine loop. The IR content ranged 6.0-52.6 ng/100 {micro}g cell membrane protein in thyroid cancer primary cultures (n = 8) and permanent cell lines (n = 6) vs. 1.2-1.7 in normal thyroid cells (n = 11 primary cultures; P < 0.0001). IR-A isoform relative abundance ranged from 36-79% in cancer cells (with the highest values in undifferentiated cancers) vs. 27-39% in normal cells. Similar results were obtained in normal vs. cancer thyroid tissue specimens. IGF-II caused IR autophosphorylation with an ED50 of 1.5-40.0 nM in cancer cells vs. more than 100 nM in normal cells; IGF-II affinity correlated with the relative abundance of IR-A (r = 0.628; P < 0.0001). IGF-II was expressed in all cancer cells, highly expressed in anaplastic cells, and less expressed in normal cells. In conclusion, malignant thyrocytes, especially when poorly differentiated, produce IGF-II and overexpress IR, predominantly as IGF-II-sensitive isoform A. A growth-promoting autocrine loop is activated, therefore, and may affect thyroid cancer biology.

Vieira, T. C., M. R. Dias da Silva, et al. (2003). "Familial Combined Pituitary Hormone Deficiency due to a Novel Mutation R99Q in the Hot Spot Region of Prophet of Pit-1 Presenting as Constitutional Growth Delay." *J. Clin. Endocrinol. Metab.* **88**(1): 38-44.

<http://jcem.endojournals.org/cgi/content/abstract/88/1/38>

Combined pituitary hormone deficiency (CPHD) is characterized by impaired production of GH and one or more of the other anterior pituitary hormones. Prophet of Pit-1 (PROP-1), one of the pituitary specific homeodomain transcription factors, is involved in the differentiation of the anterior pituitary cells (somatotrophs, lactotrophs, thyrotrophs, and gonadotrophs), and PROP-1 gene mutations may interfere with the development of these cells, resulting in CPHD. We performed molecular analyses of the PROP-1 gene in two siblings, born to consanguineous parents, who presented with short stature. The index patient, a boy, was initially diagnosed with constitutional growth delay based on familial short stature, low parental target height, normal GH secretion, and imaging of the pituitary gland. On follow-up, auxological data and pubertal delay prompted a thorough reevaluation, which documented GH, TSH, and gonadotropin deficiencies. Direct sequencing of the PROP-1 gene revealed a novel homozygous transition 296G[-&gt]A in exon 2 in the two affected siblings. The mutation substitutes a highly conserved arginine by a glutamine at codon 99 (R99Q) in the second helix of the DNA-binding domain of the PROP-1 protein. Compared with wild-type PROP-1, R99Q displays a significant decrease in DNA binding on a paired box response element (PRDQ9) and trans-activation of a luciferase reporter gene. The findings emphasize the importance of repeated evaluations and illustrate that patients with CPHD associated with PROP-1 mutations present with a phenotypic spectrum, suggesting that the consequences of distinct PROP-1 mutations may be diverse and/or that additional factors, such as modifier genes, may have an impact on their expressivity.

Wang, H.-J., F. Geller, et al. (2004). "Ghrelin Receptor Gene: Identification of Several Sequence Variants in Extremely Obese Children and Adolescents, Healthy Normal-Weight and Underweight Students, and Children with Short Normal Stature." *J. Clin. Endocrinol. Metab.* **89**(1): 157-162.

<http://jcem.endojournals.org/cgi/content/abstract/89/1/157>

GH secretagogue receptor (GHSR, ghrelin receptor) is involved in regulation of body weight and GH secretion. We initially analyzed two single-nucleotide polymorphisms of the GHSR in up to 184 extremely obese children and adolescents and up to 184 healthy underweight students. The frequency of the 171T allele of rs495225 was higher in our obese samples (75.0%) than in the underweight individuals (70.2%; nominal $P = 0.14$). This trend could not be substantiated in an additional association study in 270 obese and 145 underweight and normal weight individuals and in a transmission disequilibrium test based on 387 obesity trios (transmission rate of 171T, 51.8%; nominal $P = 0.53$). Additionally, the coding region of GHSR was systematically screened, and seven sequence variants were identified in 93 obese, 96 normal weight, and 94 underweight individuals and 43 children with short normal stature (SNS). Five silent single-nucleotide polymorphisms showed similar genotype frequencies in the different weight groups and SNS children (all nominal $P > 0.3$). Two novel missense variants were detected only in one obese carrier and one SNS child, respectively. In conclusion, we did not obtain conclusive evidence for an involvement of the ghrelin receptor gene in body weight regulation or SNS in our study groups.

Wang, H., Y. Wen, et al. (2002). "Phospholipase A2 and Cyclooxygenase Gene Expression in Human Preimplantation Embryos." *J. Clin. Endocrinol. Metab.* **87**(6): 2629-2634.

<http://jcem.endojournals.org/cgi/content/abstract/87/6/2629>

Phospholipase A2 (PLA2) and cyclooxygenase (COX) are two key enzymes in PG synthesis; the latter has two forms, COX-1 and COX-2. mRNA was extracted from single preimplantation embryos and examined for PLA2, COX-1, and COX-2 gene expression by RT-PCR to investigate whether PLA2 and COX genes are expressed in human preimplantation conceptuses from zygote to blastocyst stage and to compare COX-1 and COX-2 gene expression within the same stage of embryonic development. Expression of PLA2, COX-1, and COX-2 was detected in 48, 37, and 45%, respectively, of total embryos examined. COX-1 was expressed in approximately 66% of early human preimplantation embryos from zygote to two-cell stage, whereas COX-2 was expressed in about 58% of later stage embryos from eight-cell to blastocyst stage ($P < 0.05$). Furthermore, COX-2 mRNA and protein were localized to trophoctoderm in blastocyst stage embryos. In conclusion, PLA2, COX-1, and COX-2 are expressed during early human embryonic development and may contribute to the production of PGs such as PGE2 in human embryogenesis. COX-1 and COX-2 are differentially expressed, with COX-2 being primarily expressed by trophoctoderm in late-stage human preimplantation embryos, which may promote embryonic differentiation and implantation.

Williams, T. A., P. Mulatero, et al. (2004). "A Particular Phenotype in a Girl with Aldosterone Synthase Deficiency." *J. Clin. Endocrinol. Metab.* **89**(7): 3168-3172.

<http://jcem.endojournals.org/cgi/content/abstract/89/7/3168>

Aldosterone synthase deficiency (ASD) usually presents in infancy as a life-threatening electrolyte imbalance. A 4-wk-old child of unrelated parents was examined for failure to thrive and salt-wasting. Notable laboratory findings were hyperkalemia, high plasma renin, and low-normal aldosterone levels. Urinary metabolite ratios of corticosterone/18-hydroxycorticosterone and 18-

hydroxycorticosterone/aldosterone were intermediate between ASD type I and type II. Sequence analysis of CYP11B2, the gene encoding aldosterone synthase (P450c11AS), revealed that the patient was a compound heterozygote carrying a previously described mutation located in exon 4 causing a premature stop codon (E255X) and a further, novel mutation in exon 5 that also causes a premature stop codon (Q272X). The patient's unaffected father was a heterozygous carrier of the E255X mutation, whereas the unaffected mother was a heterozygous carrier of the Q272X mutation. Therefore, the patient's CYP11B2 encodes two truncated forms of aldosterone synthase predicted to be inactive because they lack critical active site residues as well as the heme-binding site. This case of ASD is of particular interest because despite the apparent lack of aldosterone synthase activity, the patient displays low-normal aldosterone levels, thus raising the question of its source.

Yorifuji, T., K. Kurokawa, et al. (2004). "Neonatal Diabetes Mellitus and Neonatal Polycystic, Dysplastic Kidneys: Phenotypically Discordant Recurrence of a Mutation in the Hepatocyte Nuclear Factor-1{beta} Gene Due to Germline Mosaicism." *J. Clin. Endocrinol. Metab.* **89**(6): 2905-2908.

<http://jcem.endojournals.org/cgi/content/abstract/89/6/2905>

Mutations in the gene coding for hepatocyte nuclear factor-1{beta} (HNF-1{beta}) have been known to cause a form of maturity-onset diabetes of the young (MODY5), which is usually characterized by dominantly inherited adolescence-onset diabetes mellitus associated with renal cysts. This report, however, describes recurrence of a novel missense mutation in the HNF-1{beta} gene, S148W (C443G), in two sibs, one with neonatal diabetes mellitus and the other with neonatal polycystic, dysplastic kidneys leading to early renal failure. The former patient had only a few small renal cysts with normal renal functions, and the latter had only a transient episode of hyperglycemia, which resolved spontaneously. Interestingly, both parents were clinically unaffected, and PCR restriction fragment length polymorphism analysis showed that the mother was a low-level mosaic of normal and mutant HNF-1{beta}, which suggested that the recurrence was caused by germline mosaicism. This is the first report of permanent neonatal diabetes mellitus caused by a mutation of the HNF-1{beta} gene as well as the first report of germline mosaicism of this gene. In addition, the two cases described here show that additional factors, genetic or environmental, can have a significant influence on the phenotypic expression of HNF-1{beta} mutations.

Yorifuji, T., K. Nagashima, et al. (2005). "The C42R mutation in the Kir6.2 (KCNJ11) gene as a cause of transient neonatal diabetes, childhood diabetes, or later-onset, apparently type 2 diabetes mellitus." *J. Clin. Endocrinol. Metab.*: jc.2005-0096.

<http://jcem.endojournals.org/cgi/content/abstract/jc.2005-0096v1>

[Context]: Known MODY genes account for only a fraction of families with dominantly inherited diabetes in Japan. There should be as yet unidentified genes which account for the rest of the patients[Objective]: To identify and characterize the mutation responsible for a Japanese family with dominantly inherited diabetes mellitus.[Subjects]: Members of a four-generation family with dominantly inherited diabetes mellitus observed in three generations. None of the patients in this family had permanent neonatal diabetes (PND). One with transient neonatal diabetes, one with childhood diabetes, the others with adult-onset diabetes without autoantibodies or insulin resistance.[Methods]: Screening of the chromosomal location of the gene by a genome-wide linkage analysis followed by candidate gene sequencing. Confirmation of the functional significance of the identified mutation by the population survey and the physiological analysis[Results]: We identified a novel mutation (C42R) in the KCNJ11 gene coding for the Kir6.2 subunit of the pancreatic KATP channel. The patch-clamp experiments using the mutated

KCNJ11 showed that the mutation causes increased spontaneous open probability (Po) and reduced ATP-sensitivity. The effect, however, was partially compensated by the reduction of functional KATP-channel expression at the cell surface, which could account for the milder phenotype of our patients.[Conclusions]: These results broaden the spectrum of diabetes phenotypes caused by mutations of KCNJ11, and suggest that mutations in this gene should be taken into consideration not only for PND but also for other forms of diabetes with milder phenotypes and later onset.

Young, R., F. Wu, et al. (2003). "Calcium Sensing Receptor Gene A986S Polymorphism and Responsiveness to Calcium Supplementation in Postmenopausal Women." J. Clin. Endocrinol. Metab. **88**(2): 697-700.

<http://jcem.endojournals.org/cgi/content/abstract/88/2/697>

Recently several studies in adolescent girls or premenopausal women have implicated the calcium sensing receptor (CASR) gene A986S polymorphism in calcium and bone metabolism. However, the role of this genetic variant in postmenopausal women, specifically the development of osteoporosis, is unknown. This study reports the findings of a randomized, double-blind, placebo-controlled study of healthy postmenopausal women followed for 2 yr while taking placebo or supplementary calcium. Specifically, we examined the relationship between the CASR A986S polymorphism, bone biochemical profile, and bone mineral density at baseline and after 2 yr of treatment. We found no effect of this genetic variant in postmenopausal women at baseline or in response to calcium supplementation. These results are in contrast to those in young or premenopausal women, and they provide no support for an important role for the CASR A986S polymorphism in osteoporosis.

Zalloua, P. A., H. Shbaklo, et al. (2002). "Type-2 Diabetes Family History Delays the Onset of Type-1 Diabetes." J. Clin. Endocrinol. Metab. **87**(7): 3192-3196.

<http://jcem.endojournals.org/cgi/content/abstract/87/7/3192>

Type-1 diabetes (T1D) is an autoimmune disease leading to insulin deficiency. Its occurrence is influenced by genetic and environmental factors. The human leukocyte antigen (HLA) region on chromosome 6 accounts for 45% of the genetic susceptibility for the disease, mainly the HLA-DQB1*0201 and HLA-DQB1*0302 alleles. Among the environmental factors involved, early exposure to cow's milk seems to be a trigger. In this study, we investigated the occurrence of T1D in 253 Lebanese Caucasian patients, in relation to HLA-DQB1*0201, HLA-DQB1*0302, HLA-DQB1*0602, gender, and early exposure to cow's milk, as well as to family history of T1D and type-2 diabetes (T2D). Our genetic analysis results show that in the patients studied, 77% and 40% were positive for BQ1*0201 and BQ1*0302, respectively. As for BQ1*0602, only 0.8% of patients were positive for this T1D protective allele, compared with 24% among the controls. Furthermore, our results did not show any gender preference of the disease or any effects of early intake of cow's milk on the age at onset of T1D. When family history of T2D or T1D was studied, our results show a novel finding whereby an immediate family history of T2D, but not T1D, delays the age at onset of T1D.

Amberg, G. C., A. D. Bonev, et al. (2003). "Modulation of the molecular composition of large conductance, Ca²⁺ activated K⁺ channels in vascular smooth muscle during hypertension." J. Clin. Invest. **112**(5): 717-724.

<http://www.jci.org/cgi/content/abstract/112/5/717>

Hypertension is a clinical syndrome characterized by increased vascular tone. However, the molecular mechanisms underlying vascular dysfunction during acquired hypertension remain unresolved. Localized intracellular Ca²⁺ release events through ryanodine receptors (Ca²⁺ sparks) in the sarcoplasmic reticulum are tightly coupled to the activation of large-conductance, Ca²⁺-activated K⁺ (BK) channels to provide a hyperpolarizing influence that opposes vasoconstriction. In this study we tested the hypothesis that a reduction in Ca²⁺ spark-BK channel coupling underlies vascular smooth muscle dysfunction during acquired hypertension. We found that in hypertension, expression of the {beta}1 subunit was decreased relative to the pore-forming {alpha} subunit of the BK channel. Consequently, the BK channels were functionally uncoupled from Ca²⁺ sparks. Consistent with this, the contribution of BK channels to vascular tone was reduced during hypertension. We conclude that downregulation of the {beta}1 subunit of the BK channel contributes to vascular dysfunction in hypertension. These results support the novel concept that changes in BK channel subunit composition regulate arterial smooth muscle function.

Carvalho, L. R., K. S. Woods, et al. (2003). "A homozygous mutation in HESX1 is associated with evolving hypopituitarism due to impaired repressor-corepressor interaction." J. Clin. Invest. **112**(8): 1192-1201.

<http://www.jci.org/cgi/content/abstract/112/8/1192>

The paired-like homeobox gene expressed in embryonic stem cells Hesx1/HESX1 encodes a developmental repressor and is expressed in early development in a region fated to form the forebrain, with subsequent localization to Rathke's pouch, the primordium of the anterior pituitary gland. Mutations within the gene have been associated with septo-optic dysplasia, a constellation of phenotypes including eye, forebrain, and pituitary abnormalities, or milder degrees of hypopituitarism. We identified a novel homozygous nonconservative missense mutation (I26T) in the critical Engrailed homology repressor domain (eh1) of HESX1, the first, to our knowledge, to be described in humans, in a girl with evolving combined pituitary hormone deficiency born to consanguineous parents. Neuroimaging revealed a thin pituitary stalk with anterior pituitary hypoplasia and an ectopic posterior pituitary, but no midline or optic nerve abnormalities. This I26T mutation did not affect the DNA-binding ability of HESX1 but led to an impaired ability to recruit the mammalian Groucho homolog/Transducin-like enhancer of split-1 (Gro/TLE1), a crucial corepressor for HESX1, thereby leading to partial loss of repression. Thus, the novel pituitary phenotype highlighted here appears to be a specific consequence of the inability of HESX1 to recruit Groucho-related corepressors, suggesting that other molecular mechanisms govern HESX1 function in the forebrain.

Chen, H. C., S. J. Smith, et al. (2002). "Increased insulin and leptin sensitivity in mice lacking acyl CoA:diacylglycerol acyltransferase 1." J. Clin. Invest. **109**(8): 1049-1055.

<http://www.jci.org/cgi/content/abstract/109/8/1049>

Acyl coenzyme A:diacylglycerol acyltransferase 1 (DGAT1) is one of two known DGAT enzymes that catalyze the final step in mammalian triglyceride synthesis. DGAT1-deficient mice are resistant to diet-induced obesity through a mechanism involving increased energy expenditure. Here we show that these mice have decreased levels of tissue triglycerides, as well as increased sensitivity to insulin and to leptin. Importantly, DGAT1 deficiency protects against insulin resistance and obesity in agouti yellow mice, a model of severe leptin resistance. In contrast, DGAT1 deficiency did not affect energy and glucose metabolism in leptin-deficient (ob/ob) mice, possibly due in part to a compensatory upregulation of DGAT2 expression in the absence of leptin. Our results suggest that inhibition of DGAT1 may be useful in treating insulin resistance and leptin resistance in human obesity.

Chen, H. C., S. J. Smith, et al. (2002). "Leptin modulates the effects of acyl CoA:diacylglycerol acyltransferase deficiency on murine fur and sebaceous glands." J. Clin. Invest. **109**(2): 175-181.

<http://www.jci.org/cgi/content/abstract/109/2/175>

Acyl CoA:diacylglycerol acyltransferase (DGAT) is a ubiquitously expressed enzyme that catalyzes the final reaction in the major pathways of triglyceride synthesis. Mice lacking DGAT1 (Dgat^{-/-}) demonstrate significant changes in lipid metabolism in several tissues, including the skin. Here we report the effects of DGAT1 deficiency on fur and sebaceous glands. Adult Dgat^{-/-} mice had dry fur and hair loss, which were associated with atrophic sebaceous glands and fur lipid abnormalities. As a result, Dgat^{-/-} mice had impaired water repulsion and defective thermoregulation after water immersion. These phenotypes were mostly absent in Dgat^{-/-} mice with leptin deficiency, indicating an unexpected role for leptin in modulating the skin phenotype. Our findings indicate that DGAT1 plays an important role in normal fur and sebaceous gland physiology and provide evidence that leptin modulates these processes in the skin.

Endres, M., D. Biniszkiwicz, et al. (2004). "Increased postischemic brain injury in mice deficient in uracil-DNA glycosylase." J. Clin. Invest. **113**(12): 1711-1721.

<http://www.jci.org/cgi/content/abstract/113/12/1711>

Uracil-DNA glycosylase (UNG) is involved in base excision repair of aberrant uracil residues in nuclear and mitochondrial DNA. Ung knockout mice generated by gene targeting are viable, fertile, and phenotypically normal and have regular mutation rates. However, when exposed to a nitric oxide donor, Ung^{-/-} fibroblasts show an increase in the uracil/cytosine ratio in the genome and augmented cell death. After combined oxygen-glucose deprivation, Ung^{-/-} primary cortical neurons have increased vulnerability to cell death, which is associated with early mitochondrial dysfunction. In vivo, UNG expression and activity are low in brains of naive WT mice but increase significantly after reversible middle cerebral artery occlusion and reperfusion. Moreover, major increases in infarct size are observed in Ung^{-/-} mice compared with littermate control mice. In conclusion, our results provide compelling evidence that UNG is of major importance for tissue repair after brain ischemia.

Melillo, R. M., M. D. Castellone, et al. (2005). "The RET/PTC-RAS-BRAF linear signaling cascade mediates the motile and mitogenic phenotype of thyroid cancer cells." J. Clin. Invest. **115**(4): 1068-1081.

<http://www.jci.org/cgi/content/abstract/115/4/1068>

In papillary thyroid carcinomas (PTCs), rearrangements of the RET receptor (RET/PTC) and activating mutations in the BRAF or RAS oncogenes are mutually exclusive. Here we show that the 3 proteins function along a linear oncogenic signaling cascade in which RET/PTC induces RAS-dependent BRAF activation and RAS- and BRAF-dependent ERK activation. Adoptive activation of the RET/PTC-RAS-BRAF axis induced cell proliferation and Matrigel invasion of thyroid follicular cells. Gene expression profiling revealed that the 3 oncogenes activate a common transcriptional program in thyroid cells that includes upregulation of the CXCL1 and CXCL10 chemokines, which in turn stimulate proliferation and invasion. Thus, motile and mitogenic properties are intrinsic to transformed thyroid cells and are governed by an epistatic oncogenic signaling cascade.

Park, J. O., C. A. Lopez, et al. (2002). "Transcriptional control of viral gene therapy by cisplatin." J. Clin. Invest. **110**(3): 403-410.

<http://www.jci.org/cgi/content/abstract/110/3/403>

Ionizing radiation (IR) and radical oxygen intermediates (ROIs) activate the early growth response-1 (Egr1) promoter through specific cis-acting sequences termed CArG elements. Ad.Egr.TNF.11D, a replication-deficient adenoviral vector containing CArG elements cloned upstream of the cDNA for human recombinant TNF- α was used to treat human esophageal adenocarcinoma and rat colon adenocarcinoma cells in culture and as xenografts in athymic nude mice. Cisplatin, a commonly used chemotherapeutic agent, causes tumor cell death by producing DNA damage and generating ROIs. The present studies demonstrate induction of TNF- α production in tumor cells and xenografts treated with the combination of Ad.Egr.TNF.11D and cisplatin. The results show that the Egr1 promoter is induced by cisplatin and that this induction is mediated in part through the CArG elements. These studies also demonstrate an enhanced antitumor response without an increase in toxicity following treatment with Ad.Egr.TNF.11D and cisplatin, compared with either agent alone. Chemo-inducible cancer gene therapy thus provides a means to control transgene expression while enhancing the effectiveness of commonly used chemotherapeutic agents.

Poblete-Gutierrez, P., T. Wiederholt, et al. (2004). "Allelic loss underlies type 2 segmental Hailey-Hailey disease, providing molecular confirmation of a novel genetic concept." J. Clin. Invest. **114**(10): 1467-1474.

<http://www.jci.org/cgi/content/abstract/114/10/1467>

Hailey-Hailey disease (HHD) is an autosomal dominant trait characterized by erythematous and oozing skin lesions preponderantly involving the body folds. In the present unusual case, however, unilateral segmental areas along the lines of Blaschko showing a rather severe involvement were superimposed on the ordinary symmetrical phenotype. Based on this observation and similar forms of mosaicism as reported in other autosomal dominant skin disorders, we postulated that in such cases, 2 different types of segmental involvement can be distinguished. Accordingly, the linear lesions as noted in the present case would exemplify type 2 segmental HHD. In the heterozygous embryo, loss of heterozygosity occurring at an early developmental stage would have given rise to pronounced linear lesions reflecting homozygosity or hemizyosity for the mutation. By analyzing DNA and RNA derived from blood and skin samples as well as keratinocytes of the index patient with various molecular techniques including RT-PCR, real-time PCR, and microsatellite analysis, we found a consistent loss of the paternal wild-type allele in more severely affected segmental skin regions, confirming this hypothesis for the first time, to our knowledge, at the molecular and cellular level.

Schulze-Bahr, E., A. Neu, et al. (2003). "Pacemaker channel dysfunction in a patient with sinus node disease." J. Clin. Invest. **111**(10): 1537-1545.

<http://www.jci.org/cgi/content/abstract/111/10/1537>

The cardiac pacemaker current I_f is a major determinant of diastolic depolarization in sinus nodal cells and has a key role in heartbeat generation. Therefore, we hypothesized that some forms of "idiopathic" sinus node dysfunction (SND) are related to inherited dysfunctions of cardiac pacemaker ion channels. In a candidate gene approach, a heterozygous 1-bp deletion (1631delC) in exon 5 of the human HCN4 gene was detected in a patient with idiopathic SND. The mutant HCN4 protein (HCN4-573X) had a truncated C-terminus and lacked the cyclic nucleotide-binding domain. COS-7 cells transiently transfected with HCN4-573X cDNA indicated normal intracellular trafficking and membrane integration of HCN4-573X subunits. Patch-clamp experiments showed that HCN4-573X channels mediated I_f -like currents that were insensitive to increased cellular cAMP levels. Coexpression experiments showed a dominant-negative effect of HCN4-573X subunits on wild-type subunits. These data indicate that the cardiac I_f channels are functionally expressed but with altered biophysical properties. Taken together, the clinical, genetic, and in vitro data provide a likely explanation for the patient's sinus bradycardia and the chronotropic incompetence.

Taylor, R. W., M. J. Barron, et al. (2003). "Mitochondrial DNA mutations in human colonic crypt stem cells." J. Clin. Invest. **112**(9): 1351-1360.

<http://www.jci.org/cgi/content/abstract/112/9/1351>

The mitochondrial genome encodes 13 essential subunits of the respiratory chain and has remarkable genetics based on uniparental inheritance. Within human populations, the mitochondrial genome has a high rate of sequence divergence with multiple polymorphic variants and thus has played a major role in examining the evolutionary history of our species. In recent years it has also become apparent that pathogenic mitochondrial DNA (mtDNA) mutations play an important role in neurological and other diseases. Patients harbor many different mtDNA mutations, some of which are mtDNA mutations, some of which are inherited, but others that seem to be sporadic. It has also been suggested that mtDNA mutations play a role in aging and cancer, but the evidence for a causative role in these conditions is less clear. The accumulated data would suggest, however, that mtDNA mutations occur on a frequent basis. In this article we describe a new phenomenon: the accumulation of mtDNA mutations in human colonic crypt stem cells that result in a significant biochemical defect in their progeny. These studies have important consequences not only for understanding of the finding of mtDNA mutations in aging tissues and tumors, but also for determining the frequency of mtDNA mutations within a cell.

Thebault, S., M. Roudbaraki, et al. (2003). " α_1 -adrenergic receptors activate Ca^{2+} -permeable cationic channels in prostate cancer epithelial cells." J. Clin. Invest. **111**(11): 1691-1701.

<http://www.jci.org/cgi/content/abstract/111/11/1691>

The prostate gland is a rich source of α_1 -adrenergic receptors (α_1 -ARs). α_1 -AR antagonists are commonly used in the treatment of benign prostatic hyperplasia symptoms, due to their action on smooth muscle cells. However, virtually nothing is known about the role of α_1 -ARs in epithelial cells. Here, by using two human prostate cancer epithelial (hPCE) cell

models -- primary cells from resection specimens (primary hPCE cells) and an LNCaP (lymph node carcinoma of the prostate) cell line -- we identify an α 1A subtype of adrenergic receptor (α 1A-AR) and show its functional coupling to plasmalemmal cationic channels via direct diacylglycerol (DAG) gating. In both cell types, agonist-mediated stimulation of α 1A-ARs and DAG analogues activated similar cationic membrane currents and Ca^{2+} influx. These currents were sensitive to the α 1A-AR antagonists, prazosin and WB4101, and to transient receptor potential (TRP) channel blockers, 2-aminophenyl borate and SK&F 96365. Chronic activation of α 1A-ARs enhanced LNCaP cell proliferation, which could be antagonized by α 1A-AR and TRP inhibitors. Collectively, our results suggest that α 1A-ARs play a role in promoting hPCE cell proliferation via TRP channels.

Tolosa, E., W. Li, et al. (2003). "Cathepsin V is involved in the degradation of invariant chain in human thymus and is overexpressed in myasthenia gravis." *J. Clin. Invest.* **112**(4): 517-526.

<http://www.jci.org/cgi/content/abstract/112/4/517>

Stepwise degradation of the invariant chain (Ii) is required for the binding of antigenic peptides to MHC class II molecules. Cathepsin (Cat) L in the murine thymus and Cat S in peripheral APCs have both been implicated in the last step of Ii degradation that gives rise to the class II-associated invariant chain peptides (CLIP). Cat V has been recently described as highly homologous to Cat L and exclusively expressed in human thymus and testis, but with no mouse orthologue. We report that Cat V is the dominant cysteine protease in cortical human thymic epithelial cells, while Cat L and Cat S seem to be restricted to dendritic and macrophage-like cells. Active Cat V in thymic lysosomal preparations was demonstrated by active-site labeling. Recombinant Cat V was capable of converting Ii into CLIP efficiently, suggesting that Cat V is the protease that controls the generation of α β -CLIP complexes in the human thymus, in analogy to Cat L in mouse. Comparison of Cat V expression between thymi from patients with myasthenia gravis and healthy controls revealed a significantly higher expression level in the pathological samples, suggesting a potential involvement of this protease in the immunopathogenesis of myasthenia gravis, an autoimmune disease almost invariably associated with thymic pathology.

van Berkel, V., B. Levine, et al. (2002). "Critical role for a high-affinity chemokine-binding protein in γ -herpesvirus-induced lethal meningitis." *J. Clin. Invest.* **109**(7): 905-914.

<http://www.jci.org/cgi/content/abstract/109/7/905>

Chemokines are involved in recruitment and activation of hematopoietic cells in sites of infection and inflammation. The M3 gene of the γ -herpesvirus γ HV68 encodes an abundant secreted protein that binds CC chemokines with high affinity. We report here that this gene is essential for efficient induction of lethal meningitis by γ HV68. An M3 mutant γ HV68 (γ HV68-M3.stop) was 100-fold less virulent than wild-type or marker rescue control (γ HV68-M3.MR) viruses after intracerebral inoculation. After intracerebral inoculation, γ HV68-M3.stop grew to lower titers than γ HV68 or γ HV68-M3.MR in the brain but spread to and grew normally in the spleen and lung. Expression of several CC chemokines was significantly induced in the CNS by γ HV68 infection. Consistent with M3 acting by blockade of CC chemokine action, γ HV68 induced a neutrophilic meningeal inflammatory infiltrate, while γ HV68-M3.stop induced an infiltrate in which lymphocytes and macrophages predominated. In contrast to the important role of M3 in lethal meningitis, M3 was not required for establishment or reactivation from latent infection or induction of chronic arteritis. These data suggest a role for chemokines in the protection of the nervous system from viral infection and that the M3 protein acts in a tissue-specific fashion during acute but not chronic

{gamma}HV68 infection to limit CC chemokine-induced inflammatory responses.

Weng, L., N. Kavaslar, et al. (2005). "Lack of MEF2A mutations in coronary artery disease." J. Clin. Invest. **115**(4): 1016-1020.

<http://www.jci.org/cgi/content/abstract/115/4/1016>

Mutations in MEF2A have been implicated in an autosomal dominant form of coronary artery disease (adCAD1). In this study we sought to determine whether severe mutations in MEF2A might also explain sporadic cases of coronary artery disease (CAD). To do this, we resequenced the coding sequence and splice sites of MEF2A in approximately 300 patients with premature CAD and failed to find causative mutations in the CAD cohort. However, we did identify the 21-bp MEF2A coding sequence deletion originally implicated in adCAD1 in 1 of 300 elderly control subjects without CAD. Further screening of approximately 1,500 additional individuals without CAD revealed 2 more subjects with the MEF2A 21-bp deletion. Genotyping of 19 family members of the 3 probands with the 21-bp deletion in MEF2A revealed that the mutation did not cosegregate with early CAD. These studies support that MEF2A mutations are not a common cause of CAD in white people and argue strongly against a role for the MEF2A 21-bp deletion in autosomal dominant CAD.

J. Clin. Microbiol. (237)

Ahmad, S., Z. Khan, et al. (2002). "Seminested PCR for Diagnosis of Candidemia: Comparison with Culture, Antigen Detection, and Biochemical Methods for Species Identification." J. Clin. Microbiol. **40**(7): 2483-2489.

<http://jcm.asm.org/cgi/content/abstract/40/7/2483>

The rapid detection and identification of *Candida* species in clinical laboratories are extremely important for the management of patients with hematogenous candidiasis. The presently available culture and biochemical methods for detection and species identification of *Candida* are time-consuming and lack the required sensitivity and specificity. In this study, we have established a seminested PCR (snPCR) using universal and species-specific primers for detection of *Candida* species in serum specimens. The universal outer primers amplified the 3' end of 5.8S ribosomal DNA (rDNA) and the 5' end of 28S rDNA, including the internally transcribed spacer 2 (ITS2), generating 350- to 410-bp fragments from the four commonly encountered *Candida* species, viz., *C. albicans*, *C. tropicalis*, *C. glabrata*, and *C. parapsilosis*. The species-specific primers, complementary to unique sequences within the ITS2 of each test species, amplified species-specific DNA in the reamplification step of the snPCR. The sensitivity of *Candida* detection by snPCR in spiked serum specimens was close to 1 organism/ml. Evaluation of snPCR for specific identification of *Candida* species with 76 clinical *Candida* isolates showed 99% concordant results with the Vitek and/or ID32C yeast identification system. Further evaluation of snPCR for detection of *Candida* species in sera from culture-proven ($n = 12$), suspected ($n = 16$), and superficially colonized ($n = 10$) patients and healthy subjects ($n = 12$) showed that snPCR results were consistently negative with sera from healthy individuals and colonized patients. In culture-proven candidemia patients, the snPCR results were in full agreement with blood culture results with respect to both positivity and species identity. In

addition, snPCR detected candidemia due to two *Candida* species in five patients, compared to three by blood culture. In the category of suspected candidemia with negative blood cultures for *Candida*, nine patients (56%) were positive by snPCR; two of them had dual infection with *C. albicans* and either *C. tropicalis* or *C. glabrata*. In conclusion, the snPCR developed in this study is specific and more sensitive than culture for the detection of *Candida* species in serum specimens. Moreover, the improved detection of cases of candidemia caused by more than one *Candida* species is an additional advantage.

Amonsin, A., L. L. Li, et al. (2004). "Multilocus Short Sequence Repeat Sequencing Approach for Differentiating among *Mycobacterium avium* subsp. *paratuberculosis* Strains." J. Clin. Microbiol. **42**(4): 1694-1702.

<http://jcm.asm.org/cgi/content/abstract/42/4/1694>

We describe a multilocus short sequence repeat (MLSSR) sequencing approach for the genotyping of *Mycobacterium avium* subsp. *paratuberculosis* (*M. paratuberculosis*) strains. Preliminary analysis identified 185 mono-, di-, and trinucleotide repeat sequences dispersed throughout the *M. paratuberculosis* genome, of which 78 were perfect repeats. Comparative nucleotide sequencing of the 78 loci of six *M. paratuberculosis* isolates from different host species and geographic locations identified a subset of 11 polymorphic short sequence repeats (SSRs), with an average of 3.2 alleles per locus. Comparative sequencing of these 11 loci was used to genotype a collection of 33 *M. paratuberculosis* isolates representing different multiplex PCR for IS900 loci (MPIL) or amplified fragment length polymorphism (AFLP) types. The analysis differentiated the 33 *M. paratuberculosis* isolates into 20 distinct MLSSR types, consistent with geographic and epidemiologic correlates and with an index of discrimination of 0.96. MLSSR analysis was also clearly able to distinguish between sheep and cattle isolates of *M. paratuberculosis* and easily and reproducibly differentiated strains representing the predominant MPIL genotype (genotype A18) and AFLP genotypes (genotypes Z1 and Z2) of *M. paratuberculosis* described previously. Taken together, the results of our studies suggest that MLSSR sequencing enables facile and reproducible high-resolution subtyping of *M. paratuberculosis* isolates for molecular epidemiologic and population genetic analyses.

Antonsson, A., S. Karanfilovska, et al. (2003). "General Acquisition of Human Papillomavirus Infections of Skin Occurs in Early Infancy." J. Clin. Microbiol. **41**(6): 2509-2514.

<http://jcm.asm.org/cgi/content/abstract/41/6/2509>

The human skin papillomaviruses (HPVs) represent a group of ubiquitous viruses detected at a high prevalence in the normal skin of healthy adults. In the present study, we analyzed skin swab samples from babies during their first days of life and from infants at various ages up to age 4 years. Specimens from their parents and, for the newborn babies, environmental samples were also investigated. HPV DNA was already detected on the day of birth in samples from 2 of the 16 babies, and 45% of the samples from the babies were positive for HPV in the days following birth. Seventy-seven percent of the skin samples collected from the mothers were HPV DNA positive. The prevalence of HPV DNA among children from the ages of 1 month to 4 years varied between 50 and 70%. The HPV DNA sequences detected revealed a great diversity of genotypes and putative genotypes. Among 115 samples from 38 infants and 31 parents and 7 environmental samples, a total of 73 HPV types or putative types were isolated. Of these, 26 putative HPV types have not been described before. Our data suggest that asymptomatic HPV infections of normal skin are acquired very early in infancy and are caused by a great multiplicity of HPV types.

Bash, M. C., P. Zhu, et al. (2005). "por Variable-Region Typing by DNA Probe Hybridization Is Broadly Applicable to Epidemiologic Studies of *Neisseria gonorrhoeae*." *J. Clin. Microbiol.* **43**(4): 1522-1530.

<http://jcm.asm.org/cgi/content/abstract/43/4/1522>

The porin gene (*porB*) of *Neisseria gonorrhoeae* encodes the major outer membrane protein identified as PI or Por. To examine the utility of *por* variable-region (VR) typing, *porB* from 206 isolates was characterized by using oligonucleotide probes in a checkerboard hybridization assay that identifies the sequence types of five VRs of both PIA and PIB *porB* alleles. The strains represented temporally and geographically distinct isolates, isolates from a large cluster, epidemiologically linked partner isolates, and a collection of strains from disseminated gonococcal infections. By using rigorous epidemiologic criteria for transmission of infection between sex partners, *por* VR typing was more discriminatory than serovar typing in classifying isolates from both members of 43 epidemiologically linked pairs: 39 of 43 pairs were classified as coinciding by *por* VR typing compared to 43 of 43 by serovar determination ($P = 0.058$). *porB* sequence data confirmed the accuracy of the *por* VR method. Relationships between VR type and serovar typing monoclonal antibodies were observed for all six PIB and three of six PIA antibodies. *por* VR typing is a molecular tool that appears to have broad applicability. This method can be adapted to a wide range of technologies from simple hybridization to microarray and may allow for typing from noncultured clinical specimens.

Beck, I. A., M. Mahalanabis, et al. (2002). "Rapid and Sensitive Oligonucleotide Ligation Assay for Detection of Mutations in Human Immunodeficiency Virus Type 1 Associated with High-Level Resistance to Protease Inhibitors." *J. Clin. Microbiol.* **40**(4): 1413-1419.

<http://jcm.asm.org/cgi/content/abstract/40/4/1413>

A sensitive, specific, and high-throughput oligonucleotide ligation assay (OLA) for the detection of genotypic human immunodeficiency virus type 1 (HIV-1) resistance to Food and Drug Administration-approved protease inhibitors was developed and evaluated. This ligation-based assay uses differentially modified oligonucleotides specific for wild-type or mutant sequences, allowing sensitive and simple detection of both genotypes in a single well of a microtiter plate. Oligonucleotides were designed to detect primary mutations associated with high-level resistance to amprenavir, nelfinavir, indinavir, ritonavir, saquinavir, and lopinavir, including amino acid substitutions D30N, I50V, V82A/S/T, I84V, N88D, and L90M. Plasma HIV-1 RNA from 54 infected patients was amplified by reverse transcription-PCR and sequenced by using dideoxynucleotide chain terminators for evaluation of mutations associated with drug resistance. These same amplicons were genotyped by the OLA at positions 30, 50, 82, 88, 84, and 90 for a total of 312 codons. The sensitivity of detection of drug-resistant genotypes was 96.7% (87 of 90 mutant codons) in the OLA compared to 92.2% (83 of 90) in consensus sequencing, presumably due to the increased sensitivity of the OLA. The OLA detected genetic subpopulations more often than sequencing, detecting 30 mixtures of mutant and wild-type sequences and two mixtures of drug-resistant sequences compared to 15 detected by DNA sequencing. Reproducible and semiquantitative detection of the mutant and the wild-type genomes by the OLA was observed by analysis of wild-type and mutant plasmid mixtures containing as little as 5% of either genotype in a background of the opposite genome. This rapid, simple, economical, and highly sensitive assay provides a practical alternative to dideoxy sequencing for genotypic evaluation of HIV-1 resistance to antiretrovirals.

Becker, K., A. W. Friedrich, et al. (2003). "Prevalence of Genes Encoding Pyrogenic Toxin Superantigens and Exfoliative Toxins among Strains of *Staphylococcus aureus* Isolated from Blood and Nasal Specimens." *J. Clin. Microbiol.* **41**(4): 1434-1439.

<http://jcm.asm.org/cgi/content/abstract/41/4/1434>

A total of 429 different *Staphylococcus aureus* isolates encompassing 219 blood isolates and 210 isolates taken from anterior nares were systematically searched by two multiplex PCR-DNA enzyme immunoassays (PCR-DEIA) for exfoliative toxin (ET) genes *eta* and *etb*, as well as for the classical members of the pyrogenic toxin superantigen (PTSAg) gene family comprising the staphylococcal enterotoxin (SE) genes *sea*-*see* and the toxic shock syndrome toxin 1 gene *tst*. In addition, a third PCR-DEIA was established to investigate the possession of four recently described SE genes, viz. *seg*-*sej*. The most frequent PTSAg/ET genes amplified were *seg* and *sei*, which were found strictly in combination in 55.0% of the *S. aureus* isolates tested. Other frequently detected toxin genes were *tst* (20.3%), *sea* (15.9%), and *sec* (11.2%). Only five isolates harbored ET genes. Regarding the origin of the *S. aureus* isolates, a significant difference ($P = 0.037$) was found for the possession of the *sed/sej* gene combination (10.5% of blood isolates versus 3.3% of nasal strains). Overall, about half of *S. aureus* isolates tested harbored genes of the classical members of the PTSAg family and ETs (50.8%), whereas 73.0% of *S. aureus* isolates were toxin gene positive if the recently described SE genes were included. This notable higher prevalence indicates that the possession of PTSAg genes in particular seems to be a habitual feature of *S. aureus*. Moreover, mainly due to the fixed combinations of *seg* plus *sei*, as well as *sed* plus *sej*, the possession of multiple PTSAg genes (62.9%) is more frequent than assumed so far.

Becker, M. R., B. J. Paster, et al. (2002). "Molecular Analysis of Bacterial Species Associated with Childhood Caries." *J. Clin. Microbiol.* **40**(3): 1001-1009.

<http://jcm.asm.org/cgi/content/abstract/40/3/1001>

Although substantial epidemiologic evidence links *Streptococcus mutans* to caries, the pathobiology of caries may involve more complex communities of bacterial species. Molecular methods for bacterial identification and enumeration now make it possible to more precisely study the microbiota associated with dental caries. The purpose of this study was to compare the bacteria found in early childhood caries (ECC) to those found in caries-free children by using molecular identification methods. Cloning and sequencing of bacterial 16S ribosomal DNAs from a healthy subject and a subject with ECC were used for identification of novel species or uncultivated phylotypes and species not previously associated with dental caries. Ten novel phylotypes were identified. A number of species or phylotypes that may play a role in health or disease were identified and warrant further investigation. In addition, quantitative measurements for 23 previously known bacterial species or species groups were obtained by a reverse capture checkerboard assay for 30 subjects with caries and 30 healthy controls. Significant differences were observed for nine species: *S. sanguinis* was associated with health and, in order of decreasing cell numbers, *Actinomyces gerencseriae*, *Bifidobacterium*, *S. mutans*, *Veillonella*, *S. salivarius*, *S. constellatus*, *S. parasanguinis*, and *Lactobacillus fermentum* were associated with caries. These data suggest that *A. gerencseriae* and other *Actinomyces* species may play an important role in caries initiation and that a novel *Bifidobacterium* may be a major pathogen in deep caries. Further investigation could lead to the identification of targets for biological interventions in the caries process and thereby contribute to improved prevention of and treatment for this significant public health problem.

Beld, M., R. Minnaar, et al. (2004). "Highly Sensitive Assay for Detection of Enterovirus in Clinical

Specimens by Reverse Transcription-PCR with an Armored RNA Internal Control." J. Clin. Microbiol. **42**(7): 3059-3064.

<http://jcm.asm.org/cgi/content/abstract/42/7/3059>

The objective of the present study was the development of a diagnostic reverse transcription (RT)-PCR for the specific detection of enterovirus (EV) RNA in clinical specimens controlled by an internal control (IC) RNA. The IC RNA contains the same primer binding sites as EV RNA but has a different probe region. The IC RNA was packaged into an MS2 phage core particle (armored) and was added to the clinical sample to allow monitoring of both extraction efficiency and RT-PCR efficiency. Serial dilutions of the IC RNA were made, and the detection limit of the RT-PCR was tested in a background of EV RNA-negative cerebrospinal fluid. The sensitivity and specificity of the RT-PCR assay were tested by using all 64 known EV serotypes, several non-EV serotypes, and two Quality Control for Molecular Diagnostics (QCMD) Program EV proficiency panels from 2001 and 2002. In total, 322 clinical specimens were tested by RT-PCR, and to establish the clinical utility of the RT-PCR, a comparison of the results of viral culture and RT-PCR was done with 87 clinical specimens. The lower limit of sensitivity was reached at about 150 copies of IC RNA/ml. All 64 EV serotypes were positive, while all non-EV serotypes were negative. All culture-positive samples of the 2001 QCMD proficiency panel (according to the 50% tissue culture infective doses per milliliter) were positive by RT-PCR. Invalid results, i.e., negativity for both EV RNA and IC RNA, due to inhibition of RT-PCR were observed for 33.3% of the members of the 2002 QCMD proficiency panel and 3.1% of the clinical specimens. Inhibition of RT-PCR could be relieved by the addition of 400 ng of bovine {alpha}-casein per {micro}l to both the RT reaction mixture and the PCR mixture. With this optimized protocol, the results for all samples of the 2002 QCMD proficiency panel and all clinical specimens except one fecal sample (0.3%) were valid. Evaluation of the clinical samples demonstrated that EV infection could be detected in 12 of 87 samples (13.8%) by RT-PCR, while viral culture was negative. Our data show that the RT-PCR with armored IC RNA offers a very reliable and rapid diagnostic tool for the detection of EV in clinical specimens and that the addition of bovine {alpha}-casein relieved inhibition of the RT-PCR for 99.7% of clinical specimens.

Bergmann, A. R., B. L. Schmidt, et al. (2002). "Importance of Sample Preparation for Molecular Diagnosis of Lyme Borreliosis from Urine." J. Clin. Microbiol. **40**(12): 4581-4584.

<http://jcm.asm.org/cgi/content/abstract/40/12/4581>

Urine PCR has been used for the diagnosis of *Borrelia burgdorferi* infection in recent years but has been abandoned because of its low sensitivity and the irreproducibility of the results. Our study aimed to analyze technical details related to sample preparation and detection methods. Crucial for a successful urine PCR were (i) avoidance of the first morning urine sample; (ii) centrifugation at 36,000 x g; and (iii) the extraction method, with only DNAzol of the seven different extraction methods used yielding positive results with patient urine specimens. Furthermore, storage of frozen urine samples at -80{degrees}C reduced the sensitivity of a positive urine PCR result obtained with samples from 72 untreated erythema migrans (EM) patients from 85% in the first 3 months to <30% after more than 3 months. Bands were detected at 276 bp on ethidium bromide-stained agarose gels after amplification by a nested PCR. The specificity of bands for 32 of 33 samples was proven by hybridization with a GEN-ETI-K-DEIA kit and for a 10 further positive amplicons by sequencing. By using all of these steps to optimize the urine PCR technique, *B. burgdorferi* infection could be diagnosed by using urine samples from EM patients with a sensitivity (85%) substantially better than that of serological methods (50%). This improved method could be of future importance as an additional laboratory technique for the diagnosis of unclear, unrecognized borrelia infections and diseases possibly related to Lyme borreliosis.

Bialek, R., A. Cascante Cirera, et al. (2003). "Nested PCR Assays for Detection of *Blastomyces dermatitidis* DNA in Paraffin-Embedded Canine Tissue." *J. Clin. Microbiol.* **41**(1): 205-208.

<http://jcm.asm.org/cgi/content/abstract/41/1/205>

A *Blastomyces dermatitidis* nested PCR assay targeting the gene encoding the Wisconsin 1 (WI-1) adhesin was developed and compared with a nested PCR targeting the 18S rRNA gene (rDNA) of members of the family Onygenaceae. We examined 73 paraffin-embedded tissue samples obtained from nine dogs which died of blastomycosis and nine dogs which succumbed to lymphosarcoma according to autopsy findings; amplifiable canine DNA was extracted from 25 and 33 specimens from the two groups, respectively. The *B. dermatitidis* PCR amplified DNA from 8 of 13 tissue samples in which yeast cells were detected by microscopy. Sequencing revealed that all PCR products were homologous to the *B. dermatitidis* WI-1 adhesin gene. No PCR product was amplified from 12 microscopically negative biopsy specimens from dogs with blastomycosis or from 33 biopsy specimens from dogs with lymphosarcoma. The 18S rDNA PCR amplified DNA from 10 and 9 tissue samples taken from dogs which died of blastomycosis and lymphosarcoma, respectively. Only six products were identified as being identical to *B. dermatitidis* 18S rDNA; they were exclusively obtained from specimens positive by the *B. dermatitidis* nested PCR. For specificity testing, 20 human biopsy specimens proven to have histoplasmosis were examined, and a specific *H. capsulatum* product was amplified by the 18S rDNA PCR from all specimens, whereas no product was obtained from any of the 20 samples by the *B. dermatitidis* PCR assay. In conclusion, the PCR targeting a gene encoding the unique WI-1 adhesin is as sensitive as but more specific than the PCR targeting the 18S rDNA for detection of *B. dermatitidis* in canine tissue.

Bialek, R., A. Feucht, et al. (2002). "Evaluation of Two Nested PCR Assays for Detection of *Histoplasma capsulatum* DNA in Human Tissue." *J. Clin. Microbiol.* **40**(5): 1644-1647.

<http://jcm.asm.org/cgi/content/abstract/40/5/1644>

In order to evaluate the diagnostic relevance of two nested PCR assays for diagnosis of histoplasmosis in clinical specimens, 100 paraffin-embedded biopsy specimens were examined. Upon microscopy of tissue, 50 biopsy specimens were histoplasma positive and 50 were negative. Due to destruction by formalin fixation, successful extraction of amplifiable human DNA was limited to 29 and 33 samples, respectively. A product of the *Histoplasma capsulatum* nested PCR assay targeting the gene encoding the unique fungal 100-kDa-like protein was detected in 20 histopathologically positive biopsy specimens but in none of the microscopically negative samples. Sequencing revealed that all 20 products of 210 bp were identical to the sequence of *H. capsulatum* in the GenBank database. In contrast, the nested PCR assay targeting the fungal 18S rRNA genes amplified products in 26 histopathologically positive but also in 18 microscopically negative biopsy specimens. However, sequencing revealed that only 20 of these 44 PCR products (231 bp) were identical to the sequence of *H. capsulatum*. The remaining 24 sequences were homologous to those of several *Euscomycetes*. These PCR products were detected only in tissues possibly colonized by nonpathogenic fungi, possibly causing these nonspecific amplifications. The detection limit of both *H. capsulatum* nested PCR assays was 1 to 5 fungal cells per sample. The two assays were similarly sensitive in identifying *H. capsulatum*. In this preliminary study, the novel 100-kDa-like-protein gene nested PCR revealed a specificity of 100% without requiring sequencing, which was necessary for identification of the 18S ribosomal DNA nested PCR products in order to avoid a high rate of false-positive results.

Bialek, R., J. Kern, et al. (2004). "PCR Assays for Identification of *Coccidioides posadasii* Based on the Nucleotide Sequence of the Antigen 2/Proline-Rich Antigen." *J. Clin. Microbiol.* **42**(2): 778-783.

<http://jcm.asm.org/cgi/content/abstract/42/2/778>

A conventional nested PCR and a real-time LightCycler PCR assay for detection of *Coccidioides posadasii* DNA were designed and tested in 120 clinical strains. These had been isolated from 114 patients within 10 years in Monterrey, Nuevo Leon, Mexico, known to be endemic for coccidioidomycosis. The gene encoding the specific antigen 2/proline-rich antigen (Ag2/PRA) was used as a target. All strains were correctly identified, whereas DNA from related members of the family Onygenaceae remained negative. Melting curve analysis by LightCycler and sequencing of the 526-bp product of the first PCR demonstrated either 100% identity to the GenBank sequence of the Silveira strain, now known to be *C. posadasii* (accession number AF013256), or a single silent mutation at position 1228. Length determination of two microsatellite-containing loci (GAC and 621) identified all 120 isolates as *C. posadasii*. Specific DNA was amplified by conventional nested PCR from three microscopically spherule-positive paraffin-embedded tissue samples, whereas 20 human tissue samples positive for other dimorphic fungi remained negative. Additionally, the safety of each step of a modified commercially available DNA extraction procedure was evaluated by using 10 strains. At least three steps of the protocol were demonstrated to sufficiently kill arthroconidia. This safe procedure is applicable to cultures and to clinical specimens.

Birkenheuer, A. J., M. G. Levy, et al. (2003). "Development and Evaluation of a Seminested PCR for Detection and Differentiation of *Babesia gibsoni* (Asian Genotype) and *B. canis* DNA in Canine Blood Samples." *J. Clin. Microbiol.* **41**(9): 4172-4177.

<http://jcm.asm.org/cgi/content/abstract/41/9/4172>

Canine babesiosis has recently been recognized as an emerging infectious disease of dogs in North America. We sought to develop a seminested PCR to detect and differentiate *Babesia gibsoni* (Asian genotype), *B. canis* subsp. *vogeli*, *B. canis* subsp. *canis*, and *B. canis* subsp. *rossi* DNA in canine blood samples. An outer primer pair was designed to amplify an [~]340-bp fragment of the 18S rRNA genes from *B. gibsoni* (Asian genotype), *B. canis* subsp. *vogeli*, *B. canis* subsp. *rossi*, and *B. canis* subsp. *canis* but not mammalian DNA. Forward primers were designed that would specifically amplify a smaller fragment from each organism in a seminested PCR. The practical limit of detection was 50 organisms/ml of mock-infected EDTA anticoagulated whole blood. The primer pair also amplified an [~]370-bp fragment of the *B. gibsoni* (USA/California genotype) 18S rRNA gene from the blood of an experimentally infected dog with a high percentage of parasitemia. Amplicons were not detected when DNA extracted from the blood of a dog that was naturally infected with *Theileria annae* at a low percentage of parasitemia was amplified. Due to limited sensitivity, this test is not recommended for the routine diagnosis of *B. gibsoni* (USA/California genotype) or *T. annae*. The PCR test did not amplify *Toxoplasma gondii*, *Neospora caninum*, *Leishmania infantum*, *Cryptosporidium parvum*, or canine DNA under any of the conditions tested. The seminested PCR test was able to detect and discriminate *B. gibsoni* (Asian genotype), *B. canis* subsp. *vogeli*, *B. canis* subsp. *canis*, and *B. canis* subsp. *rossi* DNA in blood samples from infected dogs.

Bischoff, K. M., D. G. White, et al. (2002). "Characterization of Chloramphenicol Resistance in Beta-Hemolytic *Escherichia coli* Associated with Diarrhea in Neonatal Swine." *J. Clin. Microbiol.* **40**(2): 389-394.

<http://jcm.asm.org/cgi/content/abstract/40/2/389>

Ninety beta-hemolytic *Escherichia coli* isolates associated with diarrhea in neonatal pigs from multiple farms in Oklahoma were investigated for known associated disease serotypes, virulence factors, ribotypes, and antimicrobial susceptibility phenotypes. Fifteen different serotypes were observed, with 58% of isolates belonging to groups that produce one of three major enterotoxins: O149, O147, and O139. Thirty percent of the swine *E. coli* isolates possessed a combination of F4 fimbriae and the heat-labile toxin and heat-stable toxin B enterotoxins. Seventy-three percent of the *E. coli* isolates were resistant to five or more antibiotics. Interestingly, 53% of swine *E. coli* isolates exhibited resistance to chloramphenicol (CHL), an antibiotic whose use in food animals has been prohibited in the United States since the mid-1980s. The *cmlA* gene, which encodes a putative CHL efflux pump, was detected by PCR in 47 of the 48 CHL-resistant isolates, and 4 of these also possessed the *cat2* gene, which encodes a chloramphenicol acetyltransferase. The one CHL-resistant isolate that did not contain either *cmlA* or *cat-2* possessed the *flo* gene, which confers resistance to both florfenicol and CHL. To determine whether CHL-resistant swine *E. coli* isolates represented dissemination of a clonal strain, all 90 isolates were analyzed by ribotyping. Seventeen distinct *E. coli* ribogroups were identified, with CHL resistance observed among the isolates in all except one of the major ribogroups. The identification of the *cmlA* gene among diverse hemolytic enterotoxigenic *E. coli* strains demonstrates its broad dissemination in the swine production environment and its persistence even in the absence of CHL selection pressure.

Bode, E., W. Hurtle, et al. (2004). "Real-Time PCR Assay for a Unique Chromosomal Sequence of *Bacillus anthracis*." *J. Clin. Microbiol.* **42**(12): 5825-5831.

<http://jcm.asm.org/cgi/content/abstract/42/12/5825>

Real-time PCR has become an important method for the rapid identification of *Bacillus anthracis* since the 2001 anthrax mailings. Most real-time PCR assays for *B. anthracis* have been developed to detect virulence genes located on the pXO1 and pXO2 plasmids. In contrast, only two published chromosomal targets exist, the *rpoB* gene and the *gyrA* gene. In the present study, subtraction-hybridization with a plasmid-cured *B. anthracis* tester strain and a *Bacillus cereus* driver was used to find a unique chromosomal sequence. By targeting this region, a real-time assay was developed with the Ruggedized Advanced Pathogen Identification Device. Further testing has revealed that the assay has 100% sensitivity and 100% specificity, with a limit of detection of 50 fg of DNA. The results of a search for sequences with homology with the BLAST program demonstrated significant alignment to the recently published *B. anthracis* Ames strain, while an inquiry for protein sequence similarities indicated homology with an abhydrolase from *B. anthracis* strain A2012. The importance of this chromosomal assay will be to verify the presence of *B. anthracis* independently of plasmid occurrence.

Boeckh, M., M. Huang, et al. (2004). "Optimization of Quantitative Detection of Cytomegalovirus DNA in Plasma by Real-Time PCR." *J. Clin. Microbiol.* **42**(3): 1142-1148.

<http://jcm.asm.org/cgi/content/abstract/42/3/1142>

Previous studies have shown that detection of cytomegalovirus (CMV) DNA in plasma is less sensitive than the antigenemia assay for CMV surveillance in blood. In 1,983 blood samples, plasma PCR assays with three different primer sets (UL125 alone, UL126 alone, and UL55/UL123-exon 4) were compared to the pp65 antigenemia assay and blood cultures. Plasma PCR detected CMV more frequently in blood specimens than either the antigenemia assay or cultures, but of the three PCR assays, the double-primer assay (UL55/UL123-exon 4) performed

best with regard to sensitivity, specificity, and predictive values compared to antigenemia: 122 of 151 antigenemia-positive samples were detected (sensitivity, 80.1%), and there were 122 samples that were PCR positive-antigenemia negative (specificity, 93%). Samples with discrepant results had a low viral load (median, 0.5 cells per slide; 1,150 copies per ml) and were often obtained from patients receiving antiviral therapy. CMV could be detected by other methods in 15 of 29 antigenemia positive-PCR negative samples compared to 121 of 122 PCR positive-antigenemia negative samples ($P < 0.001$). On a per-subject basis, 21 of 25 patients (antigenemia positive-PCR negative) and all 57 (PCR positive-antigenemia negative) could be confirmed at different time points during follow-up. The higher sensitivity of the double-primer assay resulted in earlier detection compared to antigenemia in a time-to-event analysis of 42 CMV-seropositive stem cell transplant recipients, and two of three patients with CMV disease who were antigenemia negative were detected by plasma PCR prior to the onset of disease. Interassay variability was low, and the dynamic range was $>5 \log_{10}$. Automated DNA extraction resulted in high reproducibility, accurate CMV quantitation ($R = 0.87$, $P < 0.001$), improved sensitivity, and increased speed of sample processing. Thus, primer optimization and improved DNA extraction techniques resulted in a plasma-based PCR assay that is significantly more sensitive than pp65 antigenemia and blood cultures for detection of CMV in blood specimens.

Boel, C. H. E., C. M. C. van Herk, et al. (2005). "Evaluation of Conventional and Real-Time PCR Assays Using Two Targets for Confirmation of Results of the COBAS AMPLICOR Chlamydia trachomatis/Neisseria gonorrhoeae Test for Detection of Neisseria gonorrhoeae in Clinical Samples." *J. Clin. Microbiol.* **43**(5): 2231-2235.

<http://jcm.asm.org/cgi/content/abstract/43/5/2231>

Two conventional PCR-enzyme immunoassays (PCR-EIAs) and two real-time PCR assays (LightCycler system; Roche Diagnostics) were evaluated as confirmation assays with *cppB* and 16S rRNA genes as targets. Of 765 male and female genitourinary and nasopharyngeal specimens positive for *Neisseria gonorrhoeae* in the COBAS AMPLICOR Chlamydia trachomatis/Neisseria gonorrhoeae PCR test (Roche Diagnostics), 229 (30%) were confirmed positive; 13 of these (5.7%) were lacking the *cppB* gene. Of the 534 samples (70%) that could not be confirmed, 81 (15%) showed a positive crossing point. However, melting curve analysis revealed an aberrant melting temperature in the LightCycler 16S rRNA assay; therefore, these samples were considered non-*N. gonorrhoeae* *Neisseria* species. Both of the 16S rRNA assays performed well, with positive predictive values of 99.1% and 100% for the PCR-EIAs and the real-time assays, respectively, and a negative predictive value of 99.8% for both. The *cppB* assays were compromised by the absence of the *cppB* gene in 5.7% of the *N. gonorrhoeae*-positive samples, resulting in negative predictive values of 96.8% and 97.6% for the PCR-EIAs and the real-time assays, respectively. Therefore, the 16S rRNA gene is preferable to the *cppB* gene as a target for confirmation assays. The melting curve analysis of the real-time assays provides useful additional information.

Bopp, D. J., B. D. Sauders, et al. (2003). "Detection, Isolation, and Molecular Subtyping of *Escherichia coli* O157:H7 and *Campylobacter jejuni* Associated with a Large Waterborne Outbreak." *J. Clin. Microbiol.* **41**(1): 174-180.

<http://jcm.asm.org/cgi/content/abstract/41/1/174>

The largest reported outbreak of waterborne *Escherichia coli* O157:H7 in the United States occurred in upstate New York following a county fair in August 1999. Culture methods were used to isolate *E. coli* O157:H7 from specimens from 128 of 775 patients with suspected infections. *Campylobacter jejuni* was also isolated from stools of 44 persons who developed diarrheal illness

after attending this fair. There was one case of a confirmed coinfection with *E. coli* O157:H7 and *C. jejuni*. Molecular detection of *stx1* and *stx2* Shiga toxin genes, immunomagnetic separation (IMS), and selective culture enrichment were utilized to detect and isolate *E. coli* O157:H7 from an unchlorinated well and its distribution points, a dry well, and a nearby septic tank. PCR for *stx1* and *stx2* was shown to provide a useful screen for toxin-producing *E. coli* O157:H7, and IMS subculture improved recovery. Pulsed-field gel electrophoresis (PFGE) was used to compare patient and environmental *E. coli* O157:H7 isolates. Among patient isolates, 117 of 128 (91.5%) were type 1 or 1a (three or fewer bands different). Among the water distribution system isolates, 13 of 19 (68%) were type 1 or 1a. Additionally, PFGE of *C. jejuni* isolates revealed that 29 of 35 (83%) had indistinguishable PFGE patterns. The PFGE results implicated the water distribution system as the main source of the *E. coli* O157:H7 outbreak. This investigation demonstrates the potential for outbreaks involving more than one pathogen and the importance of analyzing isolates from multiple patients and environmental samples to develop a better understanding of bacterial transmission during an outbreak.

Bosshard, P. P., S. Abels, et al. (2004). "Comparison of Conventional and Molecular Methods for Identification of Aerobic Catalase-Negative Gram-Positive Cocci in the Clinical Laboratory." *J. Clin. Microbiol.* **42**(5): 2065-2073.

<http://jcm.asm.org/cgi/content/abstract/42/5/2065>

Over a period of 18 months we have evaluated the use of 16S ribosomal DNA (rDNA) sequence analysis as a means of identifying aerobic catalase-negative gram-positive cocci in the clinical laboratory. A total of 171 clinically relevant strains were studied. The results of molecular analyses were compared with those obtained with a commercially available phenotypic identification system (API 20 Strep system; bioMerieux sa, Marcy l'Etoile, France). Phenotypic characterization identified 67 (39%) isolates to the species level and 32 (19%) to the genus level. Seventy-two (42%) isolates could not be discriminated at any taxonomic level. In comparison, 16S rDNA sequencing identified 138 (81%) isolates to the species level and 33 (19%) to the genus level. For 42 of 67 isolates assigned to a species with the API 20 Strep system, molecular analyses yielded discrepant results. Upon further analysis it was concluded that among the 42 isolates with discrepant results, 16S rDNA sequencing was correct for 32 isolates, the phenotypic identification was correct for 2 isolates, and the results for 8 isolates remained unresolved. We conclude that 16S rDNA sequencing is an effective means for the identification of aerobic catalase-negative gram-positive cocci. With the exception of *Streptococcus pneumoniae* and beta-hemolytic streptococci, we propose the use of 16S rDNA sequence analysis if adequate species identification is of concern.

Bosshard, P. P., S. Abels, et al. (2003). "Ribosomal DNA Sequencing for Identification of Aerobic Gram-Positive Rods in the Clinical Laboratory (an 18-Month Evaluation)." *J. Clin. Microbiol.* **41**(9): 4134-4140.

<http://jcm.asm.org/cgi/content/abstract/41/9/4134>

We have evaluated over a period of 18 months the use of 16S ribosomal DNA (rDNA) sequence analysis as a means of identifying aerobic gram-positive rods in the clinical laboratory. Two collections of strains were studied: (i) 37 clinical strains of gram-positive rods well identified by phenotypic tests, and (ii) 136 clinical isolates difficult to identify by standard microbiological investigations, i.e., identification at the species level was impossible. Results of molecular analyses were compared with those of conventional phenotypic identification procedures. Good overall agreement between phenotypic and molecular identification procedures was found for the collection of 37 clinical strains well identified by conventional means. For the 136 clinical strains

which were difficult to identify by standard microbiological investigations, phenotypic characterization identified 71 of 136 (52.2%) isolates at the genus level; 65 of 136 (47.8%) isolates could not be discriminated at any taxonomic level. In comparison, 16S rDNA sequencing identified 89 of 136 (65.4%) isolates at the species level, 43 of 136 (31.6%) isolates at the genus level, and 4 of 136 (2.9%) isolates at the family level. We conclude that (i) rDNA sequencing is an effective means for the identification of aerobic gram-positive rods which are difficult to identify by conventional techniques, and (ii) molecular identification procedures are not required for isolates well identified by phenotypic investigations.

Boutaga, K., A. J. van Winkelhoff, et al. (2003). "Comparison of Real-Time PCR and Culture for Detection of *Porphyromonas gingivalis* in Subgingival Plaque Samples." *J. Clin. Microbiol.* **41**(11): 4950-4954.

<http://jcm.asm.org/cgi/content/abstract/41/11/4950>

Porphyromonas gingivalis is a major pathogen in destructive periodontal disease in humans. Detection and quantification of this microorganism are relevant for diagnosis and treatment planning. The prevalence and quantity of *P. gingivalis* in subgingival plaque samples of periodontitis patients were determined by anaerobic culture and real-time PCR amplification of the 16S small-subunit rRNA gene. The PCR was performed with primers and a fluorescently labeled probe specific for the *P. gingivalis* 16S rRNA gene. By the real-time PCR assay, as few as 1 CFU of *P. gingivalis* could be detected. Subgingival plaque samples from 259 adult patients with severe periodontitis were analyzed. *P. gingivalis* was detected in 111 (43%) of the 259 subgingival plaque samples by culture and in 138 (53%) samples by PCR. The sensitivity, specificity, and positive and negative predictive values of the real-time PCR were 100, 94, 94, and 100%, respectively. We conclude that real-time PCR confirms the results of quantitative culture of *P. gingivalis* and offers significant advantages with respect to the rapidity and sensitivity of detection of *P. gingivalis* in subgingival plaque samples.

Brennan, R. E. and J. E. Samuel (2003). "Evaluation of *Coxiella burnetii* Antibiotic Susceptibilities by Real-Time PCR Assay." *J. Clin. Microbiol.* **41**(5): 1869-1874.

<http://jcm.asm.org/cgi/content/abstract/41/5/1869>

Coxiella burnetii is an obligate intracellular bacterium. The inability to cultivate this organism on axenic medium has made calculation of infectious units challenging and prevents the use of conventional antibiotic susceptibility assays. A rapid and reliable real-time PCR assay was developed to quantify *C. burnetii* cells from J774.16 mouse macrophage cells and was applied to antibiotic susceptibility testing of *C. burnetii* Nine Mile, phase I. For calculation of bacterial replication, real-time PCR performed equally as well as immunofluorescent-antibody (IFA) assay when J774.16 cells were infected with 10-fold serial dilutions of *C. burnetii* and was significantly ($P < 0.05$) more repeatable than IFA when 2-fold dilutions were used. Newly infected murine macrophage-like J774.16 cells were treated with 8 {micro}g of chloramphenicol per ml, 4 {micro}g of tetracycline per ml, 4 {micro}g of rifampin per ml, 4 {micro}g of ampicillin per ml, or 1 {micro}g of ciprofloxacin per ml. After 6 days of treatment, tetracycline, rifampin, and ampicillin significantly ($P < 0.01$) inhibited the replication of *C. burnetii*, while chloramphenicol and ciprofloxacin did not. In general, these results are consistent with those from prior reports on the efficacy of these antibiotics against *C. burnetii* Nine Mile, phase I, and indicate that a real-time PCR-based assay is an appropriate alternative to the present methodology for evaluation of the antibiotic susceptibilities of *C. burnetii*.

Briedis, D. J., A. Khamessian, et al. (2002). "Isolation of *Campylobacter fetus* subsp. *fetus* from a Patient with Cellulitis." J. Clin. Microbiol. **40**(12): 4792-4796.

<http://jcm.asm.org/cgi/content/abstract/40/12/4792>

Campylobacter fetus subsp. *fetus* is a gram-negative, slender, spirally curved bacterial pathogen. It has been isolated from human blood, spinal fluid, and abscesses, but cellulitis associated with bacteremia is rare. We report its isolation from a blood culture of a human patient with cellulitis as well as difficulties encountered in determining the identity of the subspecies of *C. fetus*.

Brinkman, J. A., W. E. Jones, et al. (2002). "Detection of Human Papillomavirus DNA in Urine Specimens from Human Immunodeficiency Virus-Positive Women." J. Clin. Microbiol. **40**(9): 3155-3161.

<http://jcm.asm.org/cgi/content/abstract/40/9/3155>

Human immunodeficiency virus (HIV)-positive women may represent one of the fastest-growing populations at risk for acquiring cervical cancer and thus require frequent screening. The purpose of the present studies was to validate a PCR-based urine assay by comparing detection and genotyping of human papillomavirus (HPV) DNA in urine samples and matching cervical swab specimens of HIV-positive women. Despite a difference in amplifiability, the prevalence of any HPV genotype (58% for the cervical swab specimens and 48% for the urine specimens) was not significantly different in this population. The levels of concordance were 70, 71, and 78% for detection of any HPV type, any high-risk HPV type, or any low-risk HPV type in the two specimen types, respectively. While instances of discordant detection were greater for the cervical swab specimens than for the urine specimens, this was not statistically significant. The distributions of HPV genotypes were similar in the cervix and the urine for the majority of types examined. Importantly, detection of HPV DNA in urine was associated with an abnormal Papanicolaou smear to the same extent that detection of HPV DNA in a cervical swab specimen was. These data provide preliminary support for the proposal to use urine testing as a primary or secondary screening tool for cervical cancer in HIV-positive women or as an epidemiological tool. Additional studies with larger sample sizes must be conducted in order to further verify these findings.

Broccolo, F., G. Locatelli, et al. (2002). "Calibrated Real-Time PCR Assay for Quantitation of Human Herpesvirus 8 DNA in Biological Fluids." J. Clin. Microbiol. **40**(12): 4652-4658.

<http://jcm.asm.org/cgi/content/abstract/40/12/4652>

Accurate laboratory tests for the diagnosis of active human herpesvirus 8 (HHV-8) infection are becoming essential to study the pathogenesis of HHV-8-associated tumors and for the clinical management of HHV-8-infected individuals. We have developed a highly sensitive, calibrated quantitative real-time PCR assay for the measurement of cell-free HHV-8 DNA in body fluids, based on the addition of a synthetic DNA calibrator prior to DNA extraction. The calibrator controls each sample for the presence of PCR inhibitors, determines a cutoff value of sensitivity for negative samples, and normalizes positive samples for the efficiency of DNA recovery. The assay shows a wide dynamic range of detection (between 1 and 10⁶ viral genome equivalents/reaction) and a high degree of accuracy even in the presence of high amounts (up to 1 {micro}g) of human genomic DNA. Moreover, the assay has a very high sensitivity (lower detection limit, 10 genome equivalents/ml) and a high degree of reproducibility and repeatability with a coefficient of variation (CV) of <15 and 23%, respectively. Furthermore, the use of the

calibrator improves the accuracy of quantitation and decreases the intersample variability (CV, 9 and 6%, respectively). The sensitivity and specificity of the assay were tested with a series of clinical specimens obtained from patients affected by various HHV-8-related diseases, as well as from a wide number of controls. In conclusion, our calibrated real-time PCR assay provides a reliable high-throughput method for quantitation of HHV-8 DNA in clinical and laboratory specimens.

Buller, R. S. and G. Storch (2004). "Evaluation of a Real-Time PCR Assay Using the LightCycler System for Detection of Parvovirus B19 DNA." J. Clin. Microbiol. **42**(7): 3326-3328.

<http://jcm.asm.org/cgi/content/abstract/42/7/3326>

We evaluated the artus RealArt Parvovirus B19 LC PCR reagent (artus biotech USA, San Francisco, Calif.) for real-time PCR detection of parvovirus B19 DNA by retesting 71 specimens previously submitted to our laboratory. The artus assay, which produces a quantitative result and provides an internal PCR control, appeared to be slightly more sensitive than our conventional qualitative PCR assay.

Campsall, P. A., N. H. C. Au, et al. (2004). "Detection and Genotyping of Varicella-Zoster Virus by TaqMan Allelic Discrimination Real-Time PCR." J. Clin. Microbiol. **42**(4): 1409-1413.

<http://jcm.asm.org/cgi/content/abstract/42/4/1409>

A proportion of individuals vaccinated with live attenuated Oka varicella-zoster virus (VZV) vaccine subsequently develop attenuated chicken pox and/or herpes zoster. To determine whether postvaccination varicella infections are caused by vaccine or wild-type virus, a simple method for distinguishing the vaccine strain from wild-type virus is required. We have developed a TaqMan real-time PCR assay to detect and differentiate wild-type virus from Oka vaccine strains of VZV. The assay utilized two fluorogenic, minor groove binding probes targeted to a single nucleotide polymorphism in open reading frame 62 that distinguishes the Oka vaccine from wild-type strains. VZV DNA could be genotyped and quantified within minutes of thermocycling completion due to real-time monitoring of PCR product formation and allelic discrimination analysis. The allelic discrimination assay was performed in parallel with two standard PCR-restriction fragment length polymorphism (RFLP) methods on 136 clinical and laboratory VZV strains from Canada, Australia, and Japan. The TaqMan assay exhibited a genotyping accuracy of 100% and, when compared to both PCR-RFLP methods, was 100 times more sensitive. In addition, the method was technically simpler and more rapid. The TaqMan assay also allows for high-throughput genotyping, making it ideal for epidemiologic study of the live attenuated varicella vaccine.

Cano, J., J. Guarro, et al. (2004). "Molecular and Morphological Identification of Colletotrichum Species of Clinical Interest." J. Clin. Microbiol. **42**(6): 2450-2454.

<http://jcm.asm.org/cgi/content/abstract/42/6/2450>

Colletotrichum species have caused human infections in recent years. Because of the difficulties in recognizing them in vitro, we have designed a quick and unambiguous molecular test, based on the amplification of a specific fragment of the internal transcribed spacer 1 region, to distinguish any Colletotrichum isolate from other fungi, including the common pathogenic species.

Analysis of the sequences of the ribosomal DNA (rDNA) fragment showed sufficient variability to clearly separate the five species of *Colletotrichum* that are of clinical interest, i.e., *Colletotrichum coccodes*, *C. crassipes*, *C. dematium*, *C. gloeosporioides*, and *C. graminicola*. Sequencing of the D1-D2 region of the large-subunit rDNA gene also supported these results. Additionally, we reviewed the most suitable morphological characteristics for the in vitro identification of these increasingly important opportunistic fungi.

Cardinali, G., A. Martini, et al. (2002). "Multicenter Comparison of Three Different Analytical Systems for Evaluation of DNA Banding Patterns from *Cryptococcus neoformans*." *J. Clin. Microbiol.* **40**(6): 2095-2100.

<http://jcm.asm.org/cgi/content/abstract/40/6/2095>

The enormous improvement of molecular typing techniques for epidemiological and clinical studies has not always been matched by an equivalent effort in applying optimal criteria for the analysis of both phenotypic and molecular data. In spite of the availability of a large collection of statistical and phylogenetic methods, the vast majority of commercial packages are limited by using only the unweighted pair group method with arithmetic mean algorithm to construct trees and by considering electrophoretic pattern only as migration distances. The latter method has serious drawbacks when different runs (separate gels) of the same molecular analysis are to be compared. This work presents a multicenter comparison of three different systems of banding pattern analysis on random amplified polymorphic DNA, (GACA)₄, and contour-clamped homogeneous electric field patterns from strains of *Cryptococcus neoformans* var. *neoformans* isolated in different clinical and geographical situations and a standard *Saccharomyces cerevisiae* strain employed as an outgroup. The systems considered were evaluated for their actual ability to (i) recognize identities, (ii) define complete differences (i.e., the ability to place *S. cerevisiae* out of the *C. neoformans* cluster), and (iii) estimate the extent of similarity among different strains. The ability to cluster strains according to the patient from which they were isolated was also evaluated. The results indicate that different algorithms do indeed produce divergent trees, both in overall topology and in clustering of individual strains, thus suggesting that care must be taken by individual investigators to use the most appropriate procedure and by the scientific community in defining a consensus system.

Castle, P. E., A. T. Lorincz, et al. (2003). "Comparison between Prototype Hybrid Capture 3 and Hybrid Capture 2 Human Papillomavirus DNA Assays for Detection of High-Grade Cervical Intraepithelial Neoplasia and Cancer." *J. Clin. Microbiol.* **41**(9): 4022-4030.

<http://jcm.asm.org/cgi/content/abstract/41/9/4022>

We compared the performance of a prototype version of the Hybrid Capture 3 (HC3) human papillomavirus (HPV) DNA assay to the current generation Hybrid Capture 2 (HC2) assay, both of which target 13 oncogenic HPV types, for the detection of cervical intraepithelial neoplasia grade 3 and cancer (CIN3+) with cervicovaginal lavage specimens collected at enrollment into a 10-year cohort study at Kaiser Permanente (Portland, Oreg.). HC3 results for a risk-stratified sample (n = 4,364) were compared to HC2 results for the entire cohort (n = 20,810) with receiver operating characteristics curves, and the optimal cut points for both tests (relative light units [RLU]/positive control [PC]) for the detection of CIN3+ were determined. Specimens were also tested for HPV16 and HPV18 with separate HC3 type-specific probes. The optimal cut point for detecting CIN3+ was 1.0 RLU/PC for HC2, as previously shown, and was 0.6 RLU/PC for HC3. At the optimal cut points, HC3 and HC2 had similar screening performance characteristics for CIN3+ diagnosed at the enrollment visit. In analyses that included cases CIN3+ at enrollment and those diagnosed during early follow-up, HC3 had nonsignificantly higher sensitivity and equal

specificity for the detection of CIN3+ compared to HC2; this increase in sensitivity was primarily the result of increased detection of CIN3+ in women who were 30 years of age or older and were cytologically negative ($P = 0.006$). We also compared the performance of the hybrid capture tests to MY09/11 L1 consensus primer PCR results ($n = 1,247$). HC3 was less likely than HC2 to test positive for specimens that tested positive by PCR for any untargeted types ($P < 0.001$). HC3 was less likely than HC2 to test positive for untargeted PCR-detected single infections with HPV53 ($P = 0.001$) and HPV66 ($P = 0.01$). There was good agreement between test positivity by PCR and by single type-specific HC3 probes for HPV16 ($\kappa = 0.76$; 95% confidence interval [CI] = 0.71 to 0.82) and for HPV18 ($\kappa = 0.73$; 95% CI = 0.68 to 0.79). In conclusion, we suggest that HC3 ([IMG]=" BORDER="0">0.6 RLU/PC) may be slightly more sensitive than and equally specific test as HC2 ([IMG]=" BORDER="0">1.0 RLU/PC) for the detection of CIN3+ over the duration of typical screening intervals.

Cerna, J. F., J. P. Nataro, et al. (2003). "Multiplex PCR for Detection of Three Plasmid-Borne Genes of Enteraggregative Escherichia coli Strains." *J. Clin. Microbiol.* **41**(5): 2138-2140.

<http://jcm.asm.org/cgi/content/abstract/41/5/2138>

We developed a novel multiplex PCR assay for enteroaggregative Escherichia coli (EAEC) detection, by using three plasmid-borne genes (the aggregative adherence [AA] probe, aap, and aggR). One or more of the loci were detected in 24 (86%) of 28 patient isolates analyzed. The multiplex PCR assay is a fast, convenient, and sensitive molecular test to detect EAEC.

Chi, X. S., A. Hu, et al. (2005). "Detection and Characterization of New Influenza B Virus Variants in 2002." *J. Clin. Microbiol.* **43**(5): 2345-2349.

<http://jcm.asm.org/cgi/content/abstract/43/5/2345>

One-hundred five influenza B-positive specimens obtained from southeast Asia in 2002 were categorized on the basis of DNA sequencing of HA1 gene as well as real-time PCR analysis of the NA gene. Phylogenetic analysis of the HA1 gene sequences showed that the majority of the viruses (96.2%) belonged to the B/Victoria/2/87 lineage, while a smaller percentage of the viruses (3.8%) belonged to the B/Yamagata/16/88 lineage. The B/Yamagata/16/88 viruses displayed significant antigenic drift in the deduced amino acid sequences of the HA1 protein, and the B/Victoria/2/87-like viruses consisted of B/Hong Kong/1351/02-like (72.3%) and B/Hong Kong/330/01-like (27.7%) viruses. The B/Hong Kong/1351/02-like viruses were reassortants with the HA gene belonging to the B/Victoria/2/87 lineage and the NA gene belonging to the B/Yamagata/16/88 lineage, whereas both the HA and NA genes of B/Hong Kong/330/01 virus belonged to the B/Victoria/2/87 lineage. In this study, however, all the B/Hong Kong/330/01-like isolates exhibited the B/Yamagata/16/88-like NA gene, which likely resulted from reassortment of B/Hong Kong/330/01 and B/Hong Kong/1351/02 viruses during coinfection. Additional molecular characterization of the six internal genes showed that the M, NS, PA, and PB2 genes of the new variants were B/Hong Kong/1351/02 in origin, whereas the NP and PA genes retained the B/Hong Kong/330/01 origin. Interestingly, these new variants all appeared late in the year 2002. These results support the notion that influenza B viruses continued to evolve through antigenic drift and shift.

Chizhikov, V., M. Wagner, et al. (2002). "Detection and Genotyping of Human Group A Rotaviruses by Oligonucleotide Microarray Hybridization." *J. Clin. Microbiol.* **40**(7): 2398-2407.

<http://jcm.asm.org/cgi/content/abstract/40/7/2398>

A rapid and reliable method for the identification of five clinically relevant G genotypes (G1 to G4 and G9) of human rotaviruses based on oligonucleotide microarray hybridization has been developed. The genotype-specific oligonucleotides immobilized on the surface of glass slides were selected to bind to the multiple target regions within the VP7 gene that are highly conserved among individual rotavirus genotypes. Rotavirus cDNA was amplified in a PCR with primers common to all group A rotaviruses. A second round of nested PCR amplification was performed in the presence of indodicarbocyanine-dCTP and another pair of degenerate primers also broadly specific for all genotypes. The use of one primer containing 5'-biotin allowed us to prepare fluorescently labeled single-stranded hybridization probe by binding of another strand to magnetic beads. The identification of rotavirus genotype was based on hybridization with several individual genotype-specific oligonucleotides. This approach combines the high sensitivity of PCR with the selectivity of DNA-DNA hybridization. The specificity of oligonucleotide microchip hybridization was evaluated by testing 20 coded rotavirus isolates from different geographic areas for which genotypes were previously determined by conventional methods. Analysis of the coded specimens showed that this microarray-based method is capable of unambiguous identification of all rotavirus strains. Because of the presence of random mutations, each individual virus isolate produced a unique hybridization pattern capable of distinguishing different isolates of the same genotype and, therefore, subgenotype differentiation. This strain information indicates one of several advantages that microarray technology has over conventional PCR techniques.

Chomel, B. B., A. C. Wey, et al. (2003). "Isolation of *Bartonella washoensis* from a Dog with Mitral Valve Endocarditis." *J. Clin. Microbiol.* **41**(11): 5327-5332.

<http://jcm.asm.org/cgi/content/abstract/41/11/5327>

We report the first documented case of *Bartonella washoensis* bacteremia in a dog with mitral valve endocarditis. *B. washoensis* was isolated in 1995 from a human patient with cardiac disease. The main reservoir species appears to be ground squirrels (*Spermophilus beecheyi*) in the western United States. Based on echocardiographic findings, a diagnosis of infective vegetative valvular mitral endocarditis was made in a spayed 12-year-old female Doberman pinscher. A year prior to presentation, the referring veterinarian had detected a heart murmur, which led to progressive dyspnea and a diagnosis of congestive heart failure the week before examination. One month after initial presentation, symptoms worsened. An emergency therapy for congestive heart failure was unsuccessfully implemented, and necropsy evaluation of the dog was not permitted. Indirect immunofluorescence tests showed that the dog was strongly seropositive (titer of 1:4,096) for several *Bartonella* antigens (*B. vinsonii* subsp. *berkhoffii*, *B. clarridgeiae*, and *B. henselae*), highly suggestive of *Bartonella* endocarditis. Standard aerobic and aerobic-anaerobic cultures were negative. However, a specific blood culture for *Bartonella* isolation grew a fastidious, gram-negative organism 7 days after being plated. Phenotypic and genotypic characterizations of the isolate, including partial sequencing of the citrate synthase (*gltA*), *groEL*, and 16S rRNA genes, indicated that this organism was identical to *B. washoensis*. The dog was seronegative for all tick-borne pathogens tested (*Anaplasma phagocytophilum*, *Ehrlichia canis*, and *Rickettsia rickettsii*), but the sample was highly positive for *B. washoensis* (titer of 1:8,192) and, according to indirect immunofluorescent-antibody assay, weakly positive for phase II *Coxiella burnetii* infection.

Collantes-Fernandez, E., A. Zaballos, et al. (2002). "Quantitative Detection of *Neospora caninum* in Bovine Aborted Fetuses and Experimentally Infected Mice by Real-Time PCR." *J. Clin. Microbiol.* **40**(4): 1194-1198.

<http://jcm.asm.org/cgi/content/abstract/40/4/1194>

We report the development of a real-time PCR assay for the quantitative detection of *Neospora caninum* in infected host tissues. The assay uses the double-stranded DNA-binding dye SYBR Green I to continuously monitor product formation. Oligonucleotide primers were designed to amplify a 76-bp DNA fragment corresponding to the Nc5 sequence of *N. caninum*. A similar method was developed to quantify the 28S rRNA host gene in order to compare the parasite load of different samples and to correct for the presence of potential PCR-inhibiting compounds in the DNA samples. A linear quantitative detection range of 6 logs with a calculated detection limit of 10-1 tachyzoite per assay was observed with excellent linearity ($R^2 = 0.998$). Assay specificity was confirmed by using DNA from the closely related parasite *Toxoplasma gondii*. The applicability of the technique was successfully tested in a variety of host brain tissues: (i) aborted bovine fetuses classified into negative or positive *Neospora*-infected animals according to the observation of compatible lesions by histopathological study and (ii) experimentally infected BALB/c mice, divided into three groups, inoculated animals with or without compatible lesions and negative controls. All samples were also tested by ITS1 *Neospora* nested PCR and a high degree of agreement was shown between both PCR techniques ($\kappa = 0.86$). This technique represents a useful quantitative diagnostic tool to be used in the study of the pathogenicity, immunophylaxis, and treatment of *Neospora* infection.

Collot, S., B. Petit, et al. (2002). "Real-Time PCR for Quantification of Human Herpesvirus 6 DNA from Lymph Nodes and Saliva." *J. Clin. Microbiol.* **40**(7): 2445-2451.

<http://jcm.asm.org/cgi/content/abstract/40/7/2445>

A real-time quantitative PCR assay has been developed to measure human herpesvirus 6 (HHV-6) DNA in biological specimens. The assay sensitivity was 10 copies of DNA per well, with a linear dynamic range of 10 to 107 copies of HHV-6 DNA. Intra- and interassay variations were, respectively, 0.88 and 0.8% for samples containing 102 DNA copies, 0.99 and 0.96% for samples containing 104 copies, and 0.76 and 0.9% for samples containing 106 copies. Among 34 saliva samples from healthy subjects, 26 were found to contain HHV-6 DNA (76.5%; median, 23,870 copies/ml), and following a single freeze-thaw cycle, 25 of the same samples were found to be positive for HHV-6 DNA, although at a statistically significantly lower concentration (median, 3,497 copies/ml). The assay enabled detection of HHV-6 DNA in lymph node biopsies from patients with Hodgkin's disease (HD) (13 of 37 patients [35.1%]), B-cell neoplasms (8 of 36 patients [22.2%]), and T- or NK-cell neoplasms (3 of 13 patients [23.1%]), with concentrations ranging from 100 to 864,640 HHV-6 copies per μ g of DNA (HHV-6B being found in every case except two). All HD patients infected with HHV-6 presented clinically with the nodular sclerosis subtype of HD. The real-time quantitative PCR assay developed here was simple to perform and was sensitive over a wide range of HHV-6 concentrations. It therefore appears to be of potential value in clinical investigation or diagnosis of HHV-6 infection.

Coutlee, F., P. Gravitt, et al. (2002). "Use of PGMY Primers in L1 Consensus PCR Improves Detection of Human Papillomavirus DNA in Genital Samples." *J. Clin. Microbiol.* **40**(3): 902-907.

<http://jcm.asm.org/cgi/content/abstract/40/3/902>

The novel PGMY L1 consensus primer pair is more sensitive than the MY09 and MY11 primer mix for detection and typing with PCR of human papillomavirus (HPV) DNA in genital specimens. We assessed the diagnostic yield of PGMY primers for the detection and typing of HPV by comparing the results obtained with PGMY09/PGMY11 and MY09/MY11/HMB01 on 299 genital

samples. Amplicons generated with PGMY primers were typed with the line blot assay (PGMY-line blot), while HPV amplicons obtained with the degenerate primer pool MY09/MY11/HMB01 were detected with type-specific radiolabeled probes in a dot blot assay (standard consensus PCR test). Cervicovaginal lavage samples (N = 272) and cervical scrape samples (N = 27) were tested in parallel with both PCR tests. The PGMY-line blot test detected the presence of HPV DNA more frequently than the standard consensus PCR assay. The concordance for HPV typing between the two assays was 84.3% (214 of 255 samples), for a good kappa value of 0.69. Of the 177 samples containing HPV DNA by at least one method, 40 samples contained at least one HPV type detected only with PGMY-line blot, whereas positivity exclusively with the standard consensus PCR test was found for only 7 samples ($P < 0.001$). HPV types 45 and 52 were especially more frequently detected with PGMY than MY primers. However, most HPV types were better amplified with PGMY primers, including HPV-16. Samples with discordant results between the two PCR assays more frequently contained multiple HPV types. Studies using PGMY instead of MY primers have the potential to report higher detection rates of HPV infection not only for newer HPV types but also for well-known genital types.

Cowan, L. S., L. Mosher, et al. (2002). "Variable-Number Tandem Repeat Typing of Mycobacterium tuberculosis Isolates with Low Copy Numbers of IS6110 by Using Mycobacterial Interspersed Repetitive Units." *J. Clin. Microbiol.* **40**(5): 1592-1602.

<http://jcm.asm.org/cgi/content/abstract/40/5/1592>

A study set of 180 Mycobacterium tuberculosis and Mycobacterium bovis isolates having low copy numbers of IS6110 were genotyped using the recently introduced method based on the variable-number tandem repeats of mycobacterial interspersed repetitive units (MIRU-VNTR). The results were compared with results of the more commonly used methods, IS6110 restriction fragment length polymorphism (RFLP) and spoligotyping. The isolates were collected in Michigan from 1996 to 1999 as part of a project to genotype all isolates from new cases of tuberculosis in the state. Twelve MIRU loci were amplified, and the amplicons were analyzed by agarose gel electrophoresis to determine the copy number at each MIRU locus. MIRU-VNTR produced more distinct patterns (80 patterns) than did IS6110 RFLP (58 patterns), as would be expected in this study set. Spoligotyping identified 59 patterns. No single method defined all unique isolates, and the combination of all three typing methods generated 112 distinct patterns identifying 90 unique isolates and 90 isolates in 22 clusters. The results confirm the potential utility of MIRU-VNTR typing and show that typing with multiple methods is required to attain maximum specificity.

Dayan, G. H., M. S. Panero, et al. (2004). "Varicella Seroprevalence and Molecular Epidemiology of Varicella-Zoster Virus in Argentina, 2002." *J. Clin. Microbiol.* **42**(12): 5698-5704.

<http://jcm.asm.org/cgi/content/abstract/42/12/5698>

There is limited data on immunity against varicella-zoster virus (VZV) in adults in different parts of Argentina, and it is not known which VZV strains are circulating in Argentina. The objectives of this study were as follows: (i) to evaluate seroprevalence of varicella among adults, assessing the accuracy of clinical history and determining the sociodemographic factors associated with seropositivity; and (ii) to determine the VZV strains circulating in Argentina. A cross-sectional serological survey enrolling 2,807 women aged 15 to 49 years attending public health-care settings in four cities in Argentina (i.e., Buenos Aires, Salta, Mendoza, and Rosario) and one rural area was conducted from August to November 2002. Specimens for identification of VZV strains were obtained from vesicular lesions from 13 pediatric patients with varicella from different areas of the country. PCR amplification was used for genotyping. The overall seroprevalence of varicella antibodies was 98.5% (95% confidence interval, 98.0 to 98.9), ranging from 97.2% in

central Buenos Aires to 99.3% in southern Buenos Aires and Salta. Varicella seroprevalence increased with age. Crowding and length of residence in the same place were associated with seropositivity. The positive predictive value of varicella history for immunity to varicella was 99.4%; however, the negative predictive value was 2.5%. The European genotype was identified in all viral specimens. In Argentina, seroprevalence in women more than 15 years old was high regardless of the area of residence. Negative or uncertain varicella history was not a good predictor of immunity. VZV genotype was stable in all areas of the country.

De Cock, H. E. V., S. L. Marks, et al. (2004). "Ileocolitis Associated with *Anaerobiospirillum* in Cats." J. Clin. Microbiol. **42**(6): 2752-2758.

<http://jcm.asm.org/cgi/content/abstract/42/6/2752>

Ileocolitis associated with spiral bacteria identified as an *Anaerobiospirillum* sp. was found in six cats. Two cats had acute onset of gastrointestinal signs characterized by vomiting and diarrhea in one cat and vomiting in another cat, one cat had chronic diarrhea that was refractory to medical therapy; one cat had acute onset of anorexia and lethargy, and two cats had clinical signs that were not related to the gastrointestinal tract. The presence of an *Anaerobiospirillum* sp. was demonstrated on the basis of ultrastructural morphology of spiral bacteria associated with intestinal lesions and PCR amplification of a genus-specific 16S rRNA gene from affected tissues from each cat. The colons of three clinically healthy cats without lesions and one cat with mild colitis not associated with spiral bacteria were negative for *Anaerobiospirillum* spp. in the same assay. Comparative nucleotide sequence analysis of cloned PCR products from three affected cats further suggested that the spiral bacteria were closely related to *Anaerobiospirillum succiniciproducens*.

del Mar Mosquera, M., F. de Ory, et al. (2002). "Simultaneous Detection of Measles Virus, Rubella Virus, and Parvovirus B19 by Using Multiplex PCR." J. Clin. Microbiol. **40**(1): 111-116.

<http://jcm.asm.org/cgi/content/abstract/40/1/111>

We describe here a multiplex reverse transcription-PCR (RTMNPCR) assay designed to detect and differentiate measles virus, rubella virus, and parvovirus B19. Serial dilution experiments with vaccine strains that compared cell culture isolation of measles in B95 cells and rubella in RK13 cells showed sensitivity rates of 0.004 50% tissue culture infective dose (TCID₅₀) for measles virus and 0.04 TCID₅₀ for rubella virus. This RTMNPCR can detect as few as 10 molecules for measles virus and rubella virus and one molecule for parvovirus B19 in dilution experiments with plasmids containing inserts of the primary reaction amplification products. Five pharyngeal exudates from measles patients and 2 of 15 cerebrospinal fluid samples from measles-related encephalitis were found to be positive for measles virus by this RTMNPCR. A total of 3 of 27 pharyngeal exudates from vaccinated children and 2 pharyngeal exudates, plus one urine sample from a case of congenital rubella syndrome, were found to be positive for rubella virus by RTMNPCR, whereas 16 of 19 sera from patients with erythema infectiosum were determined to be positive for parvovirus B19 by RTMNPCR. In view of these results, we can assess that this method is a useful tool in the diagnosis of these three viruses and could be used as an effective surveillance tool in measles eradication programs.

Domann, E., G. Hong, et al. (2003). "Culture-Independent Identification of Pathogenic Bacteria and Polymicrobial Infections in the Genitourinary Tract of Renal Transplant Recipients." J. Clin.

Microbiol. **41**(12): 5500-5510.

<http://jcm.asm.org/cgi/content/abstract/41/12/5500>

Renal transplant recipients are predisposed to urinary tract infections caused by both common uropathogens and opportunistic bacteria resulting frequently in significant polymicrobial infections. In this study, a culture-independent 16S rRNA-based approach was established to identify unusual, fastidious, or anaerobic bacteria and to investigate bacterial diversity in urinary tract specimens. Similarly sized amplicons encompassing the V6 to V8 region of the 16S rRNA were analyzed with denaturing high-performance liquid chromatography (DHPLC) (WAVE System). Artificial mixtures of single amplicons from commonly encountered uropathogenic bacteria produced distinct peak profiles whose identities were confirmed by sequencing individually collected peak products. We evaluated the application of the method on 109 urinary tract specimens from renal transplant recipients; 100% correlation was found for culture-positive specimens, and DHPLC generated peak profiles. However, for culture-negative specimens, DHPLC facilitated the detection of novel peak profiles. DNA sequencing of these individual peaks was used to identify the bacteria involved. Thus, in PCR-positive but culture-negative samples the method allowed detection of previously known uropathogens such as *Corynebacterium urealyticum* and *Gardnerella vaginalis*, but also unusual agents including *Anaerococcus lactolyticus*, *Bacteroides vulgatus*, *Dialister invisus*, *Fusobacterium nucleatum*, *Lactobacillus iners*, *Leptotrichia amnionii*, *Prevotella buccalis*, *Prevotella ruminicola*, *Rahnella aquatilis*, and *Streptococcus intermedius* were detected as single pathogens or as constituents of polymicrobial infections. The method described is reproducible and rapidly and enables both DHPLC-based profiling and sequence-based investigation of microbial communities and polymicrobial infections. A detailed understanding of infections found in recipients of renal transplants will guide antibiotic therapy regimens and provide new perspectives for decreasing the risk of graft rejection.

Eishi, Y., M. Suga, et al. (2002). "Quantitative Analysis of Mycobacterial and Propionibacterial DNA in Lymph Nodes of Japanese and European Patients with Sarcoidosis." J. Clin. Microbiol. **40**(1): 198-204.

<http://jcm.asm.org/cgi/content/abstract/40/1/198>

The cause(s) of sarcoidosis is unknown. *Mycobacterium* spp. are suspected in Europe and *Propionibacterium* spp. are suspected in Japan. The present international collaboration evaluated the possible etiological links between sarcoidosis and the suspected bacterial species. Formalin-fixed and paraffin-embedded sections of biopsy samples of lymph nodes, one from each of 108 patients with sarcoidosis and 65 patients with tuberculosis, together with 86 control samples, were collected from two institutes in Japan and three institutes in Italy, Germany, and England. Genomes of *Propionibacterium acnes*, *Propionibacterium granulosum*, *Mycobacterium tuberculosis*, *Mycobacterium avium* subsp. *paratuberculosis*, and *Escherichia coli* (as the control) were counted by quantitative real-time PCR. Either *P. acnes* or *P. granulosum* was found in all but two of the sarcoid samples. *M. avium* subsp. *paratuberculosis* was found in no sarcoid sample. *M. tuberculosis* was found in 0 to 9% of the sarcoid samples but in 65 to 100% of the tuberculosis samples. In sarcoid lymph nodes, the total numbers of genomes of *P. acnes* or *P. granulosum* were far more than those of *M. tuberculosis*. *P. acnes* or *P. granulosum* was found in 0 to 60% of the tuberculosis and control samples, but the total numbers of genomes of *P. acnes* or *P. granulosum* in such samples were less than those in sarcoid samples. *Propionibacterium* spp. are more likely than *Mycobacteria* spp. to be involved in the etiology of sarcoidosis, not only in Japanese but also in European patients with sarcoidosis.

Emanuel, P. A., R. Bell, et al. (2003). "Detection of *Francisella tularensis* within Infected Mouse Tissues

by Using a Hand-Held PCR Thermocycler." *J. Clin. Microbiol.* **41**(2): 689-693.

<http://jcm.asm.org/cgi/content/abstract/41/2/689>

The diagnosis of human cases of tularemia often relies upon the demonstration of an antibody response to *Francisella tularensis* or the direct culturing of the bacteria from the patient. Antibody response is not detectable until 2 weeks or more after infection, and culturing requires special media and suspicion of tularemia. In addition, handling live *Francisella* poses a risk to laboratory personnel due to the highly infectious nature of this pathogen. In an effort to develop a rapid diagnostic assay for tularemia, we investigated the use of TaqMan 5' hydrolysis fluorogenic PCR to detect the organism in tissues of infected mice. Mice were infected to produce respiratory tularemia. The *fopA* and *tul4* genes of *F. tularensis* were amplified from infected spleen, lung, liver, and kidney tissues sampled over a 5-day period. The samples were analyzed using the laboratory-based Applied Biosystems International 7900 and the Smiths Detection-Edgewood BioSeeq, a hand-held portable fluorescence thermocycler designed for use in the field. A comparison of culturing and PCR for detection of bacteria in infected tissues shows that culturing was more sensitive than PCR. However, the results for culture take 72 h, whereas PCR results were available within 4 h. PCR was able to detect infection in all the tissues tested. Lung tissue showed the earliest response at 2 days when tested with the ABI 7900 and in 3 days when tested with the BioSeeq. The results were in agreement between the ABI 7900 and the BioSeeq when presented with the same sample. Template preparation may account for the loss of sensitivity compared to culturing techniques. The hand-held BioSeeq thermocycler shows promise as an expedient means of forward diagnosis of infection in the field.

Eshleman, S. H., G. Crutcher, et al. (2005). "Sensitivity and Specificity of the ViroSeq Human Immunodeficiency Virus Type 1 (HIV-1) Genotyping System for Detection of HIV-1 Drug Resistance Mutations by Use of an ABI PRISM 3100 Genetic Analyzer." *J. Clin. Microbiol.* **43**(2): 813-817.

<http://jcm.asm.org/cgi/content/abstract/43/2/813>

The ViroSeq human immunodeficiency virus type 1 (HIV-1) genotyping system is an integrated system for identification of drug resistance mutations in HIV-1 protease and reverse transcriptase (RT). Reagents are included for sample preparation, reverse transcription, PCR amplification, and sequencing. Software is provided to assemble and edit sequence data and to generate a drug resistance report. We determined the sensitivity and specificity of the ViroSeq system for mutation detection using an ABI PRISM 3100 genetic analyzer with a set of clinical samples and recombinant viruses. Twenty clinical plasma samples (viral loads, 1,800 to 10,500 copies/ml) were characterized by cloning and sequencing individual viral variants. Twelve recombinant-virus samples (viral loads, approximately 2,000 to 5,000 copies/ml) were also prepared. Eleven recombinant-virus samples contained drug resistance mutations as 40% mixtures. One recombinant-virus sample contained an insertion at codon 69 in RT (100% mutant). Plasma and recombinant-virus samples were analyzed using the ViroSeq system. Each sample was analyzed on three consecutive days at each of three testing laboratories. The sensitivity of mutation detection was 99.65% for the clinical plasma samples and 99.7% for the recombinant-virus preparations. The specificity of mutation detection was 99.95% for the clinical samples and 100% for the recombinant-virus mixtures. The base calling accuracy of the 3100 instrument was 99.91%. Mutations in clinical plasma samples and recombinant-virus samples were detected with high sensitivity and specificity, including mutations present as mixtures. This report supports the use of the ViroSeq system for identification of drug resistance mutations in HIV-1 protease and RT genes.

Eshleman, S. H., J. Hackett, Jr., et al. (2004). "Performance of the Celera Diagnostics ViroSeq HIV-1 Genotyping System for Sequence-Based Analysis of Diverse Human Immunodeficiency Virus Type 1 Strains." J. Clin. Microbiol. **42**(6): 2711-2717.

<http://jcm.asm.org/cgi/content/abstract/42/6/2711>

The Celera Diagnostics ViroSeq HIV-1 Genotyping System is a Food and Drug Administration-cleared, integrated system for sequence-based analysis of drug resistance mutations in subtype B human immunodeficiency virus type 1 (HIV-1) protease and reverse transcriptase (RT). We evaluated the performance of this system for the analysis of diverse HIV-1 strains. Plasma samples were obtained from 126 individuals from Uganda, Cameroon, South Africa, Argentina, Brazil, and Thailand with viral loads ranging from 2.92 to >6.0 log₁₀ copies/ml. HIV-1 genotyping was performed with the ViroSeq system. HIV-1 subtyping was performed by using phylogenetic methods. PCR products suitable for sequencing were obtained for 125 (99%) of the 126 samples. Genotypes including protease (amino acids 1 to 99) and RT (amino acids 1 to 321) were obtained for 124 (98%) of the samples. Full bidirectional sequence data were obtained for 95 of those samples. The sequences were categorized into the following subtypes: A1/A2 (16 samples), B (12 samples), C (13 samples), D (11 samples), CRF01_AE (9 samples), F/F2 (9 samples), G (7 samples), CRF02_AG (32 samples), H (1 sample), and intersubtype recombinant (14 samples). The performances of the individual sequencing primers were examined. Genotyping of duplicate samples in a second laboratory was successful for 124 of the 126 samples. The identity level for the sequence data from two laboratories ranged from 98 to 100% (median, 99.8%). The ViroSeq system performs well for the analysis of plasma samples with diverse non-B subtypes. The availability of this genotyping system should facilitate studies of HIV-1 drug resistance in non-subtype B strains of HIV-1.

Fiebelkorn, K. R., S. A. Crawford, et al. (2003). "Practical Disk Diffusion Method for Detection of Inducible Clindamycin Resistance in *Staphylococcus aureus* and Coagulase-Negative *Staphylococci*." J. Clin. Microbiol. **41**(10): 4740-4744.

<http://jcm.asm.org/cgi/content/abstract/41/10/4740>

Resistance to macrolides in staphylococci may be due to active efflux (encoded by *msrA*) or ribosomal target modification (macrolide-lincosamide-streptogramin B [MLSB] resistance; usually encoded by *ermA* or *ermC*). MLSB resistance is either constitutive or inducible following exposure to a macrolide. Induction tests utilize closely approximated erythromycin and clindamycin disks; the flattening of the clindamycin zone adjacent to the erythromycin disk indicates inducible MLSB resistance. The present study reassessed the reliability of placing erythromycin and clindamycin disks in adjacent positions (26 to 28 mm apart) in a standard disk dispenser, compared to distances of 15 or 20 mm. A group of 130 clinical isolates of *Staphylococcus aureus* and 100 isolates of erythromycin-resistant coagulase-negative staphylococci (CNS) were examined by disk approximation; all CNS isolates and a subset of *S. aureus* isolates were examined by PCR for *ermA*, *ermC*, and *msrA*. Of 114 erythromycin-resistant *S. aureus* isolates, 39 demonstrated constitutive resistance to clindamycin, while 33 showed inducible resistance by disk approximation at all three distances. Only one isolate failed to clearly demonstrate induction at 26 mm. Of 82 erythromycin-resistant CNS isolates that contained *ermA* or *ermC*, 57 demonstrated constitutive clindamycin resistance, and 25 demonstrated inducible resistance, at 20 and 26 mm. None of the 42 *S. aureus* isolates or 18 CNS isolates containing only *msrA* and none of the erythromycin-susceptible isolates yielded positive disk approximation tests. Simple placement of erythromycin and clindamycin disks at a distance achieved with a standard disk dispenser allowed detection of 97% of *S. aureus* strains and 100% of CNS strains with inducible MLSB resistance in this study.

Fischer, A., C. Lejczak, et al. (2004). "Simple DNA Extraction Method for Dried Blood Spots and Comparison of Two PCR Assays for Diagnosis of Vertical Human Immunodeficiency Virus Type 1 Transmission in Rwanda." J. Clin. Microbiol. **42**(1): 16-20.

<http://jcm.asm.org/cgi/content/abstract/42/1/16>

Dried blood spots (DBS) on filter paper facilitate the collection, transport, and storage of blood samples for laboratory use. A rapid and simple DNA extraction procedure from DBS was developed and evaluated for the diagnosis of human immunodeficiency virus type 1 (HIV-1) infection in children by an in-house nested-PCR assay on three genome regions and by the Amplicor HIV-1 DNA prototype assay version 1.5 (Roche Molecular Systems). A total of 150 samples from children born to HIV-1-infected mothers were collected in Kigali, Rwanda, in parallel as DBS and as peripheral blood mononuclear cell (PBMC) pellets. The results obtained on DBS by the two PCR assays were compared to the results of nested PCR on PBMCs. Of 150 PBMC samples, 10 were positive, 117 were negative, and 23 were indeterminate for HIV-1 infection. In DNA extracted from filter papers and amplified by using the in-house nested PCR, 9 of these 10 positive samples (90%) were found to be positive, and 1 was found to be indeterminate (only the pol region could be amplified). All of the negative samples and all of the 23 indeterminate samples tested negative for HIV-1 infection. When we used the Amplicor DNA test on DBS, all of the 10 PBMC-positive samples were found to be positive and all of the 23 indeterminate samples were found to be negative. Of the PBMC-negative samples, 115 were found to be negative and 2 were found to be indeterminate. We conclude that this simple rapid DNA extraction method on DBS in combination with both detection methods gave a reliable molecular diagnosis of HIV-1 infection in children born to HIV-infected mothers.

Flaherty, J. D., P. N. Levett, et al. (2003). "Fatal Case of Endocarditis Due to *Weissella confusa*." J. Clin. Microbiol. **41**(5): 2237-2239.

<http://jcm.asm.org/cgi/content/abstract/41/5/2237>

This is the first reported case of endocarditis due to the *Lactobacillus*-like vancomycin-resistant gram-positive bacillus *Weissella confusa*. Full identification and susceptibility testing of *Lactobacillus*-like organisms recovered in blood culture should be performed for patients with clinical presentations that suggest endocarditis.

Fournier, P.-E., Y. Zhu, et al. (2004). "Use of Highly Variable Intergenic Spacer Sequences for Multispacer Typing of *Rickettsia conorii* Strains." J. Clin. Microbiol. **42**(12): 5757-5766.

<http://jcm.asm.org/cgi/content/abstract/42/12/5757>

By use of the nearly perfectly colinear genomes of *Rickettsia conorii* and *Rickettsia prowazekii*, we compared the usefulness of three types of sequences for typing of *R. conorii* isolates: (i) 5 variable coding genes comprising the 16S ribosomal DNA, *gltA*, *ompB*, and *sca4* (gene D) genes, which are present in both genomes, and the *ompA* gene, which is degraded in *R. prowazekii*; (ii) 28 genes degraded in *R. conorii* but intact in *R. prowazekii*, including 23 split and 5 remnant genes; and (iii) 27 conserved and 25 variable intergenic spacers. The 4 conserved and 23 split genes as well as the 27 conserved intergenic spacers each had identical sequences in 34 human and 5 tick isolates of *R. conorii*. Analysis of the *ompA* sequences identified three genotypes of *R. conorii*. The variable intergenic spacers were significantly more variable than conserved genes, split genes, remnant genes, and conserved spacers ($P < 10^{-2}$ in all cases). Four of the variable intergenic spacers (*dksA-xerC*, *mppA-purC*, *rpmE-tRNA^{fMet}*, and *tRNA^{Gly}-tRNA^{Tyr}*) had highly

variable sequences; when they were combined for typing, multispacer typing (MST) identified 27 different genotypes in the 39 *R. conorii* isolates. Two batches from the same *R. conorii* strain, Malish (Seven), with different culture passage histories were found to exhibit the same MST type. MST was more discriminatory for strain genotyping than multiple gene sequencing ($P < 10^{-2}$). Phylogenetic analysis based on MST sequences was concordant with the geographic origins of *R. conorii* isolates. Our study supports the usefulness of MST for strain genotyping. This tool may be useful for tracing a strain and identifying its source during outbreaks, including those resulting from bioterrorism.

Fukushima, M., K. Kakinuma, et al. (2003). "Detection and Identification of Mycobacterium Species Isolates by DNA Microarray." *J. Clin. Microbiol.* **41**(6): 2605-2615.

<http://jcm.asm.org/cgi/content/abstract/41/6/2605>

Rapid identification of Mycobacterium species isolates is necessary for the effective management of tuberculosis. Recently, analysis of DNA gyrase B subunit (*gyrB*) genes has been identified as a suitable means for the identification of bacterial species. We describe a microarray assay based on *gyrB* gene sequences that can be used for the identification of Mycobacteria species. Primers specific for a *gyrB* gene region common to all mycobacteria were synthesized and used for PCR amplification of DNA purified from clinical samples. A set of oligonucleotide probes for specific *gyrB* gene regions was developed for the identification of 14 Mycobacterium species. Each probe was spotted onto a silylated glass slide with an arrayer and used for hybridization with fluorescently labeled RNA derived from amplified sample DNA to yield a pattern of positive spots. This microarray produced unique hybridization patterns for each species of mycobacteria and could differentiate closely related bacterial species. Moreover, the results corresponded well with those obtained by the conventional culture method for the detection of mycobacteria. We conclude that a *gyrB*-based microarray can rapidly detect and identify closely related mycobacterial species and may be useful in the diagnosis and effective management of tuberculosis.

Fukushima, M., K. Kakinuma, et al. (2002). "Phylogenetic Analysis of Salmonella, Shigella, and Escherichia coli Strains on the Basis of the *gyrB* Gene Sequence." *J. Clin. Microbiol.* **40**(8): 2779-2785.

<http://jcm.asm.org/cgi/content/abstract/40/8/2779>

Phylogenetic analysis of about 200 strains of Salmonella, Shigella, and Escherichia coli was carried out using the nucleotide sequence of the gene for DNA gyrase B (*gyrB*), which was determined by directly sequencing PCR fragments. The results establish a new phylogenetic tree for the classification of Salmonella, Shigella, and Escherichia coli in which Salmonella forms a cluster separate from but closely related to Shigella and E. coli. In comparison with 16S rRNA analysis, the *gyrB* sequences indicated a greater evolutionary divergence for the bacteria. Thus, in screening for the presence of bacteria, the *gyrB* gene might be a useful tool for differentiating between closely related species of bacteria such as Shigella spp. and E. coli. At present, 16S rRNA sequence analysis is an accurate and rapid method for identifying most unknown bacteria to the genus level because the highly conserved 16S rRNA region is easy to amplify; however, analysis of the more variable *gyrB* sequence region can identify unknown bacteria to the species level. In summary, we have shown that *gyrB* sequence analysis is a useful alternative to 16S rRNA analysis for constructing the phylogenetic relationships of bacteria, in particular for the classification of closely related bacterial species.

Gadea, I., M. Cuenca-Estrella, et al. (2004). "Genotyping and Antifungal Susceptibility Profile of *Dipodascus capitatus* Isolates Causing Disseminated Infection in Seven Hematological Patients of a Tertiary Hospital." *J. Clin. Microbiol.* **42**(4): 1832-1836.

<http://jcm.asm.org/cgi/content/abstract/42/4/1832>

Seven cases of disseminated infection due to *Dipodascus capitatus* are reported. Infections occurred in a hematological unit of a tertiary hospital during a period of 5 years. Five cases were refractory to antifungal therapy. Antifungal susceptibility testing of seven isolates was performed, and strains were typed by PCR fingerprinting with the core sequence of phage M13 and by random amplification of polymorphic DNA with two primers, Ap12h and W-80A. A very short range of MICs of each antifungal agent was observed. The MICs of amphotericin B ranged between 0.50 and 2 {micro}g/ml. Strains were susceptible in vitro to flucytosine and susceptible (dose-dependent) to fluconazole and itraconazole. Voriconazole exhibited an activity in vitro comparable to that of itraconazole. Typing techniques allowed seven additional isolates of *D. capitatus* neither geographically nor temporally related to be classified into two different genomic patterns. The genomic type of the seven strains from the hematological unit was identical regardless of typing technique utilized. It would indicate that the seven cases of disseminated infection could be related epidemiologically.

Gafan, G. P., V. S. Lucas, et al. (2004). "Prevalence of Periodontal Pathogens in Dental Plaque of Children." *J. Clin. Microbiol.* **42**(9): 4141-4146.

<http://jcm.asm.org/cgi/content/abstract/42/9/4141>

Porphyromonas gingivalis, *Actinobacillus actinomycetemcomitans*, and *Tannerella forsythensis* have been implicated as the main etiological agents of periodontal disease. The purpose of this work was to estimate the prevalence of these organisms in plaque from children without gingivitis (group 1; n = 65) and from those with gingivitis (group 2; n = 53). Extracted DNA from plaque was subjected to two rounds of PCR targeting the 16S rRNA gene using both universal primers and species-specific primers. The results were as follows: group 1, *P. gingivalis*, 49%; *A. actinomycetemcomitans*, 55%; and *T. forsythensis*, 65%; group 2, *P. gingivalis*, 47%; *A. actinomycetemcomitans*, 59%; and *T. forsythensis*, 45%. *T. forsythensis* was detected more frequently in children with no gingivitis than in those with gingivitis ($P = 0.03$). There was no significant difference between the two groups with respect to the presence of *P. gingivalis* or *A. actinomycetemcomitans* in either group ($P > 0.05$). Logistic regression analysis revealed that the odds of a patient having gingivitis were 2.3 times greater in the absence of *T. forsythensis*. In conclusion, the results of this study have shown that the three pathogens can be detected in the dental plaque of healthy children and of those with gingivitis and that *T. forsythensis* is associated with dental plaque at sites with no gingivitis.

Gaia, V., N. K. Fry, et al. (2003). "Sequence-Based Typing of *Legionella pneumophila* Serogroup 1 Offers the Potential for True Portability in Legionellosis Outbreak Investigation." *J. Clin. Microbiol.* **41**(7): 2932-2939.

<http://jcm.asm.org/cgi/content/abstract/41/7/2932>

Seven gene loci of *Legionella pneumophila* serogroup 1 were analyzed as potential epidemiological typing markers to aid in the investigation of legionella outbreaks. The genes

chosen included four likely to be selectively neutral (acn, groES, groEL, and recA) and three likely to be under selective pressure (flaA, mompS, and proA). Oligonucleotide primers were designed to amplify 279- to 763-bp fragments from each gene. Initial sequence analysis of the seven loci from 10 well-characterized isolates of *L. pneumophila* serogroup 1 gave excellent reproducibility (R) and epidemiological concordance (E) values (R = 1.00; E = 1.00). The three loci showing greatest discrimination and nucleotide variation, flaA, mompS, and proA, were chosen for further study. Indices of discrimination (D) were calculated using a panel of 79 unrelated isolates. Single loci gave D values ranging from 0.767 to 0.857, and a combination of all three loci resulted in a D value of 0.924. When all three loci were combined with monoclonal antibody subgrouping, the D value was 0.971. Sequence-based typing of *L. pneumophila* serogroup 1 using only three loci is epidemiologically concordant and highly discriminatory and has the potential to become the new "gold standard" for the epidemiological typing of *L. pneumophila*.

Galli, R. A., B. Sattha, et al. (2003). "Sources and Magnitude of Intralaboratory Variability in a Sequence-Based Genotypic Assay for Human Immunodeficiency Virus Type 1 Drug Resistance." J. Clin. Microbiol. **41**(7): 2900-2907.

<http://jcm.asm.org/cgi/content/abstract/41/7/2900>

We assessed the intralaboratory reproducibility of a system for sequencing human immunodeficiency virus type 1 (HIV-1) protease (PR) and reverse transcriptase (RT) by using replicate subanalyses of 46 plasma samples collected from HIV-1-infected, antiretroviral-experienced patients in order to determine the relative contributions of the different procedural steps to final sequence variability. Complete sequence concordance between duplicates of each sample was 99.4%. Complete and partial mismatches occurred scattered throughout the PR-RT genome segment at >300 positions. Approximately 75% of the discordances involved mixtures, some of which appeared at key resistance sites. Most differences were the result of the first-round RT-PCR procedure. Inter-rater concordance for sequence analysis and assembly was >99.9%. There was no observed correlation between the number or frequency of mismatches and plasma viral loads. A separate longitudinal analysis of a single routine control sample sequenced 103 times over 9 months consistently gave highly reproducible sequences (median percentage of nucleotide discordances, 0.04%; range, 0 to 0.2%). Finally, sequence data from 168 sequential samples collected from 22 patients with long-term, predominantly wild type HIV showed that inpatient nucleotide concordance with individual index sequences ranged from 96.5 to 100%. Together, these results confirm that sequence-based genotyping can be a precise and reliable tool for monitoring HIV drug resistance, and they suggest that efforts to reduce variability should focus on the first RT-PCR step. Consequently, the data suggest that the composition of external quality assessment panels should be based on clinical HIV isolates rather than DNA clones.

Gault, E., P. Soussan, et al. (2003). "Evaluation of a New Serotyping Assay for Detection of Anti-Hepatitis C Virus Type-Specific Antibodies in Serum Samples." J. Clin. Microbiol. **41**(5): 2084-2087.

<http://jcm.asm.org/cgi/content/abstract/41/5/2084>

The performance of a new version (HC03) of the hepatitis C virus (HCV) serotyping 1-6 assay (Abbott Murex Laboratories), a specific test for serological determination of HCV types, was evaluated using a selected panel of 180 HCV RNA-positive sera. HC03 was more sensitive than the current HC02 version, typing 53 (37.6%) of 141 samples which were not typable with HC02. Furthermore, the HC03 specificity was 94.1% as evaluated with a panel of 22 genotyped samples. This new version of the test improves the quality of the serological approach to HCV type determination.

Gautier, A.-L., D. Dubois, et al. (2005). "Rapid and Accurate Identification of Human Isolates of *Pasteurella* and Related Species by Sequencing the *sodA* Gene." *J. Clin. Microbiol.* **43**(5): 2307-2314.

<http://jcm.asm.org/cgi/content/abstract/43/5/2307>

The identification of *Pasteurella* and related bacteria remains a challenge. Here, a 449- to 473-bp fragment (*sodA*_{int}) internal to the *sodA* gene, encoding the manganese-dependent superoxide dismutase, was amplified and sequenced with a single pair of degenerate primers from the type strains of *Pasteurella* (18 strains), *Gallibacterium* (1 strain), and *Mannheimia* (5 strains) species. The *sodA*_{int}-based phylogenetic tree was in general agreement with that inferred from the analysis of the corresponding 16S rRNA gene sequences, with members of the *Pasteurella sensu stricto* cluster (*Pasteurella multocida*, *Pasteurella canis*, *Pasteurella dagmatis*, and *Pasteurella stomatis*) forming a monophyletic group and *Gallibacterium* and *Mannheimia* being independent monophyletic genera. However, the *sodA*_{int} sequences showed a markedly higher divergence than the corresponding 16S rRNA genes, confirming that *sodA* is a potent target to differentiate related species. Thirty-three independent human clinical isolates phenotypically assigned to 13 *Pasteurella* species by a reference laboratory were successfully identified by comparing their *sodA*_{int} sequences to those of the type species. In the course of this work, we identified the first *Gallibacterium anatis* isolate ever reported from a human clinical specimen. The *sodA*_{int} sequences of the clinical isolates displayed less than 2.5% divergence from those of the corresponding type strains, except for the *Pasteurella pneumotropica* isolates, which were closely related to each other (>98% *sodA*_{int} sequence identity) but shared only 92% *sodA*_{int} identity with the type strain. The method described here provides a rapid and accurate tool for species identification of *Pasteurella* isolates when access to a sequencing facility is available.

Ghosh, M. K., L. Kuhn, et al. (2003). "Quantitation of Human Immunodeficiency Virus Type 1 in Breast Milk." *J. Clin. Microbiol.* **41**(6): 2465-2470.

<http://jcm.asm.org/cgi/content/abstract/41/6/2465>

The distribution and stability of human immunodeficiency virus type 1 (HIV-1) in breast milk (BM) components remain largely unknown. Inhibitory effects, if any, of BM on HIV RNA and DNA PCR amplification are poorly understood. We have addressed these issues by using virus-spiked BM samples from HIV-negative women. BM samples from HIV-negative women were spiked with HIV-1 virions or cells containing a single integrated copy of HIV DNA (8E5/LAV). After incubation under different experimental conditions, viral RNA was detected by the Roche Amplicor UltraSensitive assay in whole-milk, skim milk, and lipid fractions. We found excellent correlation between HIV-1 input copy and recovery in whole milk ($r = 0.965$, $P < 0.0001$), skim milk ($r = 0.972$, $P < 0.0001$), and the lipid fraction ($r = 0.905$, $P < 0.001$). PCR inhibition was observed in less than 10% of the spiked samples. Similar levels of inhibition were noted in BM samples collected from HIV-infected women. HIV proviral DNA was detected in BM samples using real-time PCR (linear correlation between the threshold cycle versus log DNA copy number, >0.982). The effects of incubation duration and temperature and repeated freeze-thaw cycles on HIV RNA recovery were analyzed. HIV RNA levels were remarkably stable in whole milk after three freeze-thaw cycles and for up to 30 h at room temperature. Our findings improve the understanding of the dynamics of HIV detection in BM and the conditions for BM sample collection, storage, and processing.

Giovannelli, L., A. Lama, et al. (2004). "Detection of Human Papillomavirus DNA in Cervical Samples: Analysis of the New PGMY-PCR Compared To the Hybrid Capture II and MY-PCR Assays and a Two-Step Nested PCR Assay." J. Clin. Microbiol. **42**(8): 3861-3864.

<http://jcm.asm.org/cgi/content/abstract/42/8/3861>

The PGMY-PCR for human papillomavirus (HPV) was evaluated, in parallel with nested PCR (nPCR), in samples with noted Hybrid Capture II (HCII) and MY-PCR results. PGMY-PCR detected HPV DNA in 2.5% of HCII-negative-MY-PCR-negative samples and in 71.7% of HCII-positive-MY-PCR-negative samples; also, it detected the MY-PCR-negative-nPCR-negative types HPV-42, HPV-44, HPV-51, HPV-87, and HPV-89.

Gookin, J. L., A. J. Birkenheuer, et al. (2002). "Single-Tube Nested PCR for Detection of *Tritrichomonas foetus* in Feline Feces." J. Clin. Microbiol. **40**(11): 4126-4130.

<http://jcm.asm.org/cgi/content/abstract/40/11/4126>

Tritrichomonas foetus, a venereal pathogen of cattle, was recently identified as an inhabitant of the large intestine in young domestic cats with chronic diarrhea. Recognition of the infection in cats has been mired by unfamiliarity with *T. foetus* in cats as well as misdiagnosis of the organisms as *Pentatrichomonas hominis* or *Giardia* sp. when visualized by light microscopy. The diagnosis of *T. foetus* presently depends on the demonstration of live organisms by direct microscopic examination of fresh feces or by fecal culturing. As *T. foetus* organisms are fastidious and fragile, routine flotation techniques and delayed examination and refrigeration of feces are anticipated to preclude the diagnosis in numerous cases. The objective of this study was to develop a sensitive and specific PCR test for the diagnosis of feline *T. foetus* infection. A single-tube nested PCR was designed and optimized for the detection of *T. foetus* in feline feces by using a combination of novel (TFITS-F and TFITS-R) and previously described (TFR3 and TFR4) primers. The PCR is based on the amplification of a conserved portion of the *T. foetus* internal transcribed spacer (ITS) region (ITS1 and ITS2) and the 5.8S rRNA gene. The absolute detection limit of the single-tube nested PCR was 1 organism, while the practical detection limit was 10 organisms per 200 mg of feces. Specificity was examined by using *P. hominis*, *Giardia lamblia*, and feline genomic DNA. Our results demonstrate that the single-tube nested PCR is ideally suited for (i) diagnostic testing of feline fecal samples that are found negative by direct microscopy and culturing and (ii) definitive identification of microscopically observable or cultivated organisms.

Gray, J., L. V. von Stedingk, et al. (2002). "Transmission Studies of *Babesia microti* in *Ixodes ricinus* Ticks and Gerbils." J. Clin. Microbiol. **40**(4): 1259-1263.

<http://jcm.asm.org/cgi/content/abstract/40/4/1259>

In order to investigate the possible role of *Ixodes ricinus* as a vector of zoonotic *Babesia microti* infection in Europe, a European rodent isolate (HK) and a zoonotic American isolate (GI) were studied in transmission experiments. PCR detected *B. microti* in the blood and spleens of infected gerbils (*Meriones unguiculatus*) and also in laboratory-induced infections of *I. ricinus* ticks. *B. microti* DNA was detected by PCR in all pooled samples of nymphs and the majority of adults that had fed as larvae and nymphs, respectively, on gerbils with acute infection of the European isolate, confirming that *I. ricinus* could serve as a vector in Europe. The American isolate, GI, proved to be equally infective for larval and nymphal *I. ricinus* as the HK strain, despite a very different appearance in gerbil erythrocytes. Nymphs infected with the HK and GI strains readily

infected gerbils. In contrast to the finding in acute infections, ticks that fed on gerbils with chronic infections of HK and GI did not become infected. It was also found that the HK strain was not transmitted transovarially. The finding that a *B. microti* strain (GI) from a distant geographical region (United States) can infect and be transmitted by *I. ricinus* suggests that other European *B. microti* strains, in addition to the HK strain used here, are probably infective for *I. ricinus*, supporting the view that infection of humans with European *B. microti* may be a regular occurrence.

Greub, G. and D. Raoult (2002). "'Actinobaculum massiliae," a New Species Causing Chronic Urinary Tract Infection." *J. Clin. Microbiol.* **40**(11): 3938-3941.

<http://jcm.asm.org/cgi/content/abstract/40/11/3938>

We report on a new *Actinobaculum* species, "*Actinobaculum massiliae*," isolated from the urine of an elderly woman with recurrent cystitis. Its phenotypic pattern was similar to those of both of the other *Actinobaculum* species described to date. On 16S rRNA sequencing, the Marseille isolate shared 95% homology with *Actinobaculum suis*, 92 to 93% homology with *Actinobaculum schaalii*, 91 to 92% homology with *Arcanobacterium* spp., and 87 to 90% homology with *Actinomyces* species. A bootstrap value of 99% supports the node separating the *Actinobaculum* sp. from its closest neighbor (*A. suis*). In conclusion, on the basis of phenotypic, genotypic, and phylogenetic assessments, we show that the Marseille isolate is a previously unrecognized organism within the *Actinobaculum* genus, and we propose placement of the organism in the taxon "*Actinobaculum massiliae*."

Guinebretiere, M.-H., V. Broussolle, et al. (2002). "Enterotoxigenic Profiles of Food-Poisoning and Food-Borne *Bacillus cereus* Strains." *J. Clin. Microbiol.* **40**(8): 3053-3056.

<http://jcm.asm.org/cgi/content/abstract/40/8/3053>

The enterotoxigenic profiles of 51 *B. cereus* food-related strains were compared to those of 37 *B. cereus* food-poisoning strains. *cytK* and association of *hbl-nhe-cytK* enterotoxin genes were more frequent among diarrheal strains (73 and 63%) than among food-borne strains (37 and 33%). Unlike diarrheal strains, food-borne strains showed frequent *nhe* and *hbl* gene polymorphisms and were often low toxin producers.

Habib-Bein, N. F., W. H. Beckwith, III, et al. (2003). "Comparison of SmartCycler Real-Time Reverse Transcription-PCR Assay in a Public Health Laboratory with Direct Immunofluorescence and Cell Culture Assays in a Medical Center for Detection of Influenza A Virus." *J. Clin. Microbiol.* **41**(8): 3597-3601.

<http://jcm.asm.org/cgi/content/abstract/41/8/3597>

A single-tube real-time (fluorogenic) reverse transcription (RT)-PCR with the SmartCycler instrument (SmartCycler RT-PCR) for influenza A virus detection was evaluated with 238 respiratory specimens. Direct immunofluorescence antibody staining (DFA) and primary rhesus monkey kidney cell culture were performed on-site at Yale-New Haven Hospital. Specimens were transported to the Connecticut Department of Public Health Laboratory for real-time RT-PCR. Cell culture detected influenza A virus in all 150 influenza A virus-positive specimens, DFA detected the virus in 148 influenza A virus-positive specimens, and SmartCycler RT-PCR detected the

virus 143 influenza A virus-positive specimens. The sensitivity and specificity of RT-PCR were 95.3 and 100%, respectively. The high sensitivity and specificity and the rapid turnaround time made the SmartCycler RT-PCR valuable for the rapid diagnosis of influenza A, especially in a public health laboratory. The closed real-time RT-PCR system avoided cross-contamination possible with RT-PCR and the excessive manipulations required for conventional RT-PCR analysis and saved time and labor as well. In a medical center, rapid diagnosis by DFA was labor intensive but was 98.7% sensitive and 100% specific compared to the results of culture and provided results within 2 h throughout operating hours, helping with bed allocation on admission and patient management.

Hall, L., K. A. Doerr, et al. (2003). "Evaluation of the MicroSeq System for Identification of Mycobacteria by 16S Ribosomal DNA Sequencing and Its Integration into a Routine Clinical Mycobacteriology Laboratory." *J. Clin. Microbiol.* **41**(4): 1447-1453.

<http://jcm.asm.org/cgi/content/abstract/41/4/1447>

An evaluation of the MicroSeq 500 microbial identification system by nucleic acid sequencing and the Mayo Clinic experience with its integration into a routine clinical laboratory setting are described. Evaluation of the MicroSeq 500 microbial identification system was accomplished with 59 American Type Culture Collection (ATCC) strains and 328 clinical isolates of mycobacteria identified by conventional and 16S ribosomal DNA sequencing by using the MicroSeq 500 microbial identification system. Nucleic acid sequencing identified 58 of 59 (98.3%) ATCC strains to the species level or to the correct group or complex level. The identification results for 219 of 243 clinical isolates (90.1%) with a distance score of <1% were concordant with the identifications made by phenotypic methods. The remaining 85 isolates had distance scores of >1%; 35 (41.1%) were identified to the appropriate species level or group or complex level; 13 (15.3%) were identified to the species level. All 85 isolates were determined to be mycobacterial species, either novel species or species that exhibited significant genotypic divergence from an organism in the database with the closest match. Integration of nucleic acid sequencing into the routine mycobacteriology laboratory and use of the MicroSeq 500 microbial identification system and Mayo Clinic databases containing additional genotypes of common species and added species significantly reduced the number of organisms that could not be identified by phenotypic methods. The turnaround time was shortened to 24 h, and results were reported much earlier. A limited number of species could not be differentiated from one another by 16S ribosomal DNA sequencing; however, the method provides for the identification of unusual species and more accurate identifications and offers the promise of being the most accurate method available.

Harper, C. G., Y. Feng, et al. (2002). "Helicobacter cetorum sp. nov., a Urease-Positive Helicobacter Species Isolated from Dolphins and Whales." *J. Clin. Microbiol.* **40**(12): 4536-4543.

<http://jcm.asm.org/cgi/content/abstract/40/12/4536>

A novel helicobacter with the proposed name *Helicobacter cetorum*, sp. nov. (type strain MIT 99-5656; GenBank accession number AF 292378), was cultured from the main stomach of two wild, stranded Atlantic white-sided dolphins (*Lagenorhynchus acutus*) and from the feces of three captive cetaceans (a Pacific white-sided dolphin [*Lagenorhynchus obliquidens*]; an Atlantic bottlenose dolphin [*Tursiops truncatus*]; and a beluga whale [*Delphinapterus leucas*]). The infected captive cetaceans were either subclinical, or clinical signs included intermittent regurgitation, inappetance, weight loss, and lethargy. Ulcers were observed in the esophagus and forestomach during endoscopic examination in two of the three captive animals. In the third animal, esophageal linear erosions were visualized endoscopically, and histopathological evaluation of the main stomach revealed multifocal lymphoplasmacytic gastritis with silver-stained

spiral-shaped bacteria. *Helicobacter cetorum* is a fusiform gram-negative bacterium with a single bipolar flagellum. The isolates grow under microaerobic conditions at 37 and 42{degrees}C but not at 25{degrees}C. *H. cetorum* is urease, catalase, and oxidase positive, and it is sensitive to cephalothin. The isolates from the wild, stranded dolphins were sensitive to nalidixic acid, whereas the isolates from the collection animals were resistant. By 16S rRNA sequencing it was determined that *H. cetorum* represented a distinct taxon that clusters most closely with *H. pylori*. Further studies are necessary to determine the role of *H. cetorum* in the development of gastric ulcers and gastritis of cetaceans. This is the first description and formal naming of a novel *Helicobacter* species from a marine mammal.

Hartman, A. B., I. I. Essiet, et al. (2003). "Epidemiology of Tetracycline Resistance Determinants in *Shigella* spp. and Enteroinvasive *Escherichia coli*: Characterization and Dissemination of tet(A)-1." J. Clin. Microbiol. **41**(3): 1023-1032.

<http://jcm.asm.org/cgi/content/abstract/41/3/1023>

To make a comprehensive study of tetracycline resistance determinant distribution in the genus *Shigella*, a collection of 577 clinical isolates of *Shigella* spp. and enteroinvasive *Escherichia coli* (EIEC) from a variety of geographical locations was screened to identify tetracycline-resistant strains. The 459 tetracycline-resistant isolates identified were then screened by PCR analysis to determine the distribution in these strains of tetracycline efflux resistance determinants belonging to classes A to E, G, and H that have been identified in gram-negative bacteria. Only classes A to D were represented in these strains. Although Tet B was the predominant determinant in all geographical locations, there were geographical and species differences in the distribution of resistance determinants. An allele of tet(A), designated tet(A)-1, was identified and sequenced, and the 8.6-kb plasmid containing determinant Tet A-1, designated pSSTA-1, was found to have homologies to portions of a *Salmonella enterica* cryptic plasmid and the broad-host-range resistance plasmid RSF1010. This allele and pSSTA-1 were used as epidemiological markers to monitor clonal and horizontal transmission of determinant Tet A-1. An analysis of serotype, distribution of tetracycline resistance determinants, and resistance profiles indicated that both clonal spread and horizontal transfer had contributed to the spread of specific tetracycline resistance determinants in these populations and demonstrated the use of these parameters as an epidemiological tool to follow the transmission of determinants and strains.

Hawkey, P. M., E. G. Smith, et al. (2003). "Mycobacterial Interspersed Repetitive Unit Typing of *Mycobacterium tuberculosis* Compared to IS6110-Based Restriction Fragment Length Polymorphism Analysis for Investigation of Apparently Clustered Cases of Tuberculosis." J. Clin. Microbiol. **41**(8): 3514-3520.

<http://jcm.asm.org/cgi/content/abstract/41/8/3514>

An evaluation of the utility of IS6110-based restriction fragment length polymorphism (RFLP) typing compared to a combination of variable number tandem repeat (VNTR) typing and mycobacterial interspersed repetitive unit (MIRU) typing was undertaken. A total of 53 patient isolates of *Mycobacterium tuberculosis* from four presumed episodes of cross-infection were examined. Genomic DNA was extracted from the isolates by a cetyl trimethylammonium bromide method. The number of copies of tandem repeats of the five loci ETRA to ETRE and 12 MIRU loci was determined by PCR amplification and agarose gel electrophoresis of the amplicons. VNTR typing identified the major clusters of strains in the three investigations in which they occurred (each representing a different evolutionary clade: 32333, 42235, and 32433). The majority of unrelated isolates (by epidemiology and RFLP typing) were also identified by VNTR typing. The concordance between the RFLP and MIRU typing was complete, with the exception

of two isolates with RFLP patterns that differed by one band each from the rest of the major epidemiologically linked groups of isolates in investigation A. All of these isolates had identical MIRU and VNTR types. A further pair of isolates differed in the number of tandem repeat copies at two MIRU alleles but had identical RFLP patterns. The speed of the combined VNTR and MIRU typing approach enabled results for some of the investigations to be supplied in "real time," influencing choices in contact tracing. The ease of comparison of results of MIRU and VNTR typing, which are recorded as single multidigit numbers, was also found to greatly facilitate investigation management and the communication of results to health care professionals.

Hazbon, M. H. and D. Alland (2004). "Hairpin Primers for Simplified Single-Nucleotide Polymorphism Analysis of Mycobacterium tuberculosis and Other Organisms." J. Clin. Microbiol. **42**(3): 1236-1242.

<http://jcm.asm.org/cgi/content/abstract/42/3/1236>

We describe a novel, simple, rapid, and highly sensitive method to detect single-nucleotide polymorphisms (SNPs) in Mycobacterium tuberculosis and other organisms. Amplification refractory mutation (ARMS) SNP assays were modified by converting the SNP-detecting linear primers in the ARMS assay to hairpin-shaped primers (HPs) through the addition of a 5' tail complementary to the 3' end of the linear primer. The improved ability of these primers to detect SNPs in M. tuberculosis was compared in a real-time PCR with SYBR-I green dye. Linear primers resulted in incorrect or indeterminate allele designation for 6 of the 13 SNP alleles tested in seven different SNP assays, while HPs determined the correct SNP in all cases. We compared the cycle threshold differences (ΔCt) between the reactions containing primer-template matches and the reactions containing primer-template mismatches (where a larger ΔCt indicates a more robust assay). The use of HPs dramatically improved the mean ΔCt values for the SNP assays (7.6 for linear primers and 11.2 for HPs). We designed 98 different HP assays for SNPs previously associated with resistance to the antibiotic isoniazid to test the large-scale utility of the HP approach. Assay design was successful in 72.4%, 83.7%, 88.8%, and 92.9% of the assays after one to four rounds of assay design, respectively. HP SNP assays are simple, sensitive, robust, and inexpensive. These advantages favor the application of this technique for SNP assays of M. tuberculosis and other organisms.

Healy, M., K. Reece, et al. (2004). "Identification to the Species Level and Differentiation between Strains of Aspergillus Clinical Isolates by Automated Repetitive-Sequence-Based PCR." J. Clin. Microbiol. **42**(9): 4016-4024.

<http://jcm.asm.org/cgi/content/abstract/42/9/4016>

A commercially available repetitive-sequence-based PCR (rep-PCR) DNA fingerprinting assay adapted to an automated format, the DiversiLab system, enables rapid microbial identification and strain typing. We explored the performance of the DiversiLab system as a molecular typing tool for 69 Aspergillus isolates (38 A. fumigatus, 15 A. flavus, and 16 A. terreus isolates) had been previously characterized by morphological analysis. Initially, 27 Aspergillus isolates (10 A. fumigatus, 9 A. flavus, and 8 A. terreus isolates) were used as controls to create a rep-PCR-based DNA fingerprint library with the DiversiLab software. Then, 42 blinded Aspergillus isolates were typed using the system. The rep-PCR-based profile revealed 98% concordance with morphology-based identification. rep-PCR-based DNA fingerprints were reproducible and were consistent for DNA from both hyphae and conidia. DiversiLab dendrogram reports correctly identified all A. fumigatus (n = 28), A. terreus (n = 8), and A. flavus (n = 6) isolates in the 42 blinded Aspergillus isolates. rep-PCR-based identification of all isolates was 100% in agreement with the contiguous internal transcribed spacer (ITS) region (ITS1-5.8S-ITS2) sequence-based

identification of the respective isolates. Additionally, the DiversiLab system could demonstrate strain-level differentiation of *A. flavus* and *A. terreus*. Automated rep-PCR may be a time-efficient, effective, easy-to-use, novel genotyping tool for identifying and determining the strain relatedness of fungi. This system may be useful for epidemiological studies, molecular typing, and surveillance of *Aspergillus* species.

Heikkila, T., I. Seppala, et al. (2002). "Species-Specific Serodiagnosis of Lyme Arthritis and Neuroborreliosis Due to *Borrelia burgdorferi* Sensu Stricto, *B. afzelii*, and *B. garinii* by Using Decorin Binding Protein A." J. Clin. Microbiol. **40**(2): 453-460.

<http://jcm.asm.org/cgi/content/abstract/40/2/453>

The antigenic potential of decorin binding protein A (DbpA) was evaluated in serodiagnosis of human Lyme borreliosis (LB). The dbpA was cloned and sequenced from the three pathogenic *Borrelia* species common in Europe. Sequence analysis revealed high interspecies heterogeneity. The identity of the predicted amino acid sequences was 43 to 62% among *Borrelia burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii*. The respective recombinant DbpAs (rDbpAs) were produced and tested as antigens by Western blotting and enzyme-linked immunosorbent assay (ELISA). One hundred percent of patients with neuroborreliosis (NB) and 93% of patients with Lyme arthritis (LA) reacted positively. Sera from the majority of patients reacted with one rDbpA only and had no or low cross-reactivity to other two variant proteins. In patients with culture-positive erythema migrans (EM), the sensitivity of rDbpA immunoglobulin G (IgG) or IgM ELISA was low. The DbpA seems to be a sensitive and specific antigen for the serodiagnosis of LA or NB, but not of EM, provided that variants from all three pathogenic borrelial species are included in the combined set of antigens.

Heininger, A., M. Binder, et al. (2003). "DNase Pretreatment of Master Mix Reagents Improves the Validity of Universal 16S rRNA Gene PCR Results." J. Clin. Microbiol. **41**(4): 1763-1765.

<http://jcm.asm.org/cgi/content/abstract/41/4/1763>

DNase I pretreatment of 16S rRNA gene PCR reagents was tested. The DNase I requirement for the elimination of false-positive results varied between 0.1 and 70 IU per master mix depending on the applied Taq polymerase. PCR sensitivity was mostly maintained when 0.1 IU of DNase I was used.

Heo, E.-j., J.-h. Park, et al. (2002). "Serologic and Molecular Detection of *Ehrlichia chaffeensis* and *Anaplasma phagocytophila* (Human Granulocytic Ehrlichiosis Agent) in Korean Patients." J. Clin. Microbiol. **40**(8): 3082-3085.

<http://jcm.asm.org/cgi/content/abstract/40/8/3082>

Sera from 491 Korean patients with acute febrile diseases were tested for *Ehrlichia chaffeensis* and *Anaplasma phagocytophila* antibodies by indirect immunofluorescence assay (IFA), Western blotting, and TaqMan real-time PCR. Overall, 0.4% of sera reacted with *E. chaffeensis*, and 1.8% reacted with *A. phagocytophila* in IFAs. This is the first report of detection of antibodies to *A. phagocytophila* and *E. chaffeensis* in Korea and suggests the presence of *A. phagocytophila* and *E. chaffeensis* or antigenically similar species.

Herpers, B. L., B. M. de Jongh, et al. (2003). "Real-Time PCR Assay Targets the 23S-5S Spacer for Direct Detection and Differentiation of *Legionella* spp. and *Legionella pneumophila*." J. Clin. Microbiol. **41**(10): 4815-4816.

<http://jcm.asm.org/cgi/content/abstract/41/10/4815>

A real-time PCR for the ABI Prism 7000 system targeting the 23S-5S spacer of *Legionella* spp. was developed. Simultaneous detection and differentiation of *Legionella* spp. and *Legionella pneumophila* within 90 min and without post-PCR melting-curve analysis was achieved using two TaqMan probes. In sputum samples from 23 controls and 17 patients with legionellosis, defined by positive culture, urinary antigen testing, or seroconversion, 94% sensitivity and 100% specificity were observed.

Hindiyeh, M., V. Levy, et al. (2005). "Evaluation of a Multiplex Real-Time Reverse Transcriptase PCR Assay for Detection and Differentiation of Influenza Viruses A and B during the 2001-2002 Influenza Season in Israel." J. Clin. Microbiol. **43**(2): 589-595.

<http://jcm.asm.org/cgi/content/abstract/43/2/589>

The ability to rapidly diagnose influenza virus infections is of the utmost importance in the evaluation of patients with upper respiratory tract infections. It is also important for the influenza surveillance activities performed by national influenza centers. In the present study we modified a multiplex real-time reverse transcriptase PCR (RT-PCR) assay (which uses TaqMan chemistry) and evaluated it for its ability to detect and concomitantly differentiate influenza viruses A and B in 370 patient samples collected during the 2001-2002 influenza season in Israel. The performance of the TaqMan assay was compared to those of a multiplex one-step RT-PCR with gel detection, a shell vial immunofluorescence assay, and virus isolation in tissue culture. The TaqMan assay had an excellent sensitivity for the detection of influenza viruses compared to that of tissue culture. The overall sensitivity and specificity of the TaqMan assay compared to the results of culture were 98.4 and 85.5%, respectively. The sensitivity and specificity of the TaqMan assay for the detection of influenza virus A alone were 100 and 91.1%, respectively. On the other hand, the sensitivity and specificity for the detection of influenza virus B alone were 95.7 and 98.7%, respectively. The rapid turnaround time for the performance of the TaqMan assay (4.5 h) and the relatively low direct cost encourage the routine use of this assay in place of tissue culture. We conclude that the multiplex TaqMan assay is highly suitable for the rapid diagnosis of influenza virus infections both in well-established molecular biology laboratories and in reference clinical laboratories.

Hu, A., M. Colella, et al. (2003). "Simultaneous Detection, Subgrouping, and Quantitation of Respiratory Syncytial Virus A and B by Real-Time PCR." J. Clin. Microbiol. **41**(1): 149-154.

<http://jcm.asm.org/cgi/content/abstract/41/1/149>

Timely diagnosis of respiratory syncytial virus (RSV) infection is critical for appropriate treatment of lower respiratory infection in young children. To facilitate diagnosis, we developed a rapid, specific, and sensitive TaqMan PCR method for detection of RSV A and RSV B. Two sets of primer-probe pairs were selected from the nucleotide sequences encoding the nucleocapsid protein--one targeting RSV A and the other targeting RSV B. The specificity of the TaqMan reverse transcription-PCR assay was evaluated by testing each primer-probe pair against various

viruses derived from laboratory virus stocks, as well as clinical respiratory specimens. Fluorescent signals were observed only in the presence of RSV A and/or RSV B. The sensitivity of our quantitative PCR assay was determined on the basis of PFU and virus particle counts. The resulting assay sensitivity was found to be 0.023 PFU, or two copies of viral RNA, for RSV A and 0.018 PFU, or nine copies of viral RNA, for RSV B. This quantitative TaqMan PCR assay was utilized to diagnose 175 nasopharyngeal aspirates obtained from children in Hong Kong with respiratory symptoms during the winter of 2000 and 2001. Among these specimens, TaqMan PCR detected 36 RSV-positive samples, 10 of which were identified as RSV A and 26 of which were identified as RSV B, whereas culture confirmation identified 21 RSV-positive specimens and immunofluorescence identified 32 RSV-positive specimens, all of which were among those identified by PCR. The results confirmed the accuracy of our TaqMan PCR assay and demonstrated its improved sensitivity versus classical methods.

Huang, F. F., G. Haqshenas, et al. (2002). "Heterogeneity and Seroprevalence of a Newly Identified Avian Hepatitis E Virus from Chickens in the United States." J. Clin. Microbiol. **40**(11): 4197-4202.

<http://jcm.asm.org/cgi/content/abstract/40/11/4197>

We recently identified and characterized a novel virus, designated avian hepatitis E virus (avian HEV), from chickens with hepatitis-splenomegaly syndrome (HS syndrome) in the United States. Avian HEV is genetically related to but distinct from human and swine HEVs. To determine the extent of genetic variation and the seroprevalence of avian HEV infection in chicken flocks, we genetically identified and characterized 11 additional avian HEV isolates from chickens with HS syndrome and assessed the prevalence of avian HEV antibodies from a total of 1,276 chickens of different ages and breeds from 76 different flocks in five states (California, Colorado, Connecticut, Virginia, and Wisconsin). An enzyme-linked immunosorbent assay using a truncated recombinant avian HEV ORF2 antigen was developed and used to determine avian HEV seroprevalence. About 71% of chicken flocks and 30% of chickens tested in the study were positive for antibodies to avian HEV. About 17% of chickens younger than 18 weeks were seropositive, whereas about 36% of adult chickens were seropositive. By using a reverse transcription-PCR (RT-PCR) assay, we tested 21 bile samples from chickens with HS syndrome in California, Connecticut, New York, and Wisconsin for the presence of avian HEV RNA. Of the 21 bile samples, 12 were positive for 30- to 35-nm HEV-like virus particles by electron microscopy (EM). A total of 11 of the 12 EM-positive bile samples and 6 of the 9 EM-negative bile samples were positive for avian HEV RNA by RT-PCR. The sequences of a 372-bp region within the helicase gene of 11 avian HEV isolates were determined. Sequence analyses revealed that the 11 field isolates of avian HEV had 78 to 100% nucleotide sequence identities to each other, 79 to 88% identities to the prototype avian HEV, 76 to 80% identities to chicken big liver and spleen disease virus, and 56 to 61% identities to other known strains of human and swine HEV. The data from this study indicated that, like swine and human HEVs, avian HEV isolates are genetically heterogenic and that avian HEV infection is enzoonotic in chicken flocks in the United States.

Huber, B. S., D. V. Allred, et al. (2002). "Random Amplified Polymorphic DNA and Amplified Fragment Length Polymorphism Analyses of *Pasteurella multocida* Isolates from Fatal Fowl Cholera Infections." J. Clin. Microbiol. **40**(6): 2163-2168.

<http://jcm.asm.org/cgi/content/abstract/40/6/2163>

Fowl cholera, a disease caused by *Pasteurella multocida*, continues to be a major problem for the poultry industry. The sources of pathogenic organisms responsible for most sporadic epidemics remain unconfirmed, although attenuated vaccines that retain a low level of virulence have occasionally been implicated in outbreaks of the disease. One of the vaccines most commonly

used to prevent fowl cholera is the M-9 strain. In the present study, 61 clinical isolates from turkeys that died of fowl cholera from 1997 to 1999 on 36 Utah farms were analyzed and compared to the M-9 vaccine strain. Genetic analyses of the isolates were done by random amplified polymorphic DNA (RAPD) analysis and amplified fragment length polymorphism (AFLP) fingerprinting. The results of these genetic analyses were correlated with the vaccination status of the flock, isolate serotype, and geographic location. Although both genetic techniques effectively identified similar subtle genomic differences, RAPD analysis provided only 77% of the detail provided by AFLP analysis. While a relationship between genetic profile and serotype was evident, no significant relationship indicating geographic influence was found ($P = 0.351$). Interestingly, organisms isolated from vaccinated flocks were significantly closer genetically to the M-9 vaccine strain than isolates from unvaccinated birds were ($P = 0.020$). Statistical analyses revealed that this relationship could not have been determined by serotyping alone ($P = 0.320$), demonstrating the value of AFLP and RAPD analyses in the characterization of disease-causing strains.

Huijsdens, X. W., R. K. Linskens, et al. (2002). "Quantification of Bacteria Adherent to Gastrointestinal Mucosa by Real-Time PCR." *J. Clin. Microbiol.* **40**(12): 4423-4427.

<http://jcm.asm.org/cgi/content/abstract/40/12/4423>

The use of real-time quantitative PCR (5' nuclease PCR assay) as a tool to study the gastrointestinal microflora that adheres to the colonic mucosa was evaluated. We developed primers and probes based on the 16S ribosomal DNA gene sequences for the detection of *Escherichia coli* and *Bacteroides vulgatus*. DNA was isolated from pure cultures and from gut biopsy specimens and quantified by the 5' nuclease PCR assay. The assay showed a very high sensitivity: as little as 1 CFU of *E. coli* and 9 CFU of *B. vulgatus* could be detected. The specificities of the primer-probe combinations were evaluated with samples that were spiked with the species most closely related to *E. coli* and *B. vulgatus* and with eight other gut microflora species. Mucosal samples spiked with known amounts of *E. coli* or *B. vulgatus* DNA showed no PCR inhibition. We conclude that the 5' nuclease PCR assay may be a useful alternative to conventional culture techniques to study the actual in vivo composition of a complex microbial community like the gut microflora.

Hurtle, W., E. Bode, et al. (2004). "Detection of the *Bacillus anthracis* gyrA Gene by Using a Minor Groove Binder Probe." *J. Clin. Microbiol.* **42**(1): 179-185.

<http://jcm.asm.org/cgi/content/abstract/42/1/179>

Identification of chromosomal markers for rapid detection of *Bacillus anthracis* is difficult because significant chromosomal homology exists among *B. anthracis*, *Bacillus cereus*, and *Bacillus thuringiensis*. We evaluated the bacterial gyrA gene as a potential chromosomal marker for *B. anthracis*. A real-time PCR assay was developed for the detection of *B. anthracis*. After analysis of the unique nucleotide sequence of the *B. anthracis* gyrA gene, a fluorescent 3' minor groove binding probe was tested with 171 organisms from 29 genera of bacteria, including 102 *Bacillus* strains. The assay was found to be specific for all 43 strains of *B. anthracis* tested. In addition, a test panel of 105 samples was analyzed to evaluate the potential diagnostic capability of the assay. The assay showed 100% specificity, demonstrating the usefulness of the gyrA gene as a specific chromosomal marker for *B. anthracis*.

Hurtle, W., L. Lindler, et al. (2003). "Detection and Identification of Ciprofloxacin-Resistant *Yersinia pestis* by Denaturing High-Performance Liquid Chromatography." *J. Clin. Microbiol.* **41**(7): 3273-3283.

<http://jcm.asm.org/cgi/content/abstract/41/7/3273>

Denaturing high-performance liquid chromatography (DHPLC) has been used extensively to detect genetic variation. We used this method to detect and identify *Yersinia pestis* KIM5 ciprofloxacin-resistant isolates by analyzing the quinolone resistance-determining region (QRDR) of the gyrase A gene. Sequencing of the *Y. pestis* KIM5 strain *gyrA* QRDR from 55 ciprofloxacin-resistant isolates revealed five mutation types. We analyzed the *gyrA* QRDR by DHPLC to assess its ability to detect point mutations and to determine whether DHPLC peak profile analysis could be used as a molecular fingerprint. In addition to the five mutation types found in our ciprofloxacin-resistant isolates, several mutations in the QRDR were generated by site-directed mutagenesis and analyzed to further evaluate this method for the ability to detect QRDR mutations. Furthermore, a blind panel of 42 samples was analyzed by screening for two mutant types to evaluate the potential diagnostic value of this method. Our results showed that DHPLC is an efficient method for detecting mutations in genes that confer antibiotic resistance.

Huy, T. T.-T., H. Ushijima, et al. (2003). "High Prevalence of Hepatitis B Virus Pre-S Mutant in Countries Where It Is Endemic and Its Relationship with Genotype and Chronicity." *J. Clin. Microbiol.* **41**(12): 5449-5455.

<http://jcm.asm.org/cgi/content/abstract/41/12/5449>

It has been reported that hepatitis B virus (HBV) mutants carrying mutations in the pre-S region can be found in infected patients. In this study, we investigated the prevalence of the HBV variant with the pre-S mutant in different geographic regions, including countries with low and high levels of endemic HBV infection, and analyzed the correlation with clinical findings. We examined 387 HBV DNA-positive serum samples from individuals among 12 countries, consisting of Vietnam, Myanmar, Thailand, China, Korea, Nepal, Japan, Russia, Spain, United States, Bolivia, and Ghana. HBV pre-S mutants were detected in 71 (18.3%) of 387 serum samples tested. This mutant was the most prevalent in Vietnam (36%), followed by Nepal (27.3%), Myanmar (23.3%), China (22.4%), Korea (14.3%), Thailand (10.5%), Japan (7.7%), and Ghana (4.3%). In contrast, no case with this mutation was found in Russia, Spain, United States, and Bolivia. Among the HBV deletion mutations, 15.5% (11 of 71) occurred in the pre-S1 and 46.5% (33 of 71) in the pre-S2 regions. Eight (11.3%) cases had a mutation in both the pre-S1 and pre-S2 regions. In addition, a point mutation at the pre-S2 starting codon was observed in 19 (26.7%) cases. The detection rate of the HBV mutant in patients with hepatocellular carcinoma was significantly higher than in other patients ($P < 0.05$). Furthermore, these mutants were found more frequently in genotype B (25%) and genotype C (24.5%) than in the other genotypes ($P < 0.05$). Our results indicated that there was a high prevalence of HBV pre-S mutation in regions of endemic HBV infection in Asia. Furthermore, the pre-S mutation appeared to be correlated with hepatocellular carcinoma and HBV genotypes.

Imada, Y., Y. Mori, et al. (2003). "Enzyme-Linked Immunosorbent Assay Employing a Recombinant Antigen for Detection of Protective Antibody against Swine Erysipelas." *J. Clin. Microbiol.* **41**(11): 5015-5021.

<http://jcm.asm.org/cgi/content/abstract/41/11/5015>

The specificities and sensitivities of five recombinant proteins of the surface protective antigen

(SpaA) of *Erysipelothrix rhusiopathiae* were examined by indirect enzyme-linked immunosorbent assay (ELISA) with the aim of developing a reliable serological test for the detection of protective antibody against *E. rhusiopathiae*. Fully mature protein and the N-terminal 416 amino acids (SpaA416) showed sufficient antigenicities, and further examination was done with SpaA416 because of its higher yield. The antibody titers of pigs experimentally immunized with commercial live vaccine and two types of inactivated vaccines clearly increased after immunization, and all pigs were completely protected against challenge with virulent strains. On the other hand, the antibody titers of nonimmunized control pigs remained very low until they were challenged, and all showed severe symptoms or subsequently died. Interference with the production of antibody against live vaccine by maternal antibody or porcine respiratory and reproductive syndrome virus infection 1 week after vaccination was also clearly detected. Because the ELISA titer correlated well with the protection results, the specificity and sensitivity of the ELISA were further evaluated with sera collected from pigs reared on 1 farm on which animals had acute septicemia, 2 farms on which the animals were infected or free from infection, and 10 farms on which the animals were vaccinated with live vaccine, among others. The ELISA titers clearly revealed the conditions of the herds. These results indicate that the SpaA416 ELISA is an effective method not only for evaluating pigs for the presence of protective antibody levels resulting from vaccination or maternal antibody but also for detecting antibody produced by natural infection. This test has important potential for the effective control of swine erysipelas.

Imada, Y., A. Takase, et al. (2004). "Serotyping of 800 Strains of *Erysipelothrix* Isolated from Pigs Affected with Erysipelas and Discrimination of Attenuated Live Vaccine Strain by Genotyping." J. Clin. Microbiol. **42**(5): 2121-2126.

<http://jcm.asm.org/cgi/content/abstract/42/5/2121>

Eight hundred *Erysipelothrix* strains isolated between 1992 and 2002 from swine with erysipelas in Japan were serotyped. Thirty-seven, 47, 73, and 643 strains were isolated from animals with acute septicemia, urticaria, chronic endocarditis, and chronic arthritis, respectively, of which 381, 146, 254, and 19 isolates belonged to serotypes 1a, 1b, and 2b and other serotypes, respectively. All serotype 1a isolates were further examined for acriflavine resistance and their genotypes to discriminate them from the attenuated live vaccine strain, defined as serotype 1a, which is resistant to 0.02% acriflavine and which shows low levels of pathogenicity in mice. Of the serotype 1a isolates, 64.6% were acriflavine resistant, with 98.4% of these acriflavine-resistant strains having been isolated from animals with chronic arthritis. By randomly amplified polymorphic DNA (RAPD) analysis, almost all the acriflavine-resistant serotype 1a strains showed the 253-bp band characteristic of vaccine strains and were easily discriminated from all 113 strains of acriflavine-sensitive serotype 1a strains from animals with acute and subacute swine erysipelas. The incidence of acriflavine-resistant strains of the distinctive RAPD type 1-2 was markedly higher than that of the other RAPD types and serotypes. RAPD type 1-2 strains also included a specific group identifiable by restriction fragment length polymorphism DNA analysis. Furthermore, the pathogenicities of 29 isolates of RAPD type 1-2 for mice were lower than those of the 21 isolates of other RAPD types. Our results indicate that RAPD type 1-2 strains are live vaccine strains and that 37% of the cases of chronic swine erysipelas detected in the past 11 years in Japan have occurred as a side effect of live vaccine use.

Ito, Y., J.-C. Grivel, et al. (2003). "Real-Time PCR Assay of Individual Human Immunodeficiency Virus Type 1 Variants in Coinfected Human Lymphoid Tissues." J. Clin. Microbiol. **41**(5): 2126-2131.

<http://jcm.asm.org/cgi/content/abstract/41/5/2126>

In the course of infection, human immunodeficiency virus type 1 (HIV-1) mutates, diverging into a

"swarm" of viral quasispecies, and the predominance of CCR5- or CXCR4-utilizing quasispecies is strongly associated with the pattern of disease progression. Quantification of CCR5- and CXCR4-utilizing viruses in viral swarms is important in the investigation of the mechanisms of this phenomenon. Here, we report on a new real-time PCR-based methodology for the evaluation of replication of individual CCR5- and CXCR4-utilizing variants. The assay is highly reproducible, with a coefficient of variation of <3%, and it accurately estimates the numbers of virus-specific RNA copies even when their difference in the mixture is 2 orders of magnitude. We demonstrate that replications of CCR5- and CXCR4-utilizing variants can be evaluated and distinguished in experimentally coinfecting human lymphoid tissue. The assay we developed may facilitate study of the mechanisms of the R5-to-X4 switch in viral swarms in human tissues infected with HIV-1.

Ivshina, A. V., G. M. Vodeiko, et al. (2004). "Mapping of Genomic Segments of Influenza B Virus Strains by an Oligonucleotide Microarray Method." *J. Clin. Microbiol.* **42**(12): 5793-5801.

<http://jcm.asm.org/cgi/content/abstract/42/12/5793>

Similar to other segmented RNA viruses, influenza viruses can exchange genome segments and form a wide variety of reassortant strains upon coreplication within a host cell. Therefore, the mapping of genome segments of influenza viruses is essential for understanding their phenotypes. In this work, we have developed an oligonucleotide microarray hybridization method for simultaneous genotyping of all genomic segments of two highly homologous strains of influenza B virus. A few strain-specific oligonucleotide probes matching each of the eight segments of the viral genomes of the B/Beijing/184/93 and B/Shangdong/7/97 strains were hybridized with PCR-amplified fluorescently labeled single-stranded DNA. Even though there were a few mismatches among the genomes of the studied virus strains, microarray hybridization showed highly significant and reproducible discrimination ability and allowed us to determine the origins of individual genomic segments in a series of reassortant strains prepared as vaccine candidates. Additionally, we were able to detect the presence of at least 5% of mixed genotypes in virus stocks even when conventional sequencing methods failed, for example, for the NS segment. Thus, the proposed microarray method can be used for (i) rapid and reliable genome mapping of highly homologous influenza B viruses and (ii) extensive monitoring of influenza B virus reassortants and the mixed genotypes. The array can be expanded by adding new oligoprobes and using more quantitative assays to determine the origin of individual genomic segments in series of reassortant strains prepared as vaccine candidates or in mixed virus populations.

Jensen, J. S., M. B. Borre, et al. (2003). "Detection of Mycoplasma genitalium by PCR Amplification of the 16S rRNA Gene." *J. Clin. Microbiol.* **41**(1): 261-266.

<http://jcm.asm.org/cgi/content/abstract/41/1/261>

In order to develop a species-specific PCR for the detection of Mycoplasma genitalium, the sequence of 1,490 bases of the 16S rRNA gene was determined for M. genitalium G37 (type strain) and four Danish isolates of M. genitalium. The sequences of the four Danish strains, mutually different with respect to their MgPa gene, were 100% homologous, although they carried a single common base substitution compared to the type strain. Among members of the Mycoplasma pneumoniae phylogenetic cluster, M. genitalium showed the most-prominent homology to the 16S rRNA sequence of M. pneumoniae (98% homology). From regions showing the least homology to the M. pneumoniae 16S rRNA gene sequence, primers were chosen to amplify DNA from M. genitalium only. Two sets of primers were selected for their ability to detect <10 to 50 M. genitalium genome copies without cross-reactions with M. pneumoniae. The performance of these primers was compared to the performance of two pairs of primers

amplifying parts of the MgPa adhesin gene; 1,030 randomly selected specimens submitted for Chlamydia trachomatis culture were screened with one of the 16S rRNA gene primer sets. A total of 41 specimens were found to be positive for this gene; 40 of these could be confirmed by one of the MgPa primer sets, whereas the other MgPa primer set detected only 21 positive specimens out of 40. These results indicate that estimates of the prevalence of M. genitalium in various populations using MgPa PCR primers could be incorrectly low if the PCR primers are located in variable regions of the MgPa gene.

Johansen, I. S., B. Lundgren, et al. (2003). "Direct Detection of Multidrug-Resistant Mycobacterium tuberculosis in Clinical Specimens in Low- and High-Incidence Countries by Line Probe Assay." J. Clin. Microbiol. **41**(9): 4454-4456.

<http://jcm.asm.org/cgi/content/abstract/41/9/4454>

The INNO-LiPA Rif.TB assay is designed for the detection of rpoB gene mutations causing rifampin resistance in isolates. We applied the method directly to 60 Lithuanian and Danish clinical specimens to detect rifampin resistance rapidly. Results were obtained in 78.3% of clinical specimens, and all were concordant with those obtained by BACTEC 460. The assay could have major impact on the management of multidrug-resistant tuberculosis.

Johansen, T. B., B. Djonje, et al. (2005). "Distribution of IS1311 and IS1245 in Mycobacterium avium Subspecies Revisited." J. Clin. Microbiol. **43**(5): 2500-2502.

<http://jcm.asm.org/cgi/content/abstract/43/5/2500>

We demonstrated that IS1245 is not present in Mycobacterium avium subsp. paratuberculosis by restriction fragment length polymorphism and that the designated three-banded bird pattern of IS1245 in M. avium subsp. avium consists of one copy of IS1245 and two copies of IS1311. Cross hybridization between the two elements can be avoided by using more specific probes.

Johnson, G., M. Ayers, et al. (2003). "Detection and Identification of Bartonella Species Pathogenic for Humans by PCR Amplification Targeting the Riboflavin Synthase Gene (ribC)." J. Clin. Microbiol. **41**(3): 1069-1072.

<http://jcm.asm.org/cgi/content/abstract/41/3/1069>

Several Bartonella species have now been implicated as human pathogens. The recovery of these fastidious organisms in the clinical microbiology laboratory remains difficult, and current methods are still relatively insensitive. Thus, the bartonellae are good candidates for detection by PCR. We have developed a PCR assay which uses a single primer pair targeting the riboflavin synthase gene (ribC) and detected six Bartonella species that have been implicated in human disease, B. henselae, B. quintana, B. bacilliformis, B. clarridgeiae, B. elizabethae, and B. vinsonii subsp. berkhoffii. Species identification is achieved simply by restriction enzyme digestion of the amplicon. This PCR assay appears to be specific for the Bartonella genus because it failed to amplify DNA from several other bacterial species.

Johnson, J. R. and T. T. O'Bryan (2004). "Detection of the Escherichia coli Group 2 Polysaccharide

Capsule Synthesis Gene kpsM by a Rapid and Specific PCR-Based Assay." J. Clin. Microbiol. **42**(4): 1773-1776.

<http://jcm.asm.org/cgi/content/abstract/42/4/1773>

A rapid and simple PCR-based assay for detection of the group 2 capsule synthesis gene kpsM of *Escherichia coli* was designed and validated. When combined with the published group 2 primers (kpsII_f, 5'-GCGCATTGCTGATACTGTTG-3'; kpsII_r, 5'-CATCCAGACGATAAGCATGAGCA-3'), the new primers (the kpsII_f primer and a new reverse primer K2_r, 5'-AGGTAGTTCAGACTCACACCT-3') allowed specific identification by exclusion of the heretofore elusive K2 kpsM variant. The primers yielded the predicted amplicon when multiplexed with other primers and used under varied assay conditions, including a range of concentrations of individual reaction mixture ingredients and of annealing temperatures (from 54 to 64{degrees}C).

Johnson, S. M., K. A. Simmons, et al. (2004). "Amplification of Coccidioidal DNA in Clinical Specimens by PCR." J. Clin. Microbiol. **42**(5): 1982-1985.

<http://jcm.asm.org/cgi/content/abstract/42/5/1982>

Coccidioides DNA was amplified from serum by a PCR using coccidioid-specific primers. A 239-bp product was visualized when 10 fg of exogenous coccidioidal DNA was subjected to amplification. This product was demonstrated in some human and mouse sera prior to the detection of coccidioidal antibodies.

Jordens, J. Z., J. N. Williams, et al. (2002). "Detection of Meningococcal Carriage by Culture and PCR of Throat Swabs and Mouth Gargles." J. Clin. Microbiol. **40**(1): 75-79.

<http://jcm.asm.org/cgi/content/abstract/40/1/75>

The standard method for detecting meningococcal carriage is culture of throat swabs on selective media, but the levels of carriage determined depend heavily on the skills of the individuals taking the swab and interpreting the cultures. This study aimed to determine the most sensitive detection method for meningococcal carriage. Throat swabs and saline mouth gargles, obtained from 89 university students, were processed in parallel by conventional culture and TaqMan c_{tr}A PCR. Carriage of meningococci, as detected by the combined methods, was 20%. The sensitivities of throat swab culture, throat swab PCR, gargle culture, and gargle PCR were 72, 56, 56, and 50%, respectively, and the probabilities that these techniques would correctly identify the absence of carriage (negative predictive value [NPV]) were 93.4, 89.9, 89.9, and 88.8%. Culturing both throat swabs and gargles increased the NPV to 98.6%. The further addition of throat swab PCR increased this to 100%. Testing gargles by both culture and PCR was as sensitive as testing throat swabs by both methods, suggesting that gargles may be a suitable alternative for large-scale screening studies when throat swabs are difficult to obtain, although they required more lengthy laboratory processing. PCR was a useful adjunct to culture for detecting nasopharyngeal carriage, but it failed to detect some nongroupable strains. For maximum sensitivity, a combination of techniques was required. This study indicates the confidence with which health care professionals involved in meningococcal screening can regard laboratory results.

Karmali, M. A., M. Mascarenhas, et al. (2003). "Association of Genomic O Island 122 of *Escherichia coli*

EDL 933 with Verocytotoxin-Producing *Escherichia coli* Seropathotypes That Are Linked to Epidemic and/or Serious Disease." *J. Clin. Microbiol.* **41**(11): 4930-4940.

<http://jcm.asm.org/cgi/content/abstract/41/11/4930>

The distribution of EDL 933 O island 122 (OI-122) was investigated in 70 strains of Verocytotoxin-producing *Escherichia coli* (VTEC) of multiple serotypes that were classified into five "seropathotypes" (A through E) based on the reported occurrence of serotypes in human disease, in outbreaks, and/or in the hemolytic-uremic syndrome (HUS). Seropathotype A comprised 10 serotype O157:H7 and 3 serotype O157:NM strains. Seropathotype B (associated with outbreaks and HUS but less commonly than serotype O157:H7) comprised three strains each of serotypes O26:H11, O103:H2, O111:NM, O121:H19, and O145:NM. Seropathotype C comprised four strains each of serotypes O91:H21 and O113:H21 and eight strains of other serotypes that have been associated with sporadic HUS but not typically with outbreaks. Seropathotype D comprised 14 strains of serotypes that have been associated with diarrhea but not with outbreaks or HUS, and seropathotype E comprised animal VTEC strains of serotypes not implicated in human disease. All strains were tested for four EDL 933 OI-122 virulence genes (Z4321, Z4326, Z4332, and Z4333) by PCR. Negative PCRs were confirmed by Southern hybridization. Overall, 28 (40%) strains contained OI-122 (positive for all four virulence genes), 27 (38.6%) contained an "incomplete" OI-122 (positive for one to three genes), and 15 (21.4%) strains did not contain OI-122. The seropathotype distribution of complete OI-122 was as follows: 100% for seropathotype A, 60% for B, 36% for C, 15% for D, and 0% for E. The differences in the frequency of OI-122 between seropathotypes A, B, and C (associated with HUS) and seropathotypes D and E (not associated with HUS) and between seropathotypes A and B (associated with epidemic disease) and seropathotypes C, D, and E (not associated with epidemic disease) were highly significant ($P < 0.0001$).

Kazor, C. E., P. M. Mitchell, et al. (2003). "Diversity of Bacterial Populations on the Tongue Dorsa of Patients with Halitosis and Healthy Patients." *J. Clin. Microbiol.* **41**(2): 558-563.

<http://jcm.asm.org/cgi/content/abstract/41/2/558>

The primary purpose of the present study was to compare the microbial profiles of the tongue dorsa of healthy subjects and subjects with halitosis by using culture-independent molecular methods. Our overall goal was to determine the bacterial diversity on the surface of the tongue dorsum as part of our ongoing efforts to identify all cultivable and not-yet-cultivated species of the oral cavity. Tongue dorsum scrapings were analyzed from healthy subjects with no complaints of halitosis and subjects with halitosis, defined as an organoleptic score of 2 or more and volatile sulfur compound levels greater than 200 ppb. 16S rRNA genes from DNA isolated from tongue dorsum scrapings were amplified by PCR with universally conserved bacterial primers and cloned into *Escherichia coli*. Typically, 50 to 100 clones were analyzed from each subject. Fifty-one strains isolated from the tongue dorsa of healthy subjects were also analyzed. Partial sequences of approximately 500 bases of cloned inserts from the 16S rRNA genes of isolates were compared with sequences of known species or phylotypes to determine species identity or closest relatives. Nearly complete sequences of about 1,500 bases were obtained for potentially novel species or phylotypes. In an analysis of approximately 750 clones, 92 different bacterial species were identified. About half of the clones were identified as phylotypes, of which 29 were novel to the tongue microbiota. Fifty-one of the 92 species or phylotypes were detected in more than one subject. Those species most associated with healthy subjects were *Streptococcus salivarius*, *Rothia mucilaginosa*, and an uncharacterized species of *Eubacterium* (strain FTB41). *Streptococcus salivarius* was the predominant species in healthy subjects, as it represented 12 to 40% of the total clones analyzed from each healthy subject. Overall, the predominant microbiota on the tongue dorsa of healthy subjects was different from that on the tongue dorsa of subjects with halitosis. Those species most associated with halitosis were *Atopobium parvulum*, a

phylotype (clone BS095) of *Dialister*, *Eubacterium sulci*, a phylotype (clone DR034) of the uncultivated phylum TM7, *Solobacterium moorei*, and a phylotype (clone BW009) of *Streptococcus*. On the basis of our ongoing efforts to obtain full 16S rRNA sequences for all cultivable and not-yet-cultivated species that colonize the oral cavity, there are now over 600 species.

Keller, A. P., M. L. Beggs, et al. (2002). "Evidence of the Presence of IS1245 and IS1311 or Closely Related Insertion Elements in Nontuberculous Mycobacteria outside of the *Mycobacterium avium* Complex." *J. Clin. Microbiol.* **40**(5): 1869-1872.

<http://jcm.asm.org/cgi/content/abstract/40/5/1869>

A PCR assay based on the simultaneous detection of IS1245 and IS1311 was developed and used to determine the host range of these insertion elements. Specific PCR products were observed in *Mycobacterium malmoense*, *Mycobacterium scrofulaceum*, and *Mycobacterium nonchromogenicum*, indicating that IS1245 and IS1311 are not limited to the *Mycobacterium avium* complex.

Key, K. F., D. K. Guenette, et al. (2003). "Development of a Heteroduplex Mobility Assay To Identify Field Isolates of Porcine Reproductive and Respiratory Syndrome Virus with Nucleotide Sequences Closely Related to Those of Modified Live-Attenuated Vaccines." *J. Clin. Microbiol.* **41**(6): 2433-2439.

<http://jcm.asm.org/cgi/content/abstract/41/6/2433>

Porcine reproductive and respiratory syndrome has been devastating the swine industry since the late 1980s. The disease has been controlled, to some extent, through the use of modified live-attenuated (MLV) vaccines once available. However, such a practice periodically resulted in isolation or detection of vaccine-like viruses from pigs as determined by a partial genomic sequencing. In this study, we developed a heteroduplex mobility assay (HMA) for quickly identifying porcine reproductive and respiratory syndrome virus (PRRSV) isolates with significant nucleotide sequence identities ($\geq 98\%$) with the modified live-attenuated vaccines. The major envelope gene (ORF5) of 51 PRRSV field isolates recovered before and after the introduction of the vaccines was amplified, denatured, and reannealed with the HMA reference vaccine strains Ingelvac PRRS MLV and Ingelvac PRRS ATP, respectively. Nine of the 51 field isolates and the VR2332 parent virus of Ingelvac PRRS MLV, which were all highly related to Ingelvac PRRS MLV with $\geq 2\%$ nucleotide sequence divergence as determined by sequence analysis, were all identified by the HMA to form homoduplexes with the reference Ingelvac PRRS MLV. No homoduplex-forming field isolate was identified when Ingelvac PRRS ATP was used as the HMA reference except for its parent virus JA142. Other field isolates with more than 2% nucleotide sequence divergence with the respective reference vaccine strain resulted in the formation of heteroduplexes with reduced mobility in polyacrylamide gel electrophoresis. The HMA results also correlated well with the results of phylogenetic analyses. The data indicated that the HMA developed in the study may be a rapid and efficient method for large-scale screening of potential vaccine-like PRRSV field isolates for further genetic characterization.

Khanna, M., J. Fan, et al. (2005). "The Pneumoplex Assays, a Multiplex PCR-Enzyme Hybridization Assay That Allows Simultaneous Detection of Five Organisms, *Mycoplasma pneumoniae*, *Chlamydia (Chlamydophila) pneumoniae*, *Legionella pneumophila*, *Legionella micdadei*, and

Bordetella pertussis, and Its Real-Time Counterpart." J. Clin. Microbiol. **43**(2): 565-571.

<http://jcm.asm.org/cgi/content/abstract/43/2/565>

Respiratory disease caused by atypical bacteria remains an important cause of morbidity and mortality for adults and children, despite the widespread use of effective antimicrobials agents. Culture remains the "gold standard" for the detection of these agents. However, culture is labor-intensive, takes several days to weeks for growth, and can be very insensitive for the detection of some of these organisms. Newer singleplex PCR diagnostic tests are sensitive and specific, but multiple assays would be needed to detect all of the common pathogens. Therefore, we developed the Pneumoplex assays, a multiplex PCR-enzyme hybridization assay (the standard assay) and a multiplex real-time assay to detect the most common atypical pathogens in a single test. Primer and probe sequences were designed from conserved regions of specific genes for each of these organisms. The limits of detection were as follows: for *Bordetella pertussis*, 2 CFU/ml; for *Legionella pneumophila* (serotypes 1 to 15) and *Legionella micdadei*, 9 and 80 CFU/ml, respectively; for *Mycoplasma pneumoniae*, 5 CFU/ml; and for *Chlamydia* (*Chlamydophila*) *pneumoniae*, 0.01 50% tissue culture infective doses. Recombinant DNA controls for each of these organisms were constructed, and the number of copies for each DNA control was calculated. The Pneumoplex could detect each DNA control down to 10 copies/ml. The analytical specificity demonstrated no cross-reactivity between 23 common respiratory pathogens. One hundred twenty-five clinical bronchoalveolar lavage fluid samples tested by the standard assay demonstrated that the Pneumoplex yielded a sensitivity and a specificity of 100 and 98.5%, respectively. This test has the potential to assist clinicians in establishing a specific etiologic diagnosis before initiating therapy, to decrease hospital costs, and to prevent inappropriate antimicrobial therapy.

Khare, S., T. A. Ficht, et al. (2004). "Rapid and Sensitive Detection of *Mycobacterium avium* subsp. paratuberculosis in Bovine Milk and Feces by a Combination of Immunomagnetic Bead Separation-Conventional PCR and Real-Time PCR." J. Clin. Microbiol. **42**(3): 1075-1081.

<http://jcm.asm.org/cgi/content/abstract/42/3/1075>

Immunomagnetic bead separation coupled with bead beating and real-time PCR was found to be a very effective procedure for the isolation, separation, and detection of *Mycobacterium avium* subsp. paratuberculosis from milk and/or fecal samples from cattle and American bison. Samples were spiked with *M. avium* subsp. paratuberculosis organisms, which bound to immunomagnetic beads and were subsequently lysed by bead beating; then protein and cellular contaminants were removed by phenol-chloroform-isopropanol extraction prior to DNA precipitation. DNA purified by this sequence of procedures was then analyzed by conventional and real-time IS900-based PCR in order to detect *M. avium* subsp. paratuberculosis in feces and milk. By use of this simple and rapid technique, 10 or fewer *M. avium* subsp. paratuberculosis organisms were consistently detected in milk (2-ml) and fecal (200-mg) samples, making this sensitive procedure very useful and cost-effective for the diagnosis of clinical and subclinical Johne's disease (paratuberculosis) compared to bacteriological culture, which is constrained by time, labor, and expense under diagnostic laboratory conditions.

Kivi, M., Y. Tindberg, et al. (2003). "Concordance of *Helicobacter pylori* Strains within Families." J. Clin. Microbiol. **41**(12): 5604-5608.

<http://jcm.asm.org/cgi/content/abstract/41/12/5604>

Helicobacter pylori infection is typically acquired in early childhood, and a predominantly intrafamilial transmission has been postulated. To what extent family members share the same strains is poorly documented. Our aim was to explore patterns of shared strains within families by using molecular typing. Family members of *H. pylori*-infected 10- to 12-year-old index children identified in a school survey were invited to undergo gastroscopy. Bacterial isolates were typed with random amplified polymorphic DNA and PCR-restriction fragment length polymorphism of the genes *ureA-B*, *glmM*, or *flaA*. The presence or absence of the *cag* pathogenicity island, a bacterial virulence factor, was determined by PCR. GelCompar II software, supplemented with visual inspection, was used in the cluster analysis. In 39 families, 104 individuals contributed 208 bacterial isolates from the antrum and corpus. A large proportion, 29 of 36 (81%) of the offspring in a sibship, harbored the same strain as at least one sibling. Mother-offspring strain concordance was detected in 10 of 18 (56%) of the families. Of 17 investigated father-offspring relations in eight families, none were strain concordant. Spouses were infected with the same strains in 5 of 23 (22%) of the couples. Different strains in the antrum and corpus were found in 8 of 104 (8%) of the subjects. Our family-based fingerprinting study demonstrates a high proportion of shared strains among siblings. Transmission between spouses seems to be appreciable. The data support mother-child and sib-sib transmission as the primary transmission pathways of *H. pylori*.

Klieforth, R., G. Maalouf, et al. (2002). "Malignant Catarrhal Fever-Like Disease in Barbary Red Deer (*Cervus elaphus barbarus*) Naturally Infected with a Virus Resembling Alcelaphine Herpesvirus 2." *J. Clin. Microbiol.* **40**(9): 3381-3390.

<http://jcm.asm.org/cgi/content/abstract/40/9/3381>

Eight Barbary red deer (*Cervus elaphus barbarus*) developed clinical signs suggestive of malignant catarrhal fever (MCF) over a 28-day period. These animals were housed outdoors with four other species of ruminants. Affected red deer had lethargy, ocular signs, and nasal discharge and were euthanatized within 48 h. Lesions included ulcers of the muzzle, lips, and oral cavity associated with infiltrates of neutrophils and lymphocytes. Serologically, six of seven red deer tested during the outbreak were positive by competitive enzyme-linked immunosorbent assay for antibodies to a shared MCF virus antigen. PCR using oligonucleotide primers designed for a conserved protein of alcelaphine herpesviruses 1 (AIHV-1) and 2 (AIHV-2) and for conserved regions of a herpesvirus DNA polymerase gene was positive for tissues from all eight clinically affected animals and negative for eight out of eight red deer without clinical signs of MCF. DNA sequencing of PCR amplicons from the diseased red deer indicated that they were infected with a novel herpesvirus closely related to AIHV-2; immunohistochemistry using polyclonal anti-AIHV-2 serum and in situ hybridization demonstrated the presence of virus within salivary glands adjacent to oral lesions of affected animals. A survey of other ruminants near the outbreak subsequently showed that normal Jackson's hartebeest (*Alcelaphus buselaphus jacksoni*) that were cohoused with the diseased red deer were infected with the same virus and were shedding the virus in nasal excretions. These findings suggest that a herpesvirus closely related to AIHV-2 caused the MCF-like disease epizootic in Barbary red deer and that the virus may have originated from Jackson's hartebeest.

Klotz, M., S. Oppen, et al. (2003). "Detection of *Staphylococcus aureus* Enterotoxins A to D by Real-Time Fluorescence PCR Assay." *J. Clin. Microbiol.* **41**(10): 4683-4687.

<http://jcm.asm.org/cgi/content/abstract/41/10/4683>

Staphylococcus aureus is one of the most significant pathogens causing nosocomial and community-acquired infections. Among the secreted staphylococcal virulence factors, there is a growing list of enterotoxins which can induce gastroenteric syndrome and toxic shock syndrome.

Here, we developed a real-time fluorescence PCR assay (TaqMan PCR) for the detection of genes encoding staphylococcal enterotoxins A, B, C1, and D (SEA, SEB, SEC1, and SED) of *S. aureus* as well as the *mecA* gene encoding methicillin resistance and the *femB* gene as a specific genomic marker for *S. aureus*. SEA to SED were selected because they are the four classically described enterotoxins of *S. aureus* and because they were detected by latex agglutination. In order to evaluate the reliability of TaqMan PCR, we investigated 93 isolates of *S. aureus* derived from patients at our hospital over 5 months and compared the results with data obtained by a commercially available reversed passive latex agglutination assay (SET-RPLA) for these isolates. Thirteen enterotoxin genes were detected by TaqMan PCR; however, no proteins expressed by these genes were detected by SET-RPLA. As a result, more isolates of *S. aureus* ($n = 44$) were found positive by TaqMan PCR for one or more enterotoxin genes than by SET-RPLA for the respective proteins expressed by these genes ($n = 40$). We conclude that TaqMan PCR is more sensitive because it offers the possibility for determining enterotoxins on a genotypic basis. Additionally, the assay allows the parallel detection of genes for SEA to SED and methicillin resistance in *S. aureus*. Furthermore, real-time PCR is well suited for screening large numbers of samples at the same time, allowing rapid, reliable, efficient, and cost-saving routine laboratory diagnosis.

Knutsson, R., C. Lofstrom, et al. (2002). "Modeling of 5' Nuclease Real-Time Responses for Optimization of a High-Throughput Enrichment PCR Procedure for *Salmonella enterica*." J. Clin. Microbiol. **40**(1): 52-60.

<http://jcm.asm.org/cgi/content/abstract/40/1/52>

The performance of a 5' nuclease real-time PCR assay was studied to optimize an automated method of detection of preenriched *Salmonella enterica* cells in buffered peptone water (BPW). The concentrations and interactions of the PCR reagents were evaluated on the basis of two detection responses, the threshold cycle (CT) and the fluorescence intensity by a normalized reporter value ($\{\Delta R_n\}$). The CT response was identified as the most suitable for detection modeling to describe the PCR performances of different samples. DNA extracted from *S. enterica* serovar Enteritidis was studied in double-distilled H₂O (ddH₂O) and in two different enrichment media (brain heart infusion and BPW) with two PCR mixtures based on AmpliTaq Gold or rTth. A descriptive model was proposed and fitted to the available experimental data. Equivalent PCR performances for the two PCR mixtures were obtained when DNA was diluted in ddH₂O. However, the level of detection of DNA was affected when BPW was present during amplification. Use of the rTth mixture generated a 1-log-unit wider linear range of amplification, and the DNA detection levels were 2×10^{-13} g/microwell for the rTth mixture and 2×10^{-12} g/microwell for the AmpliTaq Gold mixture. To verify the improved amplification capacity of the rTth mixture, BPW was inoculated with 1 CFU of *S. enterica* serovar Enteritidis per ml and the mixture was incubated at 30°C. Samples for PCR were withdrawn every 4 h during a 36-h enrichment. Use of the rTth mixture resulted in an earlier PCR detection during enrichment than use of the AmpliTaq Gold mixture. For accurate detection (CT ≤ 30) of *S. enterica* serovar Enteritidis inoculated in BPW, the rTth mixture required 8.4 h of enrichment, while the AmpliTaq Gold mixture needed 11.6 h. In conclusion, the principle applied can improve the methodology of 5' nuclease real-time PCR for numerical optimization of sample pretreatment strategies to provide automated diagnostic PCR procedures.

Konnick, E. Q., M. Erali, et al. (2005). "Evaluation of the COBAS Amplicor HBV Monitor Assay and Comparison with the Ultrasensitive HBV Hybrid Capture 2 Assay for Quantification of Hepatitis B Virus DNA." J. Clin. Microbiol. **43**(2): 596-603.

<http://jcm.asm.org/cgi/content/abstract/43/2/596>

Performance characteristics of the COBAS Amplicor HBV Monitor test (Roche Diagnostics), which measures hepatitis B virus (HBV) DNA quantitatively, were evaluated and compared with the Ultrasensitive HBV Hybrid Capture 2 (HC2; Digene Corporation) assay. Linearity and within-run precision were assessed for both methods by using eight HBV DNA-positive samples serially diluted to obtain a range of <100 to 500,000 HBV DNA copies/ml and run in triplicate. Agreement between the methods was studied with 100 clinical samples. HC2 assay performance near the limit of detection was investigated through repeat testing of 149 samples with HC2 and testing of 37 samples with HC2 results of <4,700 HBV DNA copies/ml by Amplicor assay and a qualitative PCR assay. The linearity experiment for Amplicor had regression of observed values compared to expected values ($y = 1.073x - 0.247$; $R^2 = 0.993$, $n = 32$; for HC2, $y = 0.855x + 0.759$, $R^2 = 0.729$, $n = 18$). Within-run standard deviation of log HBV DNA copies/ml ranged from 0.003 to 0.348 (Amplicor) and 0.027 to 0.253 (HC2). Agreement assessed by Deming regression was poor [Amplicor = $1.197(\text{HC2}) - 0.961$; $R^2 = 0.799$, standard error of the estimate (SEE) = 0.710, $n = 94$]. Near the lower limit of detection, 32 of 149 repeat HC2 results were <4,700 HBV DNA copies/ml. Of the 37 samples with HC2 results of <4,700 HBV DNA copies/ml, HBV DNA was not detected in 15 samples, while HBV DNA was detected by at least one PCR method in 12 samples. Amplicor is linear from 200 to 200,000 HBV DNA copies/ml with undiluted samples, and this range can be expanded through dilution. Inconsistent HC2 results near the limit of detection justify use of a grey zone.

Konomi, N., E. Lebowitz, et al. (2002). "Detection of Mycobacterial DNA in Andean Mummies." J. Clin. Microbiol. **40**(12): 4738-4740.

<http://jcm.asm.org/cgi/content/abstract/40/12/4738>

The identification of genetic material from pathogenic organisms in ancient tissues provides a powerful tool for the study of certain infectious diseases in historic populations. We have obtained tissue samples from the genital areas of 12 mummies in the American Museum of Natural History collection in New York, N.Y. The mummies were excavated in the Andes Mountain region of South America, and radiocarbon dating estimates that the mummies date from A.D. 140 to 1200. DNAs were successfully extracted from all tissues and were suitable for PCR analysis. PCRs were carried out to detect Mycobacterium tuberculosis complex and mycobacteria other than M. tuberculosis (MOTB). M. tuberculosis complex was detected in 2 out of 12 samples, and MOTB were detected in 7 samples. This study confirmed the adequate preservation of genetic material in mummified tissues and the existence of mycobacteria, including M. tuberculosis, in historic populations in South America.

Korman, T. M., A. Boers, et al. (2004). "Fatal Case of Toxic Shock-Like Syndrome Due to Group C Streptococcus Associated with Superantigen Exotoxin." J. Clin. Microbiol. **42**(6): 2866-2869.

<http://jcm.asm.org/cgi/content/abstract/42/6/2866>

Group C streptococci have been reported to cause invasive disease similar to that classically associated with group A streptococcus (GAS). We describe a fatal case of toxic shock-like syndrome due to Streptococcus equi subsp. zooepidemicus. The causative organism did not possess any known GAS superantigen exotoxin genes but did show evidence of superantigen production.

Kornegay, J. R., M. Roger, et al. (2003). "International Proficiency Study of a Consensus L1 PCR Assay

for the Detection and Typing of Human Papillomavirus DNA: Evaluation of Accuracy and Intralaboratory and Interlaboratory Agreement." J. Clin. Microbiol. **41**(3): 1080-1086.

<http://jcm.asm.org/cgi/content/abstract/41/3/1080>

The PGMY L1 consensus primer pair combined with the line blot assay allows the detection of 27 genital human papillomavirus (HPV) genotypes. We conducted an intralaboratory and interlaboratory agreement study to assess the accuracy and reproducibility of PCR for HPV DNA detection and typing using the PGMY primers and typing amplicons with the line blot (PGMY-LB) assay. A test panel of 109 samples consisting of 29 HPV-negative (10 buffer controls and 19 genital samples) and 80 HPV-positive samples (60 genital samples and 20 controls with small or large amounts of HPV DNA plasmids) were tested blindly in triplicate by three laboratories. Intralaboratory agreement ranged from 86 to 98% for HPV DNA detection. PGMY-LB assay results for samples with a low copy number of HPV DNA were less reproducible. The rate of intralaboratory agreement excluding negative results for HPV typing ranged from 78 to 96%. Interlaboratory reliability for HPV DNA positivity and HPV typing was very good, with levels of agreement of >95% and kappa values of >0.87. Again, low-copy-number samples were more prone to generating discrepant results. The accuracy varied from 91 to 100% for HPV DNA positivity and from 90 to 100% for HPV typing. HPV testing can thus be accomplished reliably with PCR by using a standardized written protocol and quality-controlled reagents. The use of validated HPV DNA detection and typing assays demonstrating excellent interlaboratory agreement will allow investigators to better compare results between epidemiological studies.

Kosoy, M., M. Murray, et al. (2003). "Bartonella Strains from Ground Squirrels Are Identical to Bartonella washoensis Isolated from a Human Patient." J. Clin. Microbiol. **41**(2): 645-650.

<http://jcm.asm.org/cgi/content/abstract/41/2/645>

The most likely animal source of a human case of cardiac disease in Washoe County, Nev., was identified by comparison of DNA sequences of three genes (citrate synthase *gltA*, 60-kDa heat shock protein gene *groEL*, and 16S rRNA gene) of *Bartonella washoensis* cultured from the human patient in question and of *Bartonella* isolates obtained from the following Nevada rodents: *Peromyscus maniculatus* (17 isolates), *Tamias minimus* (11 isolates), *Spermophilus lateralis* (3 isolates), and *Spermophilus beecheyi* (7 isolates). Sequence analyses of *gltA* amplicons obtained from *Bartonella* from the rodents demonstrated considerable heterogeneity and resulted in the identification of 16 genetic variants that were clustered within three groups in phylogenetic analysis. Each of the three groups was associated with a rodent genus, *Peromyscus*, *Tamias*, or *Spermophilus*. The *gltA*, 16S rRNA gene, and *groEL* sequences of a *Bartonella* isolate obtained from a California ground squirrel (*S. beecheyi*) were completely identical to homologous sequences of *B. washoensis*, strongly suggesting that these animals were the source of infection in the human case.

Kotlowski, R., I. C. Shamputa, et al. (2004). "PCR-Based Genotyping of *Mycobacterium tuberculosis* with New GC-Rich Repeated Sequences and IS6110 Inverted Repeats Used as Primers." J. Clin. Microbiol. **42**(1): 372-377.

<http://jcm.asm.org/cgi/content/abstract/42/1/372>

In the present study we attempted to develop a PCR-based epidemiological tool for the differentiation of *Mycobacterium tuberculosis* isolates. Use of the designed primers Mtb1 (5'-CCG-GCG-GGG-CCG-GCG-G) and Mtb2 (5'-CGG-CGG-CAA-CGG-CGG-C) targeting frequently

repeated 16-bp sequences in combination with primers sited at the inverted repeats flanking IS6110 allowed differentiation of *M. tuberculosis* isolates.

Krafft, A. E., K. L. Russell, et al. (2005). "Evaluation of PCR Testing of Ethanol-Fixed Nasal Swab Specimens as an Augmented Surveillance Strategy for Influenza Virus and Adenovirus Identification." *J. Clin. Microbiol.* **43**(4): 1768-1775.

<http://jcm.asm.org/cgi/content/abstract/43/4/1768>

Viral culture isolation has been widely accepted as the "gold standard" for laboratory confirmation of viral infection; however, it requires ultralow temperature specimen storage. Storage of specimens in ethanol at room temperature could expand our ability to conduct active surveillance and retrospective screenings of viruses with rapid and inexpensive real-time PCR tests, including isolates from remote regions where freezing specimens for culture is not feasible. Molecular methods allow for rapid identification of viral pathogens without the need to maintain viability. We hypothesized that ethanol, while inactivating viruses, can preserve DNA and RNA for PCR-based methods. To evaluate the use of ethanol-stored specimens for augmenting surveillance for detection of influenza viruses A and B and adenoviruses (AdV), paired nasal swab specimens were collected from 384 recruits with febrile respiratory illness at Fort Jackson, S.C., in a 2-year study. One swab was stored at ambient temperature in 100% ethanol for up to 6 months, and the other swab was stored at -70{degrees}C in viral medium. For viral detection, frozen specimens were cultured for a variety of respiratory viruses, and ethanol-fixed specimens were tested with TaqMan (TM) probe and LightCycler SYBR green (SG) melting curve assays with at least two different PCR targets for each virus. The sensitivities of the TM and SG assays on specimens stored in ethanol for 1 month were 75% and 58% for influenza A, 89% and 67% for influenza B, and 93 to 98% and 57% for AdV, respectively. Lower specificities of the real-time assays corresponded to the increased detection of PCR-positive but culture-negative specimens. Influenza virus RNA was detected as well or better after 6 months of storage in ethanol.

Kuboki, N., N. Inoue, et al. (2003). "Loop-Mediated Isothermal Amplification for Detection of African Trypanosomes." *J. Clin. Microbiol.* **41**(12): 5517-5524.

<http://jcm.asm.org/cgi/content/abstract/41/12/5517>

While PCR is a method of choice for the detection of African trypanosomes in both humans and animals, the expense of this method negates its use as a diagnostic method for the detection of endemic trypanosomiasis in African countries. The loop-mediated isothermal amplification (LAMP) reaction is a method that amplifies DNA with high specificity, efficiency, and rapidity under isothermal conditions with only simple incubators. An added advantage of LAMP over PCR-based methods is that DNA amplification can be monitored spectrophotometrically and/or with the naked eye without the use of dyes. Here we report our conditions for a highly sensitive, specific, and easy diagnostic assay based on LAMP technology for the detection of parasites in the *Trypanosoma brucei* group (including *T. brucei brucei*, *T. brucei gambiense*, *T. brucei rhodesiense*, and *T. evansi*) and *T. congolense*. We show that the sensitivity of the LAMP-based method for detection of trypanosomes in vitro is up to 100 times higher than that of PCR-based methods. In vivo studies in mice infected with human-infective *T. brucei gambiense* further highlight the potential clinical importance of LAMP as a diagnostic tool for the identification of African trypanosomiasis.

Kuhne, S. A., W. S. Hawes, et al. (2004). "Isolation of Recombinant Antibodies against EspA and Intimin of *Escherichia coli* O157:H7." *J. Clin. Microbiol.* **42**(7): 2966-2976.

<http://jcm.asm.org/cgi/content/abstract/42/7/2966>

Intimin, Tir, and EspA proteins are expressed by attaching-effacing *Escherichia coli*, which include enteropathogenic and enterohemorrhagic *E. coli* pathotypes. EspA proteins are part of the type three secretion system needle complex that delivers Tir to the host epithelial cell, while surface arrayed intimin docks the bacterium to the translocated Tir. This intimate attachment leads to attaching and effacing lesions. Recombinant forms of these effector proteins from enterohemorrhagic *E. coli* O157:H7 were produced by using *E. coli* expression vectors. Binding of intimin and Tir fragments in enzyme-linked immunosorbent assay (ELISAs) demonstrated the interaction of intimin fragments containing the C-terminal 282 or 188 amino acids to a Tir fragment containing amino acid residues 258 to 361. Recombinant intimin and EspA proteins were used to elicit immune responses in rabbits and immune phage-display antibody libraries were produced. Screening of these immune libraries by conventional phage-antibody panning and colony filter screening produced a panel of antibodies with specificity for EspA or intimin. Antibodies recognizing different C-terminal epitopes on intimin bound specifically to the gamma intimin of O157:H7 and not to other classes of intimin. Antibodies recognizing EspA from *E. coli* O157 also recognized the protein from the eae-deficient O157 mutant DM3 and from *E. coli* O111. Anti-intimin antibodies were also produced as fusion proteins coupled to the reporter molecule alkaline phosphatase, allowing the one-step detection of {gamma} intimin. The isolated recombinant monoclonal antibodies were functional in a range of assay formats, including ELISA, Western blotting, and dot blots, thus demonstrating their diagnostic potential.

Kwara, A., R. Schiro, et al. (2003). "Evaluation of the Epidemiologic Utility of Secondary Typing Methods for Differentiation of *Mycobacterium tuberculosis* Isolates." *J. Clin. Microbiol.* **41**(6): 2683-2685.

<http://jcm.asm.org/cgi/content/abstract/41/6/2683>

Spoligotyping and mycobacterial interspersed repetitive unit-variable-number tandem repeat analysis (MIRU-VNTR) were evaluated for the ability to differentiate 64 *Mycobacterium tuberculosis* isolates from 10 IS6110-defined clusters. MIRU-VNTR performed slightly better than spoligotyping in reducing the number of clustered isolates and the sizes of the clusters. All epidemiologically related isolates remained clustered by MIRU-VNTR but not by spoligotyping.

La Scola, B., Z. Liang, et al. (2002). "Genotypic Characteristics of Two Serotypes of *Bartonella henselae*." *J. Clin. Microbiol.* **40**(6): 2002-2008.

<http://jcm.asm.org/cgi/content/abstract/40/6/2002>

The study of 16S rRNA gene sequences of all isolates of *Bartonella henselae* obtained in our laboratory and others from human patients or cats has revealed two genotypes according to the sequence of the 16S rRNA gene. Two isolates of these genotypes have previously been related to two different serotypes, and lack of cross-protection of the two serotypes has been demonstrated in cats. We investigated the grouping of eight strains of *B. henselae* on the basis of 16S ribosomal DNA, 35-kDa protein, Pap 31 protein, and internal transcribed spacer (ITS) gene sequencing; sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profiles; and monoclonal antibody reactivity studies. Houston-1, 90-615, and SA2 strains showed the same patterns in SDS-PAGE, but they differed from the patterns of *B. henselae* isolates URBHLLY8, URBHLIE9, Cat6, Fizz, and CAL-1. Nine monoclonal antibodies derived from

BALB/c mice immunized with *B. henselae* Houston-1 strain reacted only with strains Houston-1, 90-615, and SA2, and not with any other Bartonella strains. The two serogroups corresponded with two genotypes based on differences in the sequences of the genes encoding 16S rRNA, 35-kDa protein, and Pap 31 protein. Sequences of ITS genes were highly divergent among strains, as each had a unique sequence and the subdivision was not supported by DNA-DNA relatedness study. Study of 22 additional strains of *B. henselae* isolated from French bacteremic cats demonstrated that they all belong to one or the other of the proposed serotype or genotype.

Lai, K. K.-Y., L. Cook, et al. (2003). "Evaluation of Real-Time PCR versus PCR with Liquid-Phase Hybridization for Detection of Enterovirus RNA in Cerebrospinal Fluid." *J. Clin. Microbiol.* **41**(7): 3133-3141.

<http://jcm.asm.org/cgi/content/abstract/41/7/3133>

A LightCycler and two TaqMan real-time PCR assays were evaluated against an older PCR with liquid-phase hybridization method for the detection of enterovirus RNA in 74 patient samples. The two-step LightCycler and the two-step TaqMan formats correlated well with each other ($r^2 = 0.90$) and were equally sensitive compared to the liquid-phase hybridization method, whereas the one-step recombinant Tth DNA polymerase format was rather insensitive, detecting enterovirus RNA in only about one-half of those patient samples previously positive by liquid-phase hybridization. The two-step TaqMan method was optimized utilizing 10 {micro}l of cDNA and demonstrated the highest degree of analytical sensitivity among the methods evaluated in our study, being able to reproducibly quantify down to 510 copies of enteroviral RNA/ml of cerebrospinal fluid. This new assay can be performed in 4 h, is much less labor intensive, and showed less cross-reactivity with rhinovirus than the liquid-phase hybridization assay. Thus, the two-step TaqMan assay should prove useful in the diagnosis of enteroviral meningitis versus bacterial meningitis, thereby resulting in timely and appropriate clinical management that can amount to significant cost savings to the patient and health care system.

Lan, R. and P. R. Reeves (2002). "Pandemic Spread of Cholera: Genetic Diversity and Relationships within the Seventh Pandemic Clone of *Vibrio cholerae* Determined by Amplified Fragment Length Polymorphism." *J. Clin. Microbiol.* **40**(1): 172-181.

<http://jcm.asm.org/cgi/content/abstract/40/1/172>

The seventh cholera pandemic started in 1961 and continues today. A collection of 45 seventh pandemic isolates of *V. cholerae* sampled over a 33-year period were analyzed by amplified fragment length polymorphism (AFLP) fingerprinting. All but four pairs and one set of three isolates were distinguished. AFLP revealed far more variation than ribotyping, which was until now the most useful method of revealing variation within the pandemic clone. Unfortunately, the ribotype variation observed is mainly due to recombination between the multiple copies of the *rrn* genes (R. Lan and P. R. Reeves, *Microbiology* 144:1213-1221, 1998), which makes changes susceptible to repeat occurrences and reversion. This AFLP study shows that particularly for the common ribotypes G and H, such events have indeed occurred. AFLP grouped most of the 45 isolates into two clusters. Cluster I consists mainly of strains from the 1960s and 1970s, while cluster II contains mainly strains from the 1980s and 1990s, revealing a temporal pattern of change in the clone. This is best seen in the relationships of the strains from Africa, which correlate with the epidemiology of epidemics on that continent. The data confirm independent introductions to Africa during the 1970s outbreak and reveal several other African introductions. In the 1991 cholera upsurge, isolates from the Southern and Eastern African epidemic focus are markedly different from those from the West African epidemic focus. An isolate from 1987 in Algeria was identical to the West epidemic isolates, suggesting that the strain was present in

Africa at least 3 years before causing large outbreaks. These observations have major implications for our understanding of cholera epidemiology.

Lapa, S., M. Mikheev, et al. (2002). "Species-Level Identification of Orthopoxviruses with an Oligonucleotide Microchip." *J. Clin. Microbiol.* **40**(3): 753-757.

<http://jcm.asm.org/cgi/content/abstract/40/3/753>

A method for species-specific detection of orthopoxviruses pathogenic for humans and animals is described. The method is based on hybridization of a fluorescently labeled amplified DNA specimen with the oligonucleotide DNA probes immobilized on a microchip (MAGIChip). The probes identify species-specific sites within the crmB gene encoding the viral analogue of tumor necrosis factor receptor, one of the most important determinants of pathogenicity in this genus of viruses. The diagnostic procedure takes 6 h and does not require any sophisticated equipment (a portable fluorescence reader can be used).

Laperche, S., F. Lunel, et al. (2005). "Comparison of Hepatitis C Virus NS5b and 5' Noncoding Gene Sequencing Methods in a Multicenter Study." *J. Clin. Microbiol.* **43**(2): 733-739.

<http://jcm.asm.org/cgi/content/abstract/43/2/733>

A national evaluation study was performed in 11 specialized laboratories with the objective of assessing their capacities to genotype hepatitis C virus (HCV) and define the applicability of a given genotyping method. The panel consisted of 14 samples positive for HCV RNA of different genotypes (including 3 samples with two different artificially mixed genotypes) and 1 HCV-negative sample. Seventeen sets of data were gathered from the 11 participating laboratories. The sensitivities ranged from 64.3 to 100% and from 42.7 to 85.7% for the methods that used sequencing of the NS5b region and the 5' noncoding (5' NC) region, respectively. When the data for the artificially mixed samples were excluded, NS5b genotyping gave correct results for 80% of the samples, 1.7% of the samples were misclassified, and 18.3% of the samples had false-negative results. By 5' NC-region genotyping methods, 58.3% of the results were correct, 29.7% were incomplete, 8.3% were misclassifications, 1.2% were false positive, and 2.4% were false negative. Only two procedures based on NS5b sequencing correctly identified one of the three samples with mixtures of genotypes; the other methods identified the genotype corresponding to the strain with the highest viral load in the sample. Our results suggest that HCV 5' NC-region genotyping methods give sufficient information for clinical purposes, in which the determination of the subtype is not essential, and that NS5b genotyping methods are more reliable for subtype determination, which is required in epidemiological studies.

Lasker, B. A. (2002). "Evaluation of Performance of Four Genotypic Methods for Studying the Genetic Epidemiology of *Aspergillus fumigatus* Isolates." *J. Clin. Microbiol.* **40**(8): 2886-2892.

<http://jcm.asm.org/cgi/content/abstract/40/8/2886>

In the present investigation, 49 *Aspergillus fumigatus* isolates obtained from four nosocomial outbreaks were typed by *Afu*1 restriction fragment length polymorphism (RFLP) analysis and three PCR-based molecular typing methods: random amplified polymorphic DNA (RAPD) analysis, sequence-specific DNA primer (SSDP) analysis, and polymorphic microsatellite markers (PMM) analysis. The typing methods were evaluated with respect to discriminatory power (D),

reproducibility, typeability, ease of use, and ease of interpretation to determine their performance and utility for outbreak and surveillance investigations. *Afu1* RFLP analysis detected 40 types. Thirty types were observed by RAPD analysis. PMM analysis detected 39 allelic types, but SSDP analysis detected only 14 types. All four methods demonstrated 100% typeability. PMM and RFLP analyses had comparable high degrees of discriminatory power ($D = 0.989$ and 0.988 , respectively). The discriminatory power of RAPD analysis was slightly lower ($D = 0.971$), whereas SSDP analysis had the lowest discriminatory power ($D = 0.889$). Overall, SSDP analysis was the easiest method to interpret and perform. The profiles obtained by PMM analysis were easier to interpret than those obtained by RFLP or RAPD analysis. Bands that differed in staining intensity or that were of low intensity were observed by RAPD analysis, making interpretation more difficult. The reproducibilities with repeated runs of the same DNA preparation or with different DNA preparations of the same strain were high for all the methods. A high degree of genetic variation was observed in the test population, but isolates were not always similarly divided by each method. Interpretation of band profiles requires understanding of the molecular mechanisms responsible for genetic alternations. PMM analysis and *Afu1* RFLP analysis, or their combination, appear to provide the best overall discriminatory power, reproducibility, ease of interpretation, and ease of use. This investigation will aid in planning epidemiologic and surveillance studies of *A. fumigatus*.

Lavigne, J.-P., N. Bouziges, et al. (2004). "Molecular Epidemiology of Enterobacteriaceae Isolates Producing Extended-Spectrum β -Lactamases in a French Hospital." *J. Clin. Microbiol.* **42**(8): 3805-3808.

<http://jcm.asm.org/cgi/content/abstract/42/8/3805>

In 2002, 80 isolates of Enterobacteriaceae producing extended-spectrum β -lactamases (ESBLs) were collected from infected patients in our hospital. *Enterobacter aerogenes* was the most common bacterium isolated from all specimens (36.5%). The ESBLs were predominantly (90%) TEM derivatives (TEM-24, TEM-3). Pulsed-field gel electrophoresis highlighted that *E. aerogenes*, *Klebsiella pneumoniae*, and *Citrobacter koseri* had a clonal propagation.

Lawrence, E. R., D. B. Griffiths, et al. (2003). "Evaluation of Semiautomated Multiplex PCR Assay for Determination of *Streptococcus pneumoniae* Serotypes and Serogroups." *J. Clin. Microbiol.* **41**(2): 601-607.

<http://jcm.asm.org/cgi/content/abstract/41/2/601>

A semiautomated method for the determination of five serotypes and three serogroups in *Streptococcus pneumoniae* was developed. Primers specific for serotypes 1, 3, 14, 19F, and 23F and serogroups 6, 19, and 23 were combined in three multiplex PCRs. Products were separated by capillary electrophoresis with a 7-min run time, and a serotype or serogroup was assigned on the basis of fragment size. The method was used to test 93 clinical isolates, and all isolates of the serotypes concerned were correctly detected. The strategy would allow the detection of multiple serotypes in a single sample. Detection of additional serotypes could be included as capsule locus sequences become available.

Leary, T. P., J. C. Erker, et al. (2002). "Detection of Mammalian Reovirus RNA by Using Reverse Transcription-PCR: Sequence Diversity within the λ 3-Encoding L1 Gene." *J. Clin. Microbiol.* **40**(4): 1368-1375.

<http://jcm.asm.org/cgi/content/abstract/40/4/1368>

Reoviruses infect virtually all mammalian species, and infection of humans is associated with mild gastrointestinal or upper respiratory illnesses. To improve reovirus detection strategies, we developed a reverse transcription-PCR technique to amplify a fragment of the reovirus L1 gene segment. This assay was capable of detecting 44 of 44 reovirus field isolate strains and was sufficiently sensitive to detect nearly a single viral particle (1.16 ± 0.13) per PCR of prototype strain type 3 Dearing. Pairwise comparisons of the 44 partial L1 gene sequences revealed that nucleotide variability ranged from 0 to 24.7%, with most of the nucleotide polymorphism occurring at synonymous positions. Phylogenetic trees generated from amplified L1 gene sequences suggest that multiple alleles of the L1 gene cocirculate in nature and that genetic diversity of the L1 gene is largely independent of the host species, geographic locale, or date of isolation. Phylogenetic trees constructed from the L1 gene sequences are distinct from those constructed from the four reovirus S-class gene segments, which supports the hypothesis that reovirus gene segments reassort in nature. This study establishes a new sensitive and specific technique for the identification of mammalian reoviruses and enhances our understanding of reovirus evolution.

Legoff, J., E. Guerot, et al. (2005). "High Prevalence of Respiratory Viral Infections in Patients Hospitalized in an Intensive Care Unit for Acute Respiratory Infections as Detected by Nucleic Acid-Based Assays." *J. Clin. Microbiol.* **43**(1): 455-457.

<http://jcm.asm.org/cgi/content/abstract/43/1/455>

Forty-seven bronchoalveolar lavages (BAL) were obtained from 41 patients with acute pneumonia attending an intensive care unit. By molecular diagnosis, 30% of total BAL and 63% of bacteria-negative BAL were positive for respiratory viruses. Molecular detection allows for high-rate detection of respiratory viral infections in adult patients suffering from severe pneumonia.

Li, H., N. S. Taus, et al. (2004). "Shedding of Ovine Herpesvirus 2 in Sheep Nasal Secretions: the Predominant Mode for Transmission." *J. Clin. Microbiol.* **42**(12): 5558-5564.

<http://jcm.asm.org/cgi/content/abstract/42/12/5558>

Ovine herpesvirus 2 (OvHV-2), the major causative agent of malignant catarrhal fever in ruminant species worldwide, has never been propagated *in vitro*. Using real-time PCR, a striking, short-lived, peak of viral DNA, ranging from 105 to over 108 copies/2 μ g of DNA, was detected in nasal secretions from over 60.7% of adolescent sheep ($n = 56$) at some point during the period from 6 to 9 months of age. In contrast, only about 18% of adult sheep ($n = 33$) experienced a shedding episode during the study period. The general pattern of the appearance of viral DNA in nasal secretions was a dramatic rise and subsequent fall within 24 to 36 h, implying a single cycle of viral replication. These episodes occurred sporadically and infrequently, but over the 3-month period most of the 56 lambs (33, or 60.7%) experienced at least one episode. No corresponding fluctuations in DNA levels were found in either peripheral blood leukocytes or plasma. In a DNase protection assay, complete, enveloped OvHV-2 virions were demonstrated in the nasal secretions of all sheep examined during the time when they were experiencing an intense shedding episode. OvHV-2 infectivity in nasal secretions was also demonstrated by aerosolization of the secretions into OvHV-2-negative sheep. The data herein show that nasal shedding is the major mode of OvHV-2 transmission among domestic sheep and that adolescents represent the highest risk group for transmission.

Li, J., D. S. Gerhard, et al. (2003). "Denaturing High-Performance Liquid Chromatography for Detecting and Typing Genital Human Papillomavirus." J. Clin. Microbiol. **41**(12): 5563-5571.

<http://jcm.asm.org/cgi/content/abstract/41/12/5563>

Human papillomaviruses (HPVs) are important in the development of human cancers, including cervical and oral tumors. However, most existing methods for HPV typing cannot routinely distinguish among the more than 100 distinct types of HPV or the natural HPV intratypic variants that have also been documented. To address this problem, we developed a novel method, general primer-denaturing high-performance liquid chromatography (GP-dHPLC), for the detection and typing of genital HPV using an automated 96-well plate format. GP-dHPLC uses general primer PCR (GP-PCR) to amplify the viral DNA and then analyzes the GP-PCR products by denaturing high-performance liquid chromatography (dHPLC). A number of different primer pairs with homology to most known genital HPV types were tested, and the L1C1-L1C2M pair specific for the L1 region of the viral genome was chosen. A set of HPV standard control patterns, consisting of those for HPV types 16, 18, 31, 33, 39, 45, 51, 52, 56, 58, 59, 6, and 11, was established for genital HPV typing. One hundred eighty-six frozen and formalin-fixed cervical cancer tissue samples were analyzed for the presence of HPV and the HPV type by this method, and 95.8% of them were found to contain HPV DNA. GP-dHPLC accurately discriminated among HPV variants that differed by as little as one nucleotide. Several new variants of HPV types 16, 18, 39, 45, 52, and 59 were identified. Moreover, multiple HPV infections were detected in 26.6% of the samples. Our results indicate that HPV typing by GP-dHPLC permits discrimination of common genital HPV types, detection of multiple HPV infections, and identification of HPV variants in clinical samples.

Liddell, A. M., S. L. Stockham, et al. (2003). "Predominance of Ehrlichia ewingii in Missouri Dogs." J. Clin. Microbiol. **41**(10): 4617-4622.

<http://jcm.asm.org/cgi/content/abstract/41/10/4617>

To investigate the species distribution of Ehrlichia present in Missouri dogs, we tested 78 dogs suspected of having acute ehrlichiosis and 10 healthy dogs. Blood from each dog was screened with a broad-range 16S rRNA gene PCR assay that detects known pathogenic species of Ehrlichia and Anaplasma. The species was determined by using species-specific PCR assays and nucleotide sequencing. Ehrlichia antibody testing was performed by using an indirect immunofluorescence assay with Ehrlichia chaffeensis as the antigenic substrate. The broad-range assay detected Ehrlichia or Anaplasma DNA in 20 (26%) of the symptomatic dogs and 2 (20%) of the asymptomatic dogs. E. ewingii accounted for 20 (91%), and E. chaffeensis accounted for 1 (5%) of the positives. Anaplasma phagocytophilum DNA was detected in one dog, and the sequences of regions of the 16S rRNA gene and the groESL operon amplified from the blood of this dog matched the published sequences of this organism. Antibodies reactive with E. chaffeensis were detected in 14 (67%) of the 21 PCR-positive dogs and in 12 (19%) of the 64 PCR-negative dogs. Combining the results of PCR and serology indicated that 33 (39%) of 85 evaluable dogs had evidence of past or current Ehrlichia infection. We conclude that E. ewingii is the predominant etiologic agent of canine ehrlichiosis in the areas of Missouri included in this survey. E. canis, a widely recognized agent of canine ehrlichiosis, was not detected in any animal. The finding of E. ewingii in asymptomatic dogs suggests that dogs could be a reservoir for this Ehrlichia species.

Lievano, F. A., M. A. Reynolds, et al. (2002). "Issues Associated with and Recommendations for Using PCR To Detect Outbreaks of Pertussis." J. Clin. Microbiol. **40**(8): 2801-2805.

<http://jcm.asm.org/cgi/content/abstract/40/8/2801>

Two outbreaks of respiratory tract illness associated with prolonged cough occurring in 1998 and 1999 in New York State were investigated. A PCR test for *Bordetella pertussis* was primarily used by a private laboratory to confirm 680 pertussis cases. Several clinical specimens had positive culture results for *B. pertussis* during both outbreaks, which confirmed that *B. pertussis* was circulating during the outbreaks. However, testing by the New York State Department of Health reference laboratory suggested that some of the PCR results may have been falsely positive. In addition, features of the outbreak that suggested that *B. pertussis* may not have been the primary agent of infection included a low attack rate among incompletely vaccinated children and a significant amount of illness among patients testing PCR negative for *B. pertussis*. These investigations highlight the importance of appropriate clinical laboratory quality assurance programs, of the limitations of the PCR test, and of interpreting laboratory results in context of clinical disease.

Lindecrona, R. H., T. K. Jensen, et al. (2002). "Application of a 5' Nuclease Assay for Detection of *Lawsonia intracellularis* in Fecal Samples from Pigs." *J. Clin. Microbiol.* **40**(3): 984-987.

<http://jcm.asm.org/cgi/content/abstract/40/3/984>

A 5' nuclease assay was developed to detect *Lawsonia intracellularis* in porcine fecal samples. The specific probe and primers were chosen by using the 16S ribosomal DNA gene as a target. The 5' nuclease assay was used with a total of 204 clinical samples, and the results were compared to those of immunohistochemistry (IM) on ileal sections of the same animals. There was 91% agreement between the results of IM and the 5' nuclease assay. In the 5' nuclease assay, 111 (54%) of the pigs tested positive for *L. intracellularis* infection, with a mean cycle threshold (Ct) value of 27.2, whereas 98 (48%) of the pigs tested positive by IM. On average, the Ct and ΔR_n values for the positive samples were 27.2 (standard deviation [SD], 3.7) and 1.6 (SD, 0.7), respectively. A Ct value of 27.2 corresponds to a fecal excretion of approximately 107 *L. intracellularis* cells per g of feces. Furthermore, a total of 40 fecal samples derived from a herd known to be free from infection with *L. intracellularis* all tested negative, with a Ct value of 40. By using a Ct value of 36 as the cutoff limit, the detection limit of the assay was 1 *L. intracellularis* cell per PCR tube. In conclusion, the 5' nuclease assay that has been developed represents an applicable fast method for detection of *L. intracellularis* in fecal samples, with a sensitivity and specificity comparable to those of IM.

Lindell, A. T., L. Grillner, et al. (2005). "Molecular Epidemiology of Norovirus Infections in Stockholm, Sweden, during the Years 2000 to 2003: Association of the GGIIb Genetic Cluster with Infection in Children." *J. Clin. Microbiol.* **43**(3): 1086-1092.

<http://jcm.asm.org/cgi/content/abstract/43/3/1086>

The incidence of norovirus-associated gastroenteritis and the molecular epidemiology of norovirus strains were studied during three seasons (2000-2001, 2001-2002, and 2002-2003) among patients of all ages, mainly from the Stockholm region in Sweden. A total of 3,252 fecal samples were analyzed by reverse transcription-PCR. The incidences of norovirus infection among adults were 23, 26, and 30% during the three seasons studied and 18, 11, and 15% among children 0 to 15 years of age. During the first season, all norovirus strains detected by PCR were typed either by reverse line blot hybridization or nucleotide sequence analysis. During the two successive seasons, a total of 60 norovirus-positive strains from the beginning, peak, and end of the seasons were selected for nucleotide sequence analysis. We identified two dominant

norovirus variants over the seasons: a new norovirus variant, recently described as the GGIIb genetic cluster, dominated among children during the first season, and during the following two seasons, a GGII-4 variant dominated. Our data suggest that norovirus infections are common, not only among adults, but also among children, and that some strains may predominantly affect children.

Lindstedt, B.-A., E. Heir, et al. (2003). "DNA Fingerprinting of *Salmonella enterica* subsp. *enterica* Serovar Typhimurium with Emphasis on Phage Type DT104 Based on Variable Number of Tandem Repeat Loci." *J. Clin. Microbiol.* **41**(4): 1469-1479.

<http://jcm.asm.org/cgi/content/abstract/41/4/1469>

Seventy-eight human and environmental strains of *Salmonella enterica* subsp. *enterica* serovar Typhimurium, as well as 18 isolates of other *Salmonella* serovars and 6 isolates of *Escherichia coli*, were subjected to a novel variable number of tandem repeats (VNTR)-based fingerprinting method that showed high discrimination and reproducibility for typing serovar Typhimurium isolates. The method is based on capillary separation of PCR products from fluorescence-labeled VNTR in the serovar Typhimurium genome. The serovar Typhimurium isolates displayed 54 VNTR patterns, and the VNTR assay correctly identified strains from a well-characterized outbreak. Among 37 serovar Typhimurium phage type DT104 isolates, 28 distinct VNTR patterns were found. This VNTR-based method is fast and suitable for complete automation. Our VNTR-based method was capable of high discrimination within the homogeneous serovar Typhimurium DT104 phage type and can be used to trace outbreaks and to monitor DT104 as well as other phage types. The VNTR assay was compared to XbaI pulsed-field gel electrophoresis, amplified fragment length polymorphism analysis, integron-cassette profiles and gene PCR of *int1*, *qacE*{ Δ }1, *sul1*, and *floR*. The VNTR assay showed greatly improved resolution compared to all other tested methods in this study.

Lindstrom, A., J. Odeberg, et al. (2004). "Pyrosequencing for Detection of Lamivudine-Resistant Hepatitis B Virus." *J. Clin. Microbiol.* **42**(10): 4788-4795.

<http://jcm.asm.org/cgi/content/abstract/42/10/4788>

Chronic hepatitis B virus (HBV) infection can cause severe liver disease, including cirrhosis and hepatocellular carcinoma. Lamivudine is a relatively recent alternative to alpha interferon for the treatment of HBV infection, but unfortunately, resistance to lamivudine commonly develops during monotherapy. Lamivudine-resistant HBV mutants display specific mutations in the YMDD (tyrosine, methionine, aspartate, aspartate) motif of the viral polymerase (reverse transcriptase [rt]), which is the catalytic site of the enzyme, i.e., methionine 204 to isoleucine (rtM204I) or valine (rtM204V). The latter mutation is often accompanied by a compensatory leucine-to-methionine change at codon 180 (rtL180M). In the present study, a novel sequencing method, pyrosequencing, was applied to the detection of lamivudine resistance mutations and was compared with direct Sanger sequencing. The new pyrosequencing method had advantages in terms of throughput. Experiments with mixtures of wild-type and resistant viruses indicated that pyrosequencing can detect minor sequence variants in heterogeneous virus populations. The new pyrosequencing method was evaluated with a small number of patient samples, and the results showed that the method could be a useful tool for the detection of lamivudine resistance in the clinical setting.

Lo Cascio, G., M. Ligozzi, et al. (2004). "Utility of Molecular Identification in Opportunistic Mycotic Infections: a Case of Cutaneous *Alternaria* infectoria Infection in a Cardiac Transplant Recipient." *J. Clin. Microbiol.* **42**(11): 5334-5336.

<http://jcm.asm.org/cgi/content/abstract/42/11/5334>

We report on a case of cutaneous infection caused by *Alternaria* infectoria in a cardiac transplant recipient. A rapid molecular diagnosis was obtained by sequence analysis of the internal transcribed spacer domain of the 5.8S ribosomal DNA region amplified from colonies developed on Sabouraud medium. Treatment consisted of a combination of systemic antifungal therapy, first with amphotericin B and then with itraconazole.

Lovmar, L., C. Fock, et al. (2003). "Microarrays for Genotyping Human Group A Rotavirus by Multiplex Capture and Type-Specific Primer Extension." *J. Clin. Microbiol.* **41**(11): 5153-5158.

<http://jcm.asm.org/cgi/content/abstract/41/11/5153>

Human group A rotavirus (HRV) is the major cause of severe gastroenteritis in infants worldwide. HRV shares the feature of a high degree of genetic diversity with many other RNA viruses, and therefore, genotyping of this organism is more complicated than genotyping of more stable DNA viruses. We describe a novel microarray-based method that allows high-throughput genotyping of RNA viruses with a high degree of polymorphism by multiplex capture and type-specific extension on microarrays. Denatured reverse transcription (RT)-PCR products derived from two outer capsid genes of clinical isolates of HRV were hybridized to immobilized capture oligonucleotides representing the most commonly occurring P and G genotypes on a microarray. Specific primer extension of the type-specific capture oligonucleotides was applied to incorporate the fluorescent nucleotide analogue cyanine 5-labeled dUTP as a detectable label. Laser scanning and fluorescence detection of the microarrays was followed by visual or computer-assisted interpretation of the fluorescence patterns generated on the microarrays. Initially, the method detected HRV in all 40 samples and correctly determined both the G and the P genotypes of 35 of the 40 strains analyzed. After modification by inclusion of additional capture oligonucleotides specific for the initially unassigned genotypes, all genotypes could be correctly defined. The results of genotyping with the microarray fully agreed with the results obtained by nucleotide sequence analysis and sequence-specific multiplex RT-PCR. Owing to its robustness, simplicity, and general utility, the microarray-based method may gain wide applicability for the genotyping of microorganisms, including highly variable RNA and DNA viruses.

Lovseth, A., S. Loncarevic, et al. (2004). "Modified Multiplex PCR Method for Detection of Pyrogenic Exotoxin Genes in Staphylococcal Isolates." *J. Clin. Microbiol.* **42**(8): 3869-3872.

<http://jcm.asm.org/cgi/content/abstract/42/8/3869>

A modified multiplex PCR method for detection of nine *Staphylococcus aureus* enterotoxin genes (sea, seb, sec, sed, see, seg, seh, sei, and sej) and one form of immunoreactive toxic shock syndrome toxin based on a previously published method (S. R. Monday and G. A. Bohach, *J. Clin. Microbiol.* 37:3411-3414, 1999) has been developed. The modified PCR protocol seems robust and gives reliable results.

Luo, W., H. Yang, et al. (2005). "Detection of Human Immunodeficiency Virus Type 1 DNA in Dried Blood

Spots by a Duplex Real-Time PCR Assay." *J. Clin. Microbiol.* **43**(4): 1851-1857.

<http://jcm.asm.org/cgi/content/abstract/43/4/1851>

A dried blood spot (DBS) is a well-accepted means for the collection, transport, and storage of blood samples for various epidemiologic, serologic, and molecular assays for human immunodeficiency virus (HIV) studies. It is particularly important for mother-to-infant-transmission studies of affected individuals living in remote areas. We have developed a real-time PCR method to detect HIV type 1 (HIV-1) DNA in dried blood spots. A cellular gene, RNase P, was coamplified with the HIV-1 DNA in the same tube to monitor the DNA extraction efficiency and the overall assay performance. Our assay is a one-tube, single-step closed-system assay and uses a dUTP/uracil DNA glycosidase anti-PCR contamination control. The HIV-1 primers and probe were derived from a conserved region of the long terminal repeat. The detection of RNase P is attenuated by lowering the forward and reverse primer concentrations so that its amplification will not overwhelm the HIV-1 amplification and yet will provide a semiquantitative measurement of the quality of the isolated DBS DNA. We examined 103 HIV-1-seropositive and 56 seronegative U.S. adults and found that our assay has a sensitivity of 98.1% (95% confidence interval [CI], 95.5% to 100%) and specificity of 100% (95% CI, 99% to 100%). The positive and negative predictive values are 100% and 96.6%, respectively. This duplex PCR assay may be useful in identifying HIV-1-infected persons, particularly infants born to seropositive mothers in remote areas of the world.

Ma, L. and D. H. Martin (2004). "Single-Nucleotide Polymorphisms in the rRNA Operon and Variable Numbers of Tandem Repeats in the Lipoprotein Gene among *Mycoplasma genitalium* Strains from Clinical Specimens." *J. Clin. Microbiol.* **42**(10): 4876-4878.

<http://jcm.asm.org/cgi/content/abstract/42/10/4876>

We characterized the single-nucleotide polymorphisms in the rRNA operon and variable numbers of tandem repeats in the lipoprotein gene MG309 among *Mycoplasma genitalium* strains from clinical specimens by PCR and sequencing. Analysis of 31 *M. genitalium*-infected patient specimens and 7 American Type Culture Collection strains identified six types of rRNA sequences and 11 different numbers of MG309 repeats. Examination of sequential specimens from 10 patients showed that these genotypes were stable for at least 5 weeks. These data suggest the potential usefulness of the rRNA genotypes and the MG309 repeats for genotyping of *M. genitalium*.

Maes, N., J. Magdalena, et al. (2002). "Evaluation of a Triplex PCR Assay To Discriminate *Staphylococcus aureus* from Coagulase-Negative *Staphylococci* and Determine Methicillin Resistance from Blood Cultures." *J. Clin. Microbiol.* **40**(4): 1514-1517.

<http://jcm.asm.org/cgi/content/abstract/40/4/1514>

A triplex PCR targeting the 16S rRNA, *mecA*, and *nuc* genes was developed for identification of staphylococci and detection of methicillin resistance. After validation of the assay with a collection of strains of staphylococci and enterococci ($n = 169$), the assay was evaluated with cultures of blood with gram-positive cocci from 40 patients. Accurate results were obtained for 59 (98%) of 61 cultures within 6 h of growth detection.

Maggi, R. G. and E. B. Breitschwerdt (2005). "Potential Limitations of the 16S-23S rRNA Intergenic Region for Molecular Detection of Bartonella Species." J. Clin. Microbiol. **43**(3): 1171-1176.

<http://jcm.asm.org/cgi/content/abstract/43/3/1171>

PCR targeting the 16S-23S rRNA gene intergenic transcribed spacer (ITS) region has been proposed as a rapid and reliable method for the detection of Bartonella species DNA in clinical samples. Because of variation in ITS sequences among Bartonella species, a single PCR amplification can be used to detect different species within this genus. Therefore, by targeting the ITS region, multiple PCRs or additional sample-processing steps beyond the primary amplification can be avoided when attempting to achieve molecular diagnostic detection of Bartonella species. Although PCR amplification targeting this region is considered highly sensitive, amplification specificity obviously depends on primer design. We report evidence of nonspecific PCR amplification of Mesorhizobium species with previously published primers that were designed to amplify the Bartonella consensus ITS region. Use of these or other, less species-specific, primers could lead to a false-positive diagnostic test result when evaluating clinical samples. We also report the presence of Mesorhizobium species DNA as a contaminant in molecular-grade water, a series of homologous sequences in the ITS region that are common to Bartonella and Mesorhizobium species, the amplification of Mesorhizobium DNA with unpublished primers designed in our laboratory targeting the ITS region, and the subsequent design of unambiguous ITS primers that avoid nonspecific amplification of Mesorhizobium species. Our results define some potential limitations associated with the molecular detection of Bartonella species in patient samples and indicate that primer specificity is of critical importance if the ITS region is used as a diagnostic target for detection of Bartonella species.

Maraha, B., H. Berg, et al. (2004). "Is the Perceived Association between Chlamydia pneumoniae and Vascular Diseases Biased by Methodology?" J. Clin. Microbiol. **42**(9): 3937-3941.

<http://jcm.asm.org/cgi/content/abstract/42/9/3937>

Inter- and intralaboratory inconsistencies in detection rates of Chlamydia pneumoniae in vascular specimens have been demonstrated. In this study, 66 vascular tissue specimens from 66 patients with vascular disease were tested by three PCR assays: a 16S PCR-based reverse line blot (RLB) assay, a single-step PCR, and a nested PCR. Also, we explored the impacts of different DNA polymerase enzymes on the results based on gel electrophoresis and hybridization. The PCR results by gel electrophoresis in the single-step PCR depended on which DNA polymerase was used. All samples were negative with AmpliTaq Gold DNA polymerase, and 54.5% (36 of 66) were positive with the conventional Taq DNA polymerase. All samples were negative after hybridization with a C. pneumoniae-specific probe. In the nested PCR, all specimens were negative by gel electrophoresis and after hybridization. The RLB assay failed to detect C. pneumoniae in any specimen; however, 20 specimens were Chlamydia sp. positive. The sequence analysis of six of these samples demonstrated Chlamydia-like organisms. RLB detected Chlamydia sp. DNA in water and in the elution buffer after passage of the Qiagen columns (11 of 40). This study identified factors that may influence the detection of C. pneumoniae DNA in vascular tissues and consequently bias the perception of a link between C. pneumoniae and vascular diseases. The following are strongly recommended: to use DNA polymerases that have to be activated, to decontaminate with dUTP-uracil-DNA glycosylase, to hybridize with specific probes, to include sufficient controls, and to use molecular grade water.

Marianelli, C., F. Ciuchini, et al. (2004). "Genetic Bases of the Rifampin Resistance Phenotype in Brucella spp." J. Clin. Microbiol. **42**(12): 5439-5443.

<http://jcm.asm.org/cgi/content/abstract/42/12/5439>

Rifampin is one of the most potent and broad-spectrum antibiotics against bacterial pathogens. Its bactericidal activity is due to its ability to bind to the {beta} subunit of the DNA-dependent RNA polymerase encoded by the *rpoB* gene. Mutations of the *rpoB* gene have been characterized in rifampin-resistant (Rifr) strains of *Escherichia coli* and *Mycobacterium tuberculosis*. The genetic bases of Rifr in *Brucella* spp. are still unknown. In the present study, the nucleotide sequences of the *rpoB* gene of the Rifr vaccine strain *Brucella abortus* RB51 and of 20 Rifr clones derived in our laboratory from two *Brucella melitensis* isolates were determined. These sequences were then compared to those of the respective rifampin-susceptible (Rifs) parental strains and to the published *B. melitensis* strain 16M. All Rifr strains carried one or more missense mutations mapping in two regions of the *rpoB* gene. These two "hot" regions were investigated in eight additional Rifr *Brucella* laboratory mutants and in 20 reference Rifs *Brucella* strains. *rpoB* mutations were found in all Rifr mutants. In contrast, no missense mutations were found in any analyzed Rifs strains. Our results represent the first from a study of the molecular characterization of *rpoB* mutations in resistant *Brucella* strains and provide an additional proof of the association of specific *rpoB* mutations with the development of the Rifr phenotype in prokaryotes. In addition, because of the relationship between Rifr and the attenuation of virulence in *Brucella* spp., studies of virulence in these mutants may provide useful information about the genetic basis of pathogenesis in *Brucella*.

Martella, V., M. Ciarlet, et al. (2003). "Molecular Analysis of the VP7, VP4, VP6, NSP4, and NSP5/6 Genes of a Buffalo Rotavirus Strain: Identification of the Rare P[3] Rhesus Rotavirus-Like VP4 Gene Allele." *J. Clin. Microbiol.* **41**(12): 5665-5675.

<http://jcm.asm.org/cgi/content/abstract/41/12/5665>

We report the detection and molecular characterization of a rotavirus strain, 10733, isolated from the feces of a buffalo calf affected with diarrhea in Italy. Strain 10733 was classified as a P[3] rotavirus, as the VP8* trypsin cleavage product of the VP4 protein revealed a high amino acid identity (96.2%) with that of rhesus rotavirus strain RRV (P5B[3]), used as the recipient virus in the human-simian reassortant vaccine. Analysis of the VP7 gene product revealed that strain 10733 possessed G6 serotype specificity, a type common in ruminants, with an amino acid identity to G6 rotavirus strains ranging from 88 to 98%, to Venezuelan bovine strain BRV033, and Hungarian human strain Hun4. Phylogenetic analysis based on the VP7 gene of G6 rotaviruses identified at least four lineages and an apparent linkage between each lineage and the VP4 specificity, suggesting the occurrence of repeated interspecies transmissions and genetic reassortment events between ruminant and human rotaviruses. Moreover, strain 10733 displayed a bovine-like NSP4 and NSP5/6 and a subgroup I VP6 specificity, as well as a long electropherotype pattern. The detection of the rare P[3] genotype in ruminants provides additional evidence for the wide genetic and antigenic diversity of group A rotaviruses.

Marty, F. M., D. H. Barouch, et al. (2003). "Disseminated Trichosporonosis Caused by *Trichosporon loubieri*." *J. Clin. Microbiol.* **41**(11): 5317-5320.

<http://jcm.asm.org/cgi/content/abstract/41/11/5317>

Trichosporonosis is an emerging invasive fungal infection in immunosuppressed patients; a case of disseminated infection caused by *Trichosporon loubieri* presented confirms its role as a human pathogen.

Massung, R. F. and K. G. Slater (2003). "Comparison of PCR Assays for Detection of the Agent of Human Granulocytic Ehrlichiosis, *Anaplasma phagocytophilum*." *J. Clin. Microbiol.* **41**(2): 717-722.

<http://jcm.asm.org/cgi/content/abstract/41/2/717>

Human granulocytic ehrlichiosis is an emerging infectious disease in the United States and Europe, and PCR methods have been shown to be effective for the diagnosis of acute infections. Numerous PCR assays and primer sets have been reported in the literature. The analytical sensitivities (limits of detection) of 13 published PCR primer sets were compared using DNA extracted from serial dilutions of *Anaplasma phagocytophilum*-infected HL-60 cells. The specificity of the assays that were able to detect ≤ 2.5 infected cells was tested by the use of template DNA extracted from *Ehrlichia chaffeensis*, *Rickettsia rickettsii*, and *Bartonella henselae*. The assays with the lowest limits of detection were shown to be a nested assay that amplifies the 16S rRNA gene (primer pairs ge3a-ge10 [primary] and ge9-ge3 [nested]; detects 0.25 infected cell), a direct assay that amplifies the major surface protein gene msp2 (primer pair msp2-3f-msp2-3r; detects 0.25 infected cell), and a direct assay that amplifies the 16S rRNA gene (primer pair ehr521-ehr790; detects 0.25 infected cell). The specificity and limit of detection of the MSP2 and 16S rRNA direct assays were further tested by use of *A. phagocytophilum* template DNA from both North America and Europe and from human, tick, white-footed mouse, equine, deer, bovine, and wood rat samples and of template DNA from closely related species (*Anaplasma marginale*, the white-tailed deer agent, and additional *E. chaffeensis*-positive samples). Three manufacturers' PCR kits were tested and showed distinct variations in the limit of detection, specificity, and nonspecific background amplification. The importance of these results for the molecular diagnosis of human granulocytic ehrlichiosis is discussed.

Menotti, J., G. Vilela, et al. (2003). "Comparison of PCR-Enzyme-Linked Immunosorbent Assay and Real-Time PCR Assay for Diagnosis of an Unusual Case of Cerebral Toxoplasmosis in a Stem Cell Transplant Recipient." *J. Clin. Microbiol.* **41**(11): 5313-5316.

<http://jcm.asm.org/cgi/content/abstract/41/11/5313>

A PCR-enzyme-linked immunosorbent assay and a real-time PCR assay were compared for diagnosis and follow-up of cerebral toxoplasmosis in a stem cell transplant recipient. The sensitivity of detection was similar for both assays but was higher when the assays were performed on buffy coat rather than on whole blood or serum.

Mittelholzer, C., L. Englund, et al. (2003). "Detection and Sequence Analysis of Danish and Swedish Strains of Mink Astrovirus." *J. Clin. Microbiol.* **41**(11): 5192-5194.

<http://jcm.asm.org/cgi/content/abstract/41/11/5192>

The sequences of mink astroviruses collected from 11 farms in Denmark and Sweden were analyzed and found to be homologous with one another but different from those of other astroviruses. A species-specific reverse transcriptase-PCR for mink astrovirus was established and shown to be suitable for the analysis of clinical samples.

Moberg, M., I. Gustavsson, et al. (2003). "Real-Time PCR-Based System for Simultaneous Quantification of Human Papillomavirus Types Associated with High Risk of Cervical Cancer." J. Clin. Microbiol. **41**(7): 3221-3228.

<http://jcm.asm.org/cgi/content/abstract/41/7/3221>

We have previously shown that women with a high titer of human papillomavirus type 16 (HPV16) in cervical epithelial cells have an increased risk of developing cervical carcinoma in situ. In order to study the relationship between viral DNA amount and risk of cervical carcinoma for the HPV types most commonly found in cervical tumors, we developed a real-time PCR assay for the detection and quantification of HPV16, -18, -31, -33, -35, -39, -45, -52, -58, and -67. These HPV types are analyzed in two reaction tubes, allowing for independent quantification of three viral types, or groups of viral types, in each reaction. A separate reaction is used for estimating the number of a nuclear single-copy gene and is used to calculate the HPV copy number per genomic DNA equivalent in the sample. The system has a dynamic range from 10² to 10⁷ HPV copies per assay and is applicable to both fresh clinical samples and DNA extracted from archival samples. Reconstitution experiments, made to mimic infections with several HPV types, shows that individual HPV types can be detected in a mixture as long as they represent 1 to 10% of the main type. The system was evaluated with respect to technical specificity and sensitivity, reproducibility, reagent stability, and sample preparation protocol and then used to analyze clinical samples. This homogeneous assay provides a fast and sensitive way for estimating the viral load of a series of the most frequent oncogenic HPV types in biopsies, as well as cervical smear samples.

Moissenet, D., H. Vu-Thien, et al. (2003). "DNA Fingerprinting of *Ralstonia paucula* by Infrequent-Restriction-Site PCR and Randomly Amplified Polymorphic DNA Analysis." J. Clin. Microbiol. **41**(12): 5747-5749.

<http://jcm.asm.org/cgi/content/abstract/41/12/5747>

Ralstonia paucula (formerly CDC group IV c-2) is an environmental organism that can cause serious human infections, occasionally clusters of nosocomial infections. In the present work, 26 strains of *R. paucula* (4 from the American Centers for Disease Control and Prevention collection, 10 from the Belgian Laboratorium voor Microbiologie [LMG] collection, and 12 French clinical isolates) were analyzed with infrequent-restriction-site PCR and randomly amplified polymorphic DNA analysis. Both techniques accurately distinguished between collection strains. Two close patterns obtained for all the French isolates suggested a clonal strain. Two LMG collection strains originating from human sources in the United States also showed patterns close to those of French isolates.

Molano, M., C. J. L. M. Meijer, et al. (2004). "Combination of PCR Targeting the VD2 of *omp1* and Reverse Line Blot Analysis for Typing of Urogenital *Chlamydia trachomatis* Serovars in Cervical Scrape Specimens." J. Clin. Microbiol. **42**(7): 2935-2939.

<http://jcm.asm.org/cgi/content/abstract/42/7/2935>

In this study we developed and evaluated a new PCR-based typing assay, directed to the VD2 region of the *omp1* gene, for the detection and typing of urogenital *Chlamydia trachomatis* infections. A nested VD2 PCR-reverse line blot (RLB) assay was developed for the typing of nine different urogenital serovars of *C. trachomatis*. The assay developed was tested with reference strains of *C. trachomatis* serovars and cervical scrapes of 86 Colombian women previously found

to be positive for *C. trachomatis* by using plasmid PCR. Two sets of primers directed to the VD2 region of the *omp1* gene of *C. trachomatis* were designed, and fragments of 220 and 166 bp were generated in the primary and nested PCRs, respectively. In addition, an RLB assay was developed to identify nine different urogenital serovars of *C. trachomatis* (Ba, D, E, F, G, H, I, J, and K) and group controls, including group B (Ba, D, and E), group C (I, J, K, and H), and an intermediate group (F and G). Using this assay, we were able to type 81 of the 86 samples (94.2%). Of these samples, 91.3% were single *C. trachomatis* infections, and 8.7% were multiple infections. The most common serovars identified were serovars D (22.2%), F (18.5%), G (13.6%), and E (12.3%). Of the women with multiple *C. trachomatis* infections, >50% contained both serovars D and E. The nested VD2 PCR-RLB developed is a simple, fast, and specific method for the identification of individual urogenital *C. trachomatis* serovars previously detected by using plasmid PCR. Moreover, it is an appropriate method for studying multiple *C. trachomatis* infections and for use in large epidemiological studies.

Mothershed, E. A., P. K. Cassiday, et al. (2002). "Development of a Real-Time Fluorescence PCR Assay for Rapid Detection of the Diphtheria Toxin Gene." *J. Clin. Microbiol.* **40**(12): 4713-4719.

<http://jcm.asm.org/cgi/content/abstract/40/12/4713>

We developed and evaluated a real-time fluorescence PCR assay for detecting the A and B subunits of diphtheria toxin (*tox*) gene. When 23 toxigenic *Corynebacterium diphtheriae* strains, 9 nontoxigenic *C. diphtheriae* strains, and 44 strains representing the diversity of pathogens and normal respiratory flora were tested, this real-time PCR assay exhibited 100% sensitivity and specificity. It allowed for the detection of both subunits of the *tox* gene at 750 times greater sensitivity (2 CFU) than the standard PCR (1,500 CFU). When used directly on specimens collected from patients with clinical diphtheria, one or both subunits of the *tox* gene were detected in 34 of 36 specimens by using the real-time PCR assay; only 9 specimens were found to be positive by standard PCR. Reamplification by standard PCR and DNA sequencing of the amplification product confirmed all real-time PCR *tox*-positive reactions. This real-time PCR format is a more sensitive and rapid alternative to standard PCR for detection of the *tox* gene in clinical material.

Muyldermans, G., D. Pierard, et al. (2004). "Simple Algorithm for Identification of *Bordetella pertussis* Pertactin Gene Variants." *J. Clin. Microbiol.* **42**(4): 1614-1619.

<http://jcm.asm.org/cgi/content/abstract/42/4/1614>

Studies performed in several countries have demonstrated the recent emergence and subsequent dominance of circulating *Bordetella pertussis* strains harboring pertactin and pertussis toxin variants not included in pertussis vaccines. Determination of the pertactin gene variants is commonly performed using a time-consuming and expensive sequence analysis. We developed a simple and reliable pertactin typing algorithm suitable for large-scale screening. The assay correctly identified all pertactin alleles in representative strains. The typing of 231 clinical strains of *B. pertussis* routinely isolated in Belgium showed that this algorithm was adequate to identify less-frequent prn types like prn9 and prn11.

Nadkarni, M. A., C. E. Caldon, et al. (2004). "Carious Dentine Provides a Habitat for a Complex Array of Novel *Prevotella*-Like Bacteria." *J. Clin. Microbiol.* **42**(11): 5238-5244.

<http://jcm.asm.org/cgi/content/abstract/42/11/5238>

Previous analysis of the microbiology of advanced caries by culture and real-time PCR emphasized the high incidence and abundance of gram-negative anaerobic species, particularly Prevotella-like bacteria. The diversity of Prevotella-like bacteria was further explored by analyzing pooled bacterial DNA from lesions of carious dentine. This was achieved by amplification of a region of the 16S ribosomal DNA with a Prevotella genus-specific forward primer and a universal bacterial reverse primer, followed by cloning and sequencing. Cultured Prevotella species commonly associated with oral tissues constituted only 12% of the Prevotella clones isolated from advanced carious lesions. The remaining 88% consisted of a diverse range of phylotypes. These included five clusters of previously recognized but uncultured oral Prevotella spp. and a major cluster containing Prevotella-like bacteria most closely related to uncharacterized rumen bacteria. Cluster-specific primers were designed, and the numbers of bacteria within clusters were quantified by real-time PCR, confirming the abundance of these organisms. The data indicated that advanced dental caries provides a unique environment for a complex array of novel and uncultured Prevotella and Prevotella-like bacteria which, in some cases, may dominate the diverse polymicrobial community associated with the disease.

Nagai, T., H. Sobajima, et al. (2003). "Neonatal Sudden Death Due to Legionella Pneumonia Associated with Water Birth in a Domestic Spa Bath." J. Clin. Microbiol. **41**(5): 2227-2229.

<http://jcm.asm.org/cgi/content/abstract/41/5/2227>

We report the first case of neonatal Legionnaires' disease associated with water birth in a spa bath at home. Legionella pneumophila serogroup 6 was detected from postmortem lung tissue.

Nakano, K., R. Nomura, et al. (2004). "Demonstration of Streptococcus mutans with a Cell Wall Polysaccharide Specific to a New Serotype, k, in the Human Oral Cavity." J. Clin. Microbiol. **42**(1): 198-202.

<http://jcm.asm.org/cgi/content/abstract/42/1/198>

Streptococcus mutans organisms are occasionally isolated from the blood of patients with infective endocarditis, though the mechanisms of invasion and survival remain to be elucidated. Two of four blood isolates from patients with bacteremia or infective endocarditis (strains TW295 and TW871) were serologically untypeable by immunodiffusion testing, which was due to a lack of the glucose side chain of the serotype-specific polysaccharide antigen of S. mutans. Immunodiffusion analyses using antisera against these strains demonstrated that 2 of 100 isolates from 100 subjects showed a positive reaction, while further analysis of 2,500 isolates from 50 subjects revealed that all 50 isolates from a single subject were not reactive with anti-c, -e, and -f antisera, though they were reactive with anti-TW295 and -TW871 antisera. The oral isolates showed biological properties similar to those of the reference S. mutans strain MT8148, including high levels of sucrose-dependent adhesion and cellular hydrophobicity, along with expression of glucosyltransferases and a protein antigen, PA. We designated these organisms serotype k. A glucose side chain-defective mutant strain was then constructed by insertional inactivation of the gluA gene of strain MT8148, which showed biological properties similar to those of serotype k of S. mutans. Serotype k oral isolates were less susceptible to phagocytosis, as were the gluA-inactivated mutant of strain MT8148 and blood isolates. These results indicate that S. mutans serotype k strains are present in the oral cavity in humans and may be able to survive longer in blood owing to their low susceptibility to phagocytosis.

Naser, S., F. L. Thompson, et al. (2005). "Phylogeny and Identification of Enterococci by atpA Gene Sequence Analysis." *J. Clin. Microbiol.* **43**(5): 2224-2230.

<http://jcm.asm.org/cgi/content/abstract/43/5/2224>

The relatedness among 91 *Enterococcus* strains representing all validly described species was investigated by comparing a 1,102-bp fragment of *atpA*, the gene encoding the alpha subunit of ATP synthase. The relationships observed were in agreement with the phylogeny inferred from 16S rRNA gene sequence analysis. However, *atpA* gene sequences were much more discriminatory than 16S rRNA for species differentiation. All species were differentiated on the basis of *atpA* sequences with, at a maximum, 92% similarity. Six members of the *Enterococcus faecium* species group (*E. faecium*, *E. hirae*, *E. durans*, *E. villorum*, *E. mundtii*, and *E. ratti*) showed >99% 16S rRNA gene sequence similarity, but the highest value of *atpA* gene sequence similarity was only 89.9%. The intraspecies *atpA* sequence similarities for all species except *E. faecium* strains varied from 98.6 to 100%; the *E. faecium* strains had a lower *atpA* sequence similarity of 96.3%. Our data clearly show that *atpA* provides an alternative tool for the phylogenetic study and identification of enterococci.

Nguyen, T. V., P. Le Van, et al. (2005). "Detection and Characterization of Diarrheagenic *Escherichia coli* from Young Children in Hanoi, Vietnam." *J. Clin. Microbiol.* **43**(2): 755-760.

<http://jcm.asm.org/cgi/content/abstract/43/2/755>

Diarrhea continues to be one of the most common causes of morbidity and mortality among infants and children in developing countries. *Escherichia coli* is an emerging agent among pathogens that cause diarrhea. The development of a highly applicable technique for the detection of different categories of diarrheagenic *E. coli* is important. We have used multiplex PCR by combining eight primer pairs specific for enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli*, enteropathogenic *E. coli* (EPEC), and enterotoxigenic *E. coli* (ETEC). This facilitates the identification of five different categories of diarrheagenic *E. coli* from stool samples in a single reaction simultaneously. The prevalences of diarrheagenic *E. coli* were 22.5 and 12% in the diarrhea group and the control group, respectively. Among 587 fecal samples from Vietnamese children under 5 years of age with diarrhea, this technique identified 132 diarrheagenic *E. coli* strains. This included 68 samples (11.6%) with EAEC, 12 samples (2.0%) with EIEC, 39 samples (6.6%) with EPEC, and 13 samples (2.2%) with ETEC. Among the 249 age-matched controls, 30 samples were positive for diarrheagenic *E. coli*. The distribution was 18 samples (7.2%) with EAEC, 11 samples (4.4%) with EPEC, and 1 sample (0.4%) with ETEC.

Nicolas, M. M., I. H. Stalis, et al. (2005). "Systemic Disease in Vaal Rhebok (*Pelea capreolus*) Caused by *Mycoplasmas* in the *Mycoides* Cluster." *J. Clin. Microbiol.* **43**(3): 1330-1340.

<http://jcm.asm.org/cgi/content/abstract/43/3/1330>

In the winter of 2002, an outbreak of mycoplasma infection in Vaal rhebok (*Pelea capreolus*) originating from South Africa occurred 15 weeks after their arrival in San Diego, Calif. Three rhebok developed inappetence, weight loss, lethargy, signs related to pulmonary or arthral dysfunction, and sepsis. All three rhebok died or were euthanized. Primary postmortem findings were erosive tracheitis, pleuropneumonia, regional cellulitis, and necrotizing lymphadenitis.

Mycoplasmas were detected in numerous tissues by electron microscopy, immunohistochemistry, and PCR. The three deceased rhebok were coinfecting with ovine herpesvirus-2, and two animals additionally had a novel gammaherpesvirus. However, no lesions indicative of herpesvirus were seen microscopically in any animal. The rheboks' mycoplasmas were characterized at the level of the 16S rRNA gene, the 16S-23S intergenic spacer region, and the fructose biphosphate aldolase gene. Denaturing gradient gel electrophoresis was carried out to address the possibility of infection with multiple strains. Two of the deceased rhebok were infected with a single strain of *Mycoplasma capricolum* subsp. *capricolum*, and the third animal had a single, unique strain most closely related to *Mycoplasma mycoides* subsp. *mycoides* large-colony. A PCR survey of DNA samples from 46 other ruminant species demonstrated the presence of several species of mycoplasmas in the mycoides cluster, including a strain of *M. capricolum* subsp. *capricolum* identical to that found in two of the rhebok. These findings demonstrate the pervasiveness of mycoplasmas in the mycoides cluster in small ruminants and the potential for interspecies transmission and disease when different animal taxa come in contact.

Nielsen, E. M. and M. T. Andersen (2003). "Detection and Characterization of Verocytotoxin-Producing *Escherichia coli* by Automated 5' Nuclease PCR Assay." *J. Clin. Microbiol.* **41**(7): 2884-2893.

<http://jcm.asm.org/cgi/content/abstract/41/7/2884>

In recent years increased attention has been focused on infections caused by isolates of verocytotoxin-producing *Escherichia coli* (VTEC) serotypes other than O157. These non-O157 VTEC isolates are commonly present in food and food production animals. Easy detection, isolation, and characterization of non-O157 VTEC isolates are essential for improving our knowledge of these organisms. In the present study, we detected VTEC isolates in bovine fecal samples by a duplex 5' nuclease PCR assay (real-time PCR) that targets *vtx1* and *vtx2*. VTEC isolates were obtained by colony replication by use of hydrophobic-grid membrane filters and DNA probe hybridization. Furthermore, we have developed 5' nuclease PCR assays for the detection of virulence factors typically present in VTEC isolates, including subtypes of three genes of the locus of enterocyte effacement (LEE) pathogenicity island. The 22 assays included assays for the detection of verocytotoxin genes (*vtx1*, *vtx2*), pO157-associated genes (*ehxA*, *katP*, *espP*, and *etpD*), a recently identified adhesin (*saa*), intimin (*eae*, all variants), seven subtypes of *eae*, four subtypes of *tir*, and three subtypes of *espD*. A number of reference strains (VTEC and enteropathogenic *E. coli* strains) and VTEC strains isolated from calves were tested to validate the PCR assays. The expected virulence profiles were detected for all reference strains. In addition, new information on the subtypes of LEE genes was obtained. For reference strains as well as bovine isolates, a consistent relationship between subtypes of the LEE genes was found, so that a total of seven different combinations of these were recognized (corresponding to the seven subtypes of *eae*). Isolates with 15 different serogroup-virulence profiles were isolated from 16 calves. Among these, 53% harbored LEE and 73% harbored factors carried by the large virulence plasmid. One LEE-negative isolate had the gene for the adhesin *Saa*. The most common virulence profile among the bovine isolates was *vtx1*, *eae*-{ ζ }, *tir*-{ α }, *ehxA*, and *espP*. This panel of assays offers an easy method for the extensive characterization of VTEC isolates.

Nijhuis, M., N. van Maarseveen, et al. (2002). "Rapid and Sensitive Routine Detection of All Members of the Genus Enterovirus in Different Clinical Specimens by Real-Time PCR." *J. Clin. Microbiol.* **40**(10): 3666-3670.

<http://jcm.asm.org/cgi/content/abstract/40/10/3666>

We developed a rapid and sensitive method for the routine detection of all members of the

enterovirus genus in different clinical specimens by using real-time TaqMan quantitative PCR. Multiple primer and probe sets were selected in the highly conserved 5'-untranslated region of the enterovirus genome. Our assay detected all 60 different enterovirus species tested, whereas no reactivity was observed with the viruses from the other genera of the picornaviridae family, e.g., hepatovirus and parechovirus. Weak cross-reactivity was observed with 7 of the 90 different high-titer rhinovirus stocks but not with rhinovirus-positive clinical isolates. Analysis of a well-characterized reference panel containing different enteroviruses at various concentrations demonstrated that the enterovirus real-time TaqMan PCR is as sensitive as most of the currently used molecular detection assays. Evaluation of clinical isolates demonstrated that the assay is more sensitive than the "gold standard" method, i.e., viral culture. Moreover, the PCR assay can be used on different clinical specimens, such as plasma, serum, nose and throat swabs, cerebrospinal fluid, and bronchoalveolar lavage, without apparent inhibition. Our data demonstrate that the real-time TaqMan PCR is a rapid and sensitive assay for the detection of enterovirus infection. The assay has a robust character and is easily standardized, which makes it an excellent alternative for the conventional time-consuming viral culture.

Nilsson, H.-O., I.-S. Ouis, et al. (2004). "High Prevalence of Helicobacter Species Detected in Laboratory Mouse Strains by Multiplex PCR-Denaturing Gradient Gel Electrophoresis and Pyrosequencing." J. Clin. Microbiol. **42**(8): 3781-3788.

<http://jcm.asm.org/cgi/content/abstract/42/8/3781>

Rodent models have been developed to study the pathogenesis of diseases caused by *Helicobacter pylori*, as well as by other gastric and intestinal *Helicobacter* spp., but some murine enteric *Helicobacter* spp. cause hepatobiliary and intestinal tract diseases in specific inbred strains of laboratory mice. To identify these murine *Helicobacter* spp., we developed an assay based on PCR-denaturing gradient gel electrophoresis and pyrosequencing. Nine strains of mice, maintained in four conventional laboratory animal houses, were assessed for *Helicobacter* sp. carriage. Tissue samples from the liver, stomach, and small intestine, as well as feces and blood, were collected; and all specimens (n = 210) were screened by a *Helicobacter* genus-specific PCR. Positive samples were identified to the species level by multiplex denaturing gradient gel electrophoresis, pyrosequencing, and a *H. ganmani*-specific PCR assay. Histologic examination of 30 tissue samples from 18 animals was performed. All mice of eight of the nine strains tested were *Helicobacter* genus positive; *H. bilis*, *H. hepaticus*, *H. typhlonius*, *H. ganmani*, *H. rodentium*, and a *Helicobacter* sp. flexispira-like organism were identified. *Helicobacter* DNA was common in fecal (86%) and gastric tissue (55%) specimens, whereas samples of liver tissue (21%), small intestine tissue (17%), and blood (14%) were less commonly positive. Several mouse strains were colonized with more than one *Helicobacter* spp. Most tissue specimens analyzed showed no signs of inflammation; however, in one strain of mice, hepatitis was diagnosed in livers positive for *H. hepaticus*, and in another strain, gastric colonization by *H. typhlonius* was associated with gastritis. The diagnostic setup developed was efficient at identifying most murine *Helicobacter* spp.

Nurpeisov, V., S. J. Hurwitz, et al. (2003). "Fluorescent Dye Terminator Sequencing Methods for Quantitative Determination of Replication Fitness of Human Immunodeficiency Virus Type 1 Containing the Codon 74 and 184 Mutations in Reverse Transcriptase." J. Clin. Microbiol. **41**(7): 3306-3311.

<http://jcm.asm.org/cgi/content/abstract/41/7/3306>

The fluorescent dye-labeled dideoxynucleotide automated DNA sequencing system has been routinely used for monitoring the development of resistance mutations in human

immunodeficiency virus type 1 reverse transcriptase (RT) and protease genes during therapy. This system has provided information regarding the presence of mixtures of nucleotides in the clinical samples but has not previously been validated for the quantitative determination between peak heights and relative DNA concentration. We evaluated this system by using various ratios of wild-type and mutated DNA fragments and by performing sequencing reactions at actual melting temperatures of specific primers. Several different ratios of purified DNA fragments containing mixtures of L74/V74 and M184/V184 were sequenced, and peak heights were measured. Regression analysis between ratios of peak heights and DNA concentration demonstrated a statistically significant linear correlation, suggesting that the quantification of two different species of DNA in a mixture could be achieved with the fluorescent dye-labeled dideoxynucleotide system. These strategies have broader implications for the quantification of replication fitness of viruses, particularly those containing RT mutations at codons 74 and 184.

O'Donnell, K., D. A. Sutton, et al. (2004). "Genetic Diversity of Human Pathogenic Members of the *Fusarium oxysporum* Complex Inferred from Multilocus DNA Sequence Data and Amplified Fragment Length Polymorphism Analyses: Evidence for the Recent Dispersion of a Geographically Widespread Clonal Lineage and Nosocomial Origin." *J. Clin. Microbiol.* **42**(11): 5109-5120.

<http://jcm.asm.org/cgi/content/abstract/42/11/5109>

Fusarium oxysporum is a phylogenetically diverse monophyletic complex of filamentous ascomycetous fungi that are responsible for localized and disseminated life-threatening opportunistic infections in immunocompetent and severely neutropenic patients, respectively. Although members of this complex were isolated from patients during a pseudoepidemic in San Antonio, Tex., and from patients and the water system in a Houston, Tex., hospital during the 1990s, little is known about their genetic relatedness and population structure. This study was conducted to investigate the global genetic diversity and population biology of a comprehensive set of clinically important members of the *F. oxysporum* complex, focusing on the 33 isolates from patients at the San Antonio hospital and on strains isolated in the United States from the water systems of geographically distant hospitals in Texas, Maryland, and Washington, which were suspected as reservoirs of nosocomial fusariosis. In all, 18 environmental isolates and 88 isolates from patients spanning four continents were genotyped. The major finding of this study, based on concordant results from phylogenetic analyses of multilocus DNA sequence data and amplified fragment length polymorphisms, is that a recently dispersed, geographically widespread clonal lineage is responsible for over 70% of all clinical isolates investigated, including all of those associated with the pseudoepidemic in San Antonio. Moreover, strains of the clonal lineage recovered from patients were conclusively shown to genetically match those isolated from the hospital water systems of three U.S. hospitals, providing support for the hypothesis that hospitals may serve as a reservoir for nosocomial fusarial infections.

Okitsu, N., S. Kaieda, et al. (2005). "Characterization of *ermB* Gene Transposition by Tn1545 and Tn917 in Macrolide-Resistant *Streptococcus pneumoniae* Isolates." *J. Clin. Microbiol.* **43**(1): 168-173.

<http://jcm.asm.org/cgi/content/abstract/43/1/168>

In *Streptococcus pneumoniae*, the *ermB* gene is carried by transposons, such as Tn917 and Tn1545. This study investigated the relationship between macrolide resistance and the presence of the *ermB* gene on Tn917 or Tn1545 in 84 Japanese pneumococcal isolates. Macrolide-resistant strains were classified into two groups as follows. Group 1 (19 strains) showed a tendency to high resistance to erythromycin (MIC at which 50% of isolates are inhibited, 4 mg/liter; MIC at which 90% of isolates are inhibited [MIC₉₀], 128 mg/liter) but susceptibility to

rokitamycin (MIC90, 1 mg/liter), with the *ermB* gene located on Tn1545. Group 2 (65 strains) showed a tendency to high resistance to both antibiotics (MIC90s for both erythromycin and rokitamycin, >128 mg/liter), with the *ermB* gene located on Tn917. There were no strains with constitutive macrolide resistance in either group. All of the strains in group 2 had a deletion in the promoter region of *ermB* and an insertion of the TAAA motif in the leader peptide. The results of pulsed-field gel electrophoresis and serogrouping showed that Tn1545 spread clonally while Tn917 spread both horizontally and clonally. In conclusion, in Japanese macrolide-resistant *S. pneumoniae* isolates, the *ermB* gene is carried and spread primarily by Tn917.

On, S. L. W. and P. J. Jordan (2003). "Evaluation of 11 PCR Assays for Species-Level Identification of *Campylobacter jejuni* and *Campylobacter coli*." *J. Clin. Microbiol.* **41**(1): 330-336.

<http://jcm.asm.org/cgi/content/abstract/41/1/330>

We examined the sensitivity and specificity of 11 PCR assays described for the species identification of *Campylobacter jejuni* and *Campylobacter coli* by using 111 type, reference, and field strains of *C. jejuni*, *C. coli*, and *Campylobacter lari*. For six assays, an additional 21 type strains representing related *Campylobacter*, *Arcobacter*, and *Helicobacter* species were also included. PCR tests were initially established in the laboratory by optimizing conditions with respect to five type and reference strains of *C. jejuni*, *C. coli*, and *C. lari*. One PCR test for *C. coli* failed to give appropriate results during this initial setup phase and was not evaluated further. The remaining 10 assays were used to examine heated lysate and purified DNA templates as appropriate of well-characterized type, reference, and field strains of *C. jejuni* (n = 62), *C. coli* (n = 34), and *C. lari* (n = 15). The tests varied considerably in their sensitivity and specificity for their respective target species. No assay was found to be 100% sensitive and/or specific for all *C. jejuni* strains tested, but four assays for *C. coli* gave appropriate responses for all strains examined. Between one and six strains of *C. jejuni* gave amplicons in four of seven *C. jejuni* PCR tests only where purified DNA was used as the template; corresponding results were seen with one strain of *C. coli* in each of three assays for the latter species. Our findings indicate that a polyphasic strategy for PCR-based identification should be used to identify *C. jejuni* and *C. coli* strains. The data may assist laboratories in selecting assays suited for their needs and in designing evaluations of future PCR tests aimed to identify these species.

Osiowy, C. (2002). "Sensitive Detection of HBsAg Mutants by a Gap Ligase Chain Reaction Assay." *J. Clin. Microbiol.* **40**(7): 2566-2571.

<http://jcm.asm.org/cgi/content/abstract/40/7/2566>

Hepatitis B virus (HBV) surface gene variants have been associated with diagnostic escape and immune escape following vaccination. The most common mutation observed in these variants is a glycine-to-arginine substitution at amino acid 145 (G145R). In order to sensitively detect the presence of this mutant in serum, a new molecular detection system was developed; in this new system, a gap ligase chain reaction (gLCR) assay was coupled with electrochemiluminescence detection of reaction products. The gLCR assay could detect approximately 10 copies of mutant DNA and could discriminate low levels of mutant DNA in the presence of excess wild-type DNA. Detection of the G145R mutant in clinical specimens was evaluated by testing 56 suspect serum specimens. The G145R mutation was observed in 18 of 28 HBV-DNA-positive samples. The approximate percentage of mutant present in each specimen was calculated by comparison with a standard curve of an increasing ratio of mutant DNA to wild-type DNA. Most samples contained a very low percentage of mutant virus (approximately 5%), with an observed range of approximately 3 to 74%. The G145R mutation was most frequently observed in specimens producing a diagnostic anomaly or from transplant patients but was also observed in specimens

from vaccinated individuals and specimens in which HBsAg diagnostic escape was suspected. Therefore, the gLCR assay is a sensitive and specific method for detection of G145R mutants, which could be modified to include the detection of other HBV mutants.

Osiowy, C. and E. Giles (2003). "Evaluation of the INNO-LiPA HBV Genotyping Assay for Determination of Hepatitis B Virus Genotype." J. Clin. Microbiol. **41**(12): 5473-5477.

<http://jcm.asm.org/cgi/content/abstract/41/12/5473>

Specific genotypes of hepatitis B virus (HBV) are increasingly recognized for their clinical significance and association with particular viral mutations. Although many HBV genotyping methods exist, there has been no standardized or commercially available method for direct molecular typing of the HBV genome. A newly available line probe assay (INNO-LiPA HBV Genotyping assay; Innogenetics N.V., Ghent, Belgium) that allows the identification of HBV genotypes A to G was assessed by comparison with pre-S1/pre-S2 sequence analysis of the isolates in 188 serum specimens. All seven genotypes were detected by the line probe assay (LiPA), and complete concordance between LiPA and sequence analysis was observed for 152 specimens (81%). LiPA was able to detect 19 mixed genotype infections not detected by amplicon sequencing, which for the most part were confirmed by cloning and sequencing of the pre-S1/pre-S2 amplicon. Four specimens had discrepant results between the two methods, and five specimens had indeterminate results by LiPA. The HBV DNA in four specimens was unable to be amplified by the nested INNO-LiPA HBV DR amplification primers; however, the HBV DNA in six specimens unable to be genotyped by sequencing was clearly genotyped by LiPA. The INNO-LiPA HBV Genotyping assay appears to be useful for the rapid genotyping of HBV, particularly for the sensitive detection of mixed genotype infections.

Ott, S. J., M. Musfeldt, et al. (2004). "Quantification of Intestinal Bacterial Populations by Real-Time PCR with a Universal Primer Set and Minor Groove Binder Probes: a Global Approach to the Enteric Flora." J. Clin. Microbiol. **42**(6): 2566-2572.

<http://jcm.asm.org/cgi/content/abstract/42/6/2566>

The composition of the human intestinal flora is important for the health status of the host. The global composition and the presence of specific pathogens are relevant to the effects of the flora. Therefore, accurate quantification of all major bacterial populations of the enteric flora is needed. A TaqMan real-time PCR-based method for the quantification of 20 dominant bacterial species and groups of the intestinal flora has been established on the basis of 16S ribosomal DNA taxonomy. A PCR with conserved primers was used for all reactions. In each real-time PCR, a universal probe for quantification of total bacteria and a specific probe for the species in question were included. PCR with conserved primers and the universal probe for total bacteria allowed relative and absolute quantification. Minor groove binder probes increased the sensitivity of the assays 10- to 100-fold. The method was evaluated by cross-reaction experiments and quantification of bacteria in complex clinical samples from healthy patients. A sensitivity of 101 to 103 bacterial cells per sample was achieved. No significant cross-reaction was observed. The real-time PCR assays presented may facilitate understanding of the intestinal bacterial flora through a normalized global estimation of the major contributing species.

Palacios, G., O. Jabado, et al. (2005). "Molecular Identification of Mumps Virus Genotypes from Clinical Samples: Standardized Method of Analysis." J. Clin. Microbiol. **43**(4): 1869-1878.

<http://jcm.asm.org/cgi/content/abstract/43/4/1869>

A sensitive nested reverse transcription-PCR assay, targeting a short fragment of the gene encoding the small hydrophobic protein (SH gene), was developed to allow rapid characterization of mumps virus in clinical samples. The sensitivity and specificity of the assay were established using representative genotypes A, B, C, D, E, and F. Mumps virus RNA was characterized directly from cerebrospinal fluid (CSF) samples and in extracts of mumps virus isolates from patients with various clinical syndromes. Direct sequencing of products and subsequent phylogenetic analysis enabled genetic classification. A simple web-based system of sequence analysis was established. The study also allowed characterization of mumps virus strains from Argentina as part of a new subgenotype. This PCR assay for characterization of mumps infections coupled to a web-based analytical program provides a rapid method for identification of known and novel strains.

Palmer, S., A. P. Wiegand, et al. (2003). "New Real-Time Reverse Transcriptase-Initiated PCR Assay with Single-Copy Sensitivity for Human Immunodeficiency Virus Type 1 RNA in Plasma." J. Clin. Microbiol. **41**(10): 4531-4536.

<http://jcm.asm.org/cgi/content/abstract/41/10/4531>

More sensitive assays for human immunodeficiency virus type 1 (HIV-1) RNA are needed to detect, quantify, and characterize persistent viremia in patients who are receiving antiretroviral therapy and whose plasma HIV-1 RNA levels are suppressed to less than 50 to 75 copies/ml. We therefore developed an internally controlled real-time reverse transcriptase-initiated PCR assay that quantifies HIV-1 RNA concentrations down to 1 copy per ml of plasma. This assay with single-copy sensitivity (the single-copy assay) generates a reproducible linear regression plot of input copy number versus threshold cycle by using HIV-1 RNA transcripts at copy numbers ranging from 1 to 106 per reaction mixture. The single-copy assay was compared to the ultrasensitive AMPLICOR HIV-1 MONITOR assay and a more sensitive modification of the ultrasensitive assay by repeatedly testing a low-copy-number panel containing 200 to 0.781 copies of HIV-1 RNA per ml of plasma. This comparison showed that the single-copy assay had a greater sensitivity than the other assays and was the only assay that detected HIV-1 RNA at levels as low as 0.781 copies/ml. Testing of plasma samples from 15 patients who were receiving antiretroviral therapy and who had <75 HIV-1 RNA copies/ml revealed persistent viremia in all 15 patients, with HIV-1 RNA levels ranging from 1 to 32 copies/ml (median, 13 copies/ml). The greater sensitivity of the single-copy assay should allow better characterization of persistent viremia in patients who are receiving antiretroviral therapy and whose HIV-1 RNA levels are suppressed to below the detection limits of present assays.

Pang, X., B. Lee, et al. (2004). "Evaluation and Validation of Real-Time Reverse Transcription-PCR Assay Using the LightCycler System for Detection and Quantitation of Norovirus." J. Clin. Microbiol. **42**(10): 4679-4685.

<http://jcm.asm.org/cgi/content/abstract/42/10/4679>

We developed an assay for the detection and quantitation of norovirus with the LightCycler SYBR Green-based real-time reverse transcription-PCR (real-time LC RT-PCR) and previously published primers in the capsid and the polymerase gene. One hundred thirty-two stool specimens from the Provincial Laboratory for Public Health (Microbiology), Alberta, Canada, and the Centers for Disease Control and Prevention, Atlanta, Ga., were used to validate the new assay. The samples were collected from patients involved in outbreaks of acute gastroenteritis or

children who presented with sporadic gastroenteritis. The real-time LC RT-PCR assay detected norovirus strains from three genogroup I (G-I) clusters (G-I/1, G-I/2, and G-I/3) and 10 genogroup II (G-II) clusters (G-II/1, G-II/2, G-II/3, G-II/4, G-II/6, G-II/7, G-II/10, G-II/12, G-II/15, and G-II/16). There was 100% concordance with the results from 58 stool specimens which tested positive by conventional RT-PCR assays. By dilution analysis, the real-time LC RT-PCR was 10,000 times more sensitive than the conventional RT-PCR. The new assay increased the number of samples in which noroviruses were detected by 19%. The real-time LC RT-PCR had a wide dynamic range, detecting from 5 to 5×10^6 copies of RNA per reaction, resulting in a theoretical lower limit of detection of 25,000 copies of RNA per g of stool. No cross-reactions were found with specimens containing sapovirus, rotavirus, astrovirus, and adenovirus. Because of the high sensitivity and specificity of the assay with a relatively rapid and simple procedure, the real-time LC RT-PCR will be useful as a routine assay for the clinical diagnosis of norovirus infection.

Park, S.-W., C.-S. Lee, et al. (2005). "Rapid Identification of the Coxsackievirus A24 Variant by Molecular Serotyping in an Outbreak of Acute Hemorrhagic Conjunctivitis." *J. Clin. Microbiol.* **43**(3): 1069-1071.

<http://jcm.asm.org/cgi/content/abstract/43/3/1069>

We evaluated the clinical applicability of a molecular serotyping method for determination of the cause of epidemic acute hemorrhagic conjunctivitis. Seventy conjunctival swab specimens from individuals involved in a nationwide acute hemorrhagic conjunctivitis outbreak were tested. Viral culture and a molecular biology-based assay were compared by directly using clinical specimens. On the one hand, virus culture was done to isolate the enteroviruses, and serotyping was done by a coxsackievirus A24 variant-specific PCR. On the other hand, the original clinical specimens were directly screened for enterovirus by reverse transcription (RT)-PCR with panenterovirus-specific primers. Enterovirus screening-positive specimens were subjected to RT-PCR for detection of the VP1 region of enterovirus, and the amplicons were sequenced. Molecular serotyping was done by calculating the pairwise identity scores for the sequences with the maximum identities to the sequences of known prototype enteroviruses. Thirty-two specimens (45.7%) were culture positive, whereas 37 specimens (52.8%) were screening PCR positive ($P < 0.001$). The VP1 regions were amplified from 21 of the 37 specimens (56.8%), and the products amplified from 9 specimens were appropriately sequenced. These nine sequences were homologous with the sequence of the coxsackievirus A24 variant. Molecular serotyping by direct use of clinical specimens without cell culture could be applied for the rapid identification of the causative agent of epidemic acute hemorrhagic conjunctivitis.

Paster, B. J., W. A. Falkler, Jr., et al. (2002). "Prevalent Bacterial Species and Novel Phylotypes in Advanced Noma Lesions." *J. Clin. Microbiol.* **40**(6): 2187-2191.

<http://jcm.asm.org/cgi/content/abstract/40/6/2187>

The purpose of this study was to determine the bacterial diversity in advanced noma lesions using culture-independent molecular methods. 16S ribosomal DNA bacterial genes from DNA isolated from advanced noma lesions of four Nigerian children were PCR amplified with universally conserved primers and spirochetal selective primers and cloned into *Escherichia coli*. Partial 16S rRNA sequences of approximately 500 bases from 212 cloned inserts were used initially to determine species identity or closest relatives by comparison with sequences of known species or phylotypes. Nearly complete sequences of approximately 1,500 bases were obtained for most of the potentially novel species. A total of 67 bacterial species or phylotypes were detected, 25 of which have not yet been grown in vitro. Nineteen of the species or phylotypes, including *Propionibacterium acnes*, *Staphylococcus* spp., and the opportunistic pathogens

Stenotrophomonas maltophilia and *Ochrobactrum anthropi* were detected in more than one subject. Other known species that were detected included *Achromobacter* spp., *Afipia* spp., *Brevundimonas diminuta*, *Capnocytophaga* spp., *Cardiobacterium* sp., *Eikenella corrodens*, *Fusobacterium* spp., *Gemella haemolyans*, and *Neisseria* spp. Phylotypes that were unique to noma infections included those in the genera *Eubacterium*, *Flavobacterium*, *Kocuria*, *Microbacterium*, and *Porphyromonas* and the related *Streptococcus salivarius* and genera *Sphingomonas* and *Treponema*. Since advanced noma lesions are infections open to the environment, it was not surprising to detect species not commonly associated with the oral cavity, e.g., from soil. Several species previously implicated as putative pathogens of noma, such as spirochetes and *Fusobacterium* spp., were detected in at least one subject. However, due to the limited number of available noma subjects, it was not possible at this time to associate specific species with the disease.

Paule, S. M., W. E. Trick, et al. (2003). "Comparison of PCR Assay to Culture for Surveillance Detection of Vancomycin-Resistant Enterococci." *J. Clin. Microbiol.* **41**(10): 4805-4807.

<http://jcm.asm.org/cgi/content/abstract/41/10/4805>

Direct multiplex PCR assay using *vanA* and *vanB* primers, which provides rapid results, was more sensitive than culture on selective media for samples collected by rectal swab (20 of 46 versus 8 of 46; $P < 0.001$) or perianal swab (17 of 58 versus 12 of 58; $P = 0.059$) for the detection of gastrointestinal colonization by vancomycin-resistant enterococci.

Perandin, F., N. Manca, et al. (2004). "Development of a Real-Time PCR Assay for Detection of *Plasmodium falciparum*, *Plasmodium vivax*, and *Plasmodium ovale* for Routine Clinical Diagnosis." *J. Clin. Microbiol.* **42**(3): 1214-1219.

<http://jcm.asm.org/cgi/content/abstract/42/3/1214>

A TaqMan-based real-time PCR qualitative assay for the detection of three species of malaria parasites--*Plasmodium falciparum*, *P. ovale*, and *P. vivax*--was devised and evaluated using 122 whole-blood samples from patients who had traveled to areas where malaria is endemic and who presented with malaria-like symptoms and fever. The assay was compared to conventional microscopy and to an established nested-PCR assay. The specificity of the new assay was confirmed by sequencing the PCR products from all the positive samples and by the lack of cross-reactivity with *Toxoplasma gondii* and *Leishmania infantum* DNA. Real-time PCR assay showed a detection limit (analytical sensitivity) of 0.7, 4, and 1.5 parasites/ μ l for *P. falciparum*, *P. vivax*, and *P. ovale*, respectively. Real-time PCR, like nested PCR, brought to light errors in the species identification by microscopic examination and revealed the presence of mixed infections (*P. falciparum* plus *P. ovale*). Real-time PCR can yield results within 2 h, does not require post-PCR processing, reduces sample handling, and minimizes the risks of contamination. The assay can therefore be easily implemented in routine diagnostic malaria tests. Future studies are warranted to investigate the clinical value of this technique.

Perilli, M., E. Dell'Amico, et al. (2002). "Molecular Characterization of Extended-Spectrum β -Lactamases Produced by Nosocomial Isolates of Enterobacteriaceae from an Italian Nationwide Survey." *J. Clin. Microbiol.* **40**(2): 611-614.

<http://jcm.asm.org/cgi/content/abstract/40/2/611>

Extended-spectrum β -lactamases (ESBLs) are widespread in hospital settings worldwide. The present investigation was undertaken to assess the distribution and prevalence of ESBLs belonging to the TEM and SHV families in 448 ESBL-producing clinical isolates of Enterobacteriaceae collected from 10 different Italian hospitals. The natures of TEM and SHV determinants were identified by direct sequencing of PCR-amplified genes. TEM-52 and SHV-12 were the most common variants, and they were found in most hospitals and in several different species. Other less frequent variants included TEM-5, TEM-12, TEM-15, TEM-19, TEM-20, TEM-24, TEM-26, TEM-43, TEM-60, TEM-72, TEM-87, SHV-2a, SHV-5, and SHV-11. *Proteus mirabilis* was the most common producer of TEM-type ESBLs, while *Klebsiella pneumoniae* was the most common producer of SHV-type ESBLs. The distribution of TEM- and SHV-type ESBL variants in Enterobacteriaceae from Italian hospitals exhibited notable differences from those from other geographical settings.

Pillay, A., J. Lewis, et al. (2004). "Evaluation of Xenostrip-Tv, a Rapid Diagnostic Test for *Trichomonas vaginalis* Infection." *J. Clin. Microbiol.* **42**(8): 3853-3856.

<http://jcm.asm.org/cgi/content/abstract/42/8/3853>

An immunochromatographic strip test, Xenostrip-Tv, was compared to wet mount and PCR for the diagnosis of *Trichomonas vaginalis* infection in women. Of 428 specimens tested, 54 (12.6%) were positive by an "expanded gold standard," defined as either a positive wet mount and PCR test with primers TVK3 and TVK7 and/or a positive PCR test confirmed by a second PCR assay with primers TVA5-1 and TVA6; 26 (6%) were positive by wet mount, and 36 (8.4%) were positive by Xenostrip-Tv test. Since the Xenostrip-Tv test is rapid and easy to perform and proved to be more sensitive than wet mount, it should be considered as an alternative to wet mount for point-of-care diagnosis of trichomoniasis, especially in settings where microscopy is impractical.

Plantier, J.-C., L. Vergne, et al. (2002). "Development and Evaluation of a DNA Enzyme Immunoassay Method for env Genotyping of Subtypes A through G of Human Immunodeficiency Virus Type 1 Group M, with Discrimination of the Circulating Recombinant Forms CRF01_AE and CRF02_AG." *J. Clin. Microbiol.* **40**(3): 1010-1022.

<http://jcm.asm.org/cgi/content/abstract/40/3/1010>

The tools currently available for genetic subtyping of human immunodeficiency virus type 1 are laborious or can be used only for the analysis of a limited number of samples and/or subtypes. We developed and evaluated a molecular biology-based method using subtype-specific oligonucleotide probes for env genotyping of subtypes A through G, CRF01_AE, and CRF02_AG. DNA enzyme immunoassay (DEIA) genotyping is based on nested PCR amplification of the 5' end of the env gene (proviral DNA), followed by subtype-specific hybridization and immunoenzymatic detection on microplates. DEIA genotyping was validated with a large number of samples (n = 128) collected in Europe (France; n = 47), West-Central Africa (Cameroon; n = 36), and West Africa (Senegal; n = 45). Three different formats, depending on the distribution of subtypes in the three countries, were developed. The results were compared with those obtained by sequencing of the V3-V5 region and phylogenetic analysis or an env heteroduplex mobility assay. Additional sequencing and phylogenetic analyses of the DEIA region (the first codon of the env coding sequence to the middle of conserved region C1 of gp120) were performed to investigate the reasons for discrepancies. Intense and highly specific reactions between the oligonucleotide probes and the corresponding samples were observed. Overall, correct identification was achieved for 107 of 128 samples (83.6%). One sample was not amplified, 10 (8%) were nontypeable (NT), and 10 (8%) were misidentified. Six of the 10 discordant samples were further investigated by phylogenetic analysis, which indicated that these samples

corresponded to recombinants involving the env 5' end and the V3 and V5 regions of the two parental clades. Sequencing of NT samples showed numerous differences between sample and probe sequences, resulting in a lack of hybridization, and revealed the limitations of the selected probes in terms of specificity and sensitivity. We demonstrated the feasibility of DEIA genotyping: six subtypes plus the two most prevalent circulating recombinant forms were discriminated by using the 5' end of the env gene. This method can be adapted to the local situation by including only probes that correspond to the prevalent strains.

Pottumarthy, S., A. P. Limaye, et al. (2003). "Nocardia veterana, a New Emerging Pathogen." J. Clin. Microbiol. **41**(4): 1705-1709.

<http://jcm.asm.org/cgi/content/abstract/41/4/1705>

Nocardia veterana is a newly described species named after the veteran's hospital where it was first isolated. This initial type strain was not thought to be clinically significant. We describe three cases of pulmonary disease attributable to *N. veterana*: two cases in patients presenting with multiple pulmonary nodules in a setting of immunocompromise and one case of exacerbation of chronic pulmonary disease. The isolates were susceptible to ampicillin, imipenem, gentamicin, amikacin, and trimethoprim-sulfamethoxazole and had reduced susceptibilities to ceftriaxone, cefotaxime, minocycline, and ciprofloxacin. The MICs of amoxicillin-clavulanate were higher than that of ampicillin alone, and the bacteria produced a β -lactamase detectable only after induction with clavulanic acid. Phenotypically, the isolates could not be characterized beyond the *Nocardia* genus level. All three isolates were definitively identified as *N. veterana* by PCR and sequencing of the 16S rRNA gene. On the basis of their susceptibility and restriction enzyme analysis profiles, our findings indicate that they could potentially be misidentified as *N. nova*. These cases illustrate the pathogenic potential of this newly described species and emphasize the importance of accurate identification of *Nocardia* isolates to the species level by integrated use of phenotypic and genotypic methods.

Pounder, J. I., S. Williams, et al. (2005). "Repetitive-Sequence-PCR-Based DNA Fingerprinting Using the DiversiLab System for Identification of Commonly Encountered Dermatophytes." J. Clin. Microbiol. **43**(5): 2141-2147.

<http://jcm.asm.org/cgi/content/abstract/43/5/2141>

The performance of repetitive-sequence-based PCR (rep-PCR) using the DiversiLab system for identification of dermatophytes commonly isolated in a clinical laboratory was assessed by comparing results to those of conventional tests (colony morphology, microscopic examination of slide cultures, and, for suspected *Trichophyton* species, use of additional media). Sixty-one cultures were tested in phase 1, the feasibility portion of the study; 64 additional cultures were tested in phase 2, the validation portion conducted to assess reproducibility and confirm accuracy. Discrepancies were resolved by repeating rep-PCR and conventional tests and, in phase 2, sequencing the internal transcribed spacers. After initial testing of the cultures in phase 1 (excluding one contaminated culture), agreement between conventional tests and rep-PCR was 90% (54 of 60). Agreement was 98.3% after resolution of discrepancies, and in all but one case the initial rep-PCR result was correct. After initial testing of cultures in phase 2 (excluding one discarded and one contaminated culture), agreement between rep-PCR and conventional testing was 88.7% (55 of 62). After discrepancies were resolved, agreement was 100%. Initial rep-PCR results were correct, except for one *Microsporum canis* culture containing two colony variants, which could not be initially identified by rep-PCR. The performance of the DiversiLab system for identification of the dermatophytes commonly encountered in a clinical mycology laboratory--*Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton tonsurans*, and *M. canis*--was

excellent. Moreover, the DiversiLab system is technically simple and provides results in <24 h once a pure culture is available for testing, which is considerably more rapid than conventional identification tests.

Prager, R., W. Rabsch, et al. (2003). "Molecular Properties of Salmonella enterica Serotype Paratyphi B Distinguish between Its Systemic and Its Enteric Pathovars." J. Clin. Microbiol. **41**(9): 4270-4278.

<http://jcm.asm.org/cgi/content/abstract/41/9/4270>

Salmonella enterica serotype O1,4,5,12:Hb:1,2, designated according to the current Kauffmann-White scheme as S. enterica serotype Paratyphi B, is a very diverse serotype with respect to its clinical and microbiological properties. PCR and blot techniques, which identify the presence, polymorphism, and expression of various effector protein genes, help to distinguish between strains with systemic and enteric outcomes of disease. All serotype Paratyphi B strains from systemic infections have been found to be somewhat genetically related with respect to the pattern of their virulence genes sopB, sopD, sopE1, avrA, and sptP as well as other molecular properties (multilocus enzyme electrophoresis type, pulsed-field gel electrophoresis [PFGE] type, ribotype, and IS200 type). They have been classified as members of the systemic pathovar (SPV). All these SPV strains possess a new sopE1-carrying bacteriophage (designated {Phi}SopE309) with high SopE1 protein expression but lack the commonly occurring avrA determinant. They exhibit normal SopB protein expression but lack SopD protein production. In contrast, strains from enteric infections classified as belonging to the enteric pathovar possess various combinations of the respective virulence genes, PFGE pattern, and ribotypes. We propose that the PCR technique for testing for the presence of the virulence genes sopE1 and avrA be used as a diagnostic tool for identifying both pathovars of S. enterica serotype Paratyphi B. This will be of great public health importance, since strains of serotype Paratyphi B have recently reemerged worldwide.

Pratelli, A., N. Decaro, et al. (2004). "Two Genotypes of Canine Coronavirus Simultaneously Detected in the Fecal Samples of Dogs with Diarrhea." J. Clin. Microbiol. **42**(4): 1797-1799.

<http://jcm.asm.org/cgi/content/abstract/42/4/1797>

Sixty-nine fecal samples from diarrheic puppies were examined by reverse transcription-PCR assays for the M and the S genes of canine coronaviruses (CCoVs). The isolates in 10 samples were recognized as CCoV type I, and the isolates in 6 samples were recognized as CCoV type II, while isolates of both genotypes were simultaneously detected in 53 samples.

Priestnall, S. L., B. Wiinberg, et al. (2004). "Evaluation of "Helicobacter heilmannii" Subtypes in the Gastric Mucosas of Cats and Dogs." J. Clin. Microbiol. **42**(5): 2144-2151.

<http://jcm.asm.org/cgi/content/abstract/42/5/2144>

Infection with candidate "Helicobacter heilmannii" is associated with gastritis and mucosa-associated lymphoid tissue lymphoma in people. Infection with "H. heilmannii" type 1 predominates (80%) and is thought to be acquired from dogs, cats, or pigs. We further examined the zoonotic potential of dogs and cats by amplifying gastric DNA from cats (n = 45) and dogs (n = 10) with primers against "H. heilmannii" ureB and 16S rRNA genes and sequencing the products. Fluorescence in situ hybridization (FISH) with eubacterial and "H. heilmannii"-specific

probes was employed to directly visualize "H. heilmannii" types and their intragastric distribution. ureB sequences of "H. heilmannii" amplicons clustered with human and feline isolates of "H. heilmannii" and were distinct from the "H. heilmannii"-like organisms (HHLO) *H. felis*, *H. salomonis*, and *H. bizzozzeronii*. 16S ribosomal DNA sequences in 20 "H. heilmannii"-infected cats and dogs were distinct from "H. heilmannii" type 1 and "H. suis" and clustered with "H. heilmannii" types 2 and 4. FISH confirmed the presence of "H. heilmannii" types 2 and 4 in dogs but failed to definitively characterize the "H. heilmannii" types present in cats. In infected dogs, "H. heilmannii" inhabited the gastric mucus and glands, and in dogs coinfecting with other HHLO it shared the same gastric niche. The results indicate that dogs and cats are predominantly colonized by "H. heilmannii" bacteria that are distinct from type 1 and from "H. suis." As "H. heilmannii" type 1 predominates in people, the zoonotic risk posed by dogs and cats is likely small.

Probert, W. S., K. N. Schrader, et al. (2004). "Real-Time Multiplex PCR Assay for Detection of *Brucella* spp., *B. abortus*, and *B. melitensis*." J. Clin. Microbiol. **42**(3): 1290-1293.

<http://jcm.asm.org/cgi/content/abstract/42/3/1290>

The identification of *Brucella* can be a time-consuming and labor-intensive process that places personnel at risk for laboratory-acquired infection. Here, we describe a real-time PCR assay for confirmation of presumptive *Brucella* isolates. The assay was designed in a multiplex format that will allow the rapid identification of *Brucella* spp., *B. abortus*, and *B. melitensis* in a single test.

Ratcliff, R. M., J. C. Doherty, et al. (2002). "Sensitive Detection of RNA Viruses Associated with Gastroenteritis by a Hanging-Drop Single-Tube Nested Reverse Transcription-PCR Method." J. Clin. Microbiol. **40**(11): 4091-4099.

<http://jcm.asm.org/cgi/content/abstract/40/11/4091>

The detection of the human RNA viruses, calicivirus and astrovirus, requires high sensitivity and broad reactivity. A novel single-tube nested reverse transcription-PCR (RT-PCR) method is described here, in which all of the required reagents are included in the one tube; however, those required for the nested amplification are separated in a "hanging drop" in the cap to be introduced by centrifugation after the RT and first-round cDNA amplification steps. Broad reactivity was obtained by using primer cocktails covering the published sequence variation in the primer targets. The method was evaluated with clinical fecal samples from outbreak and sporadic cases. Norwalk-like virus types 1 and 2 and rotavirus were the causal agents in 10 of 12 outbreaks. A viral agent was detected in 44% of 197 samples from sporadic infections in patients presenting to community health centers and a children's hospital. Interestingly, whereas rotavirus was more common than astrovirus in patients presenting to the hospital (33 and 7.6%, respectively), the reverse was true for patients presenting to community health centers (4.2 and 34%, respectively).

Rose, C., M. Green, et al. (2002). "Detection of Epstein-Barr Virus Genomes in Peripheral Blood B Cells from Solid-Organ Transplant Recipients by Fluorescence In Situ Hybridization." J. Clin. Microbiol. **40**(7): 2533-2544.

<http://jcm.asm.org/cgi/content/abstract/40/7/2533>

Resolution of Epstein-Barr Virus (EBV) infection in pediatric solid-organ transplant recipients often leads to an asymptomatic carrier state characterized by a persistently elevated circulating

EBV load that is 2 to 4 orders of magnitude greater than the load typical of healthy latently infected individuals. Elevated EBV loads in immunosuppressed individuals are associated with an increased risk for development of posttransplant lymphoproliferative disease. We have performed fluorescence in situ hybridization (FISH) studies with peripheral blood B cells from carriers of persistent EBV loads in order to directly quantitate the number of EBV genomes per infected cell. Patients were assigned to two groups on the basis of the level of the persistent load (low-load carriers, 8 to 200 genomes/10⁵ peripheral blood lymphocytes; high-load carriers, >200 genomes/10⁵ peripheral blood lymphocytes). FISH analysis revealed that the low-load carriers predominantly had circulating virus-infected cells harboring one or two genome copies/cell. High-load carriers also had cells harboring one or two genome copies/cell; in addition, however, they carried a distinct population of cells with high numbers of viral genome copies. The increased viral loads correlated with an increase in the frequency of cells containing high numbers of viral genomes. We conclude that low-load carriers possess EBV-infected cells that are in a state similar to normal latency, whereas high-load carriers possess two populations of virus-positive B cells, one of which carries an increased number of viral genomes per cell and is not typical of normal latency.

Sako, Y., M. Nakao, et al. (2002). "Alveolar Echinococcosis: Characterization of Diagnostic Antigen Em18 and Serological Evaluation of Recombinant Em18." *J. Clin. Microbiol.* **40**(8): 2760-2765.

<http://jcm.asm.org/cgi/content/abstract/40/8/2760>

The *Echinococcus multilocularis* protein Em18 is one of the most promising antigens for use in serodiagnosis of alveolar echinococcosis in human patients. Here we identify an antigenic relationship between Em18 and a 65-kDa immunodominant *E. multilocularis* surface protein previously identified as either EM10 or EmII/3. The NH₂-terminal sequence of native Em18 was determined, revealing it to be a fragment of EM10. Experiments were undertaken to investigate the effect of proteinase inhibitors on the degradation of EM10 in crude extracts of *E. multilocularis* protoscoleces. Em18 was found to be the product of degradation of EM10 by cysteine proteinase. A recombinant Em18 (RecEm18, derived from 349K to 508K of EM10) was successfully expressed by using *Escherichia coli* expression system and then evaluated for use in serodiagnosis of alveolar echinococcosis. RecEm18 was recognized by 27 (87.1%) and 28 (90.3%) of 31 serum samples from clinically and/or pathologically confirmed alveolar echinococcosis patients by enzyme-linked immunosorbent assay and immunoblotting, respectively. Of 33 serum samples from cystic echinococcosis patients, 1 was recorded as having a weak positive reaction to RecEm18; however, none of the serum samples which were tested from neurocysticercosis patients (n = 10) or healthy people (n = 15) showed positive reactions. RecEm18 has the potential for use in the differential serodiagnosis of alveolar echinococcosis.

Sanchez, J. L. and G. A. Storch (2002). "Multiplex, Quantitative, Real-Time PCR Assay for Cytomegalovirus and Human DNA." *J. Clin. Microbiol.* **40**(7): 2381-2386.

<http://jcm.asm.org/cgi/content/abstract/40/7/2381>

We created a multiplex, quantitative, real-time PCR assay that amplifies cytomegalovirus (CMV) and human DNA in the same reaction tube, allowing for a viral load determination that is normalized to measured human DNA. The assay targets a conserved region of the CMV DNA polymerase gene that is not affected by known drug resistance mutations. All 36 strains of CMV detected by culture or qualitative PCR in a population of lung transplant recipients were detected. The assay detected 1 to 10 copies of CMV plasmid DNA. The analytic sensitivity was not affected by the presence of DNA from 10⁶ human cells but was reduced approximately 10-fold by alkaline lysates of leukocyte preparations. CMV quantitation was linear over a range of 10¹ to 10⁶ copies.

The intraassay and interassay coefficients of variation were 29 and 40%. Human DNA was regularly detected in patient plasma samples, and the amount was increased by storage of blood at room temperature before plasma separation and by plasma separation techniques that allowed leukocyte contamination. Applied to whole blood, the assay provides a measurement of CMV DNA in relation to cellular content without a need for cell counting procedures. Applied to plasma, the assay can reveal artifactual increases in plasma CMV levels resulting from leukocyte contamination. Further study of the utility of this assay to monitor patient populations at risk for CMV disease is warranted.

Schauer, E., S. Webber, et al. (2004). "Surface Immunoglobulin-Deficient Epstein-Barr Virus-Infected B Cells in the Peripheral Blood of Pediatric Solid-Organ Transplant Recipients." J. Clin. Microbiol. **42**(12): 5802-5810.

<http://jcm.asm.org/cgi/content/abstract/42/12/5802>

Epstein-Barr virus (EBV), a ubiquitous human herpesvirus, normally causes an asymptomatic latent infection with very low levels of circulating virus in the peripheral blood of infected individuals. However, EBV does have pathogenic potential and has been linked to several diseases, including posttransplant lymphoproliferative disease (PTLD), which involves very high circulating viral loads. As a consequence of immunosuppression associated with transplantation, children in particular are at risk for PTLD. Even in the absence of symptoms of PTLD, very high viral loads are often observed in these patients. EBV-infected B cells in the circulations of 16 asymptomatic pediatric solid-organ transplant recipients from Children's Hospital of Pittsburgh were simultaneously characterized for their surface immunoglobulin (slg) isotypes and EBV genome copy numbers. Patients were characterized as having high and low viral loads on the basis of their stable levels of circulating virus. Patients with high viral loads had both high- and low-copy-number cells. Cells with a high numbers of viral episomes (>20/cell) were predominantly Ig null, and cells with low numbers of episomes were predominantly slgM positive. Patients with low viral loads carried the vast majority of their viral load in low-copy-number cells, which were predominantly IgM positive. The very rare high-copy-number cells detected in carriers with low viral loads were also predominantly Ig-null cells. This suggests that two distinct types of B-lineage cells contribute to the viral load in transplant recipients, with cells bearing high genome copy numbers having an aberrant Ig-null cellular phenotype.

Schlicht, M. J., S. D. Lovrich, et al. (2004). "High Prevalence of Genital Mycoplasmas among Sexually Active Young Adults with Urethritis or Cervicitis Symptoms in La Crosse, Wisconsin." J. Clin. Microbiol. **42**(10): 4636-4640.

<http://jcm.asm.org/cgi/content/abstract/42/10/4636>

Sexually active young adults in the small college town of La Crosse, Wisconsin, were evaluated for conventional sexually transmitted pathogens and tested for infections with mycoplasmas. The prevalence in 65 symptomatic men or women and 137 healthy volunteers (67 men and 70 women) was compared. Urine specimens from both cohorts were tested by ligase chain reaction for Chlamydia trachomatis or Neisseria gonorrhoeae. In addition, the urethral or cervical swabs from the symptomatic subjects were tested by PCR for Mycoplasma genitalium and cultured for Mycoplasma hominis and the ureaplasmas. The results confirmed a relatively low prevalence of gonorrhea among symptomatic men (12%) and chlamydia among symptomatic men (15%) and normal women (3%). In contrast, infections with mycoplasmas, especially the ureaplasmas (57%), were common and the organisms were the only potential sexually transmitted pathogen detected in 40 (62%) symptomatic subjects. Because of the high prevalence, we also evaluated urethral swabs from an additional 25 normal female volunteers and recovered ureaplasmas from

4 (16%) subjects. Additionally, the participants rarely used protection during sexual intercourse and some symptomatic subjects apparently acquired their infections despite using condoms regularly. The findings demonstrate a strong association between abnormal urogenital findings and detection of myoplasmas, particularly ureaplasmas, and suggest the infections will remain common.

Schulz, A., K. Mellenthin, et al. (2003). "Detection, Differentiation, and Quantitation of Pathogenic Leishmania Organisms by a Fluorescence Resonance Energy Transfer-Based Real-Time PCR Assay." *J. Clin. Microbiol.* **41**(4): 1529-1535.

<http://jcm.asm.org/cgi/content/abstract/41/4/1529>

Real-time technology eliminates many of the pitfalls of diagnostic PCR, but this method has not been applied to differentiation of Leishmania organisms so far. We have developed a real-time PCR that simultaneously detects, quantitates, and categorizes Leishmania organisms into three relevant groups causing distinct clinical pictures. The analytical sensitivity (detection rate of $\geq 95\%$ at 94.1 parasites/ml of blood) was within a range that has been determined previously to facilitate the confirmation of visceral leishmaniasis from peripheral blood. Parasites were successfully detected in 12 different clinical samples (blood, bone marrow, skin, and liver). The Leishmania donovani complex, the Leishmania brasiliensis complex, and species other than these could be clearly discriminated by means of distinct melting temperatures obtained with fluorescence resonance energy transfer probes (melting points, 72.7, 67.1, and 65.0°C, respectively). All three groups could be quantified within equal ranges. As in other real-time PCRs, the variability in the quantification of DNA was small (coefficient of variation [CV], <2%). However, human samples containing low levels of parasites (100 parasites per ml of blood) showed higher variation (CV, 60.89%). Therefore, despite its superior analytical performance, care must be taken when real-time PCR is utilized for therapy monitoring.

Schuurman, T., R. F. de Boer, et al. (2004). "Prospective Study of Use of PCR Amplification and Sequencing of 16S Ribosomal DNA from Cerebrospinal Fluid for Diagnosis of Bacterial Meningitis in a Clinical Setting." *J. Clin. Microbiol.* **42**(2): 734-740.

<http://jcm.asm.org/cgi/content/abstract/42/2/734>

We have evaluated the use of a broad-range PCR aimed at the 16S rRNA gene in detecting bacterial meningitis in a clinical setting. To achieve a uniform DNA extraction procedure for both gram-positive and gram-negative organisms, a combination of physical disruption (bead beating) and a silica-guanidiniumthiocyanate procedure was used for nucleic acid preparation. To diminish the risk of contamination as much as possible, we chose to amplify almost the entire 16S rRNA gene. The analytical sensitivity of the assay was approximately 1×10^2 to 2×10^2 CFU/ml of cerebrospinal fluid (CSF) for both gram-negative and gram-positive bacteria. In a prospective study of 227 CSF samples, broad-range PCR proved to be superior to conventional methods in detecting bacterial meningitis when antimicrobial therapy had already started. Overall, our assay showed a sensitivity of 86%, a specificity of 97%, a positive predictive value of 80%, and a negative predictive value of 98% compared to culture. We are currently adapting the standard procedures in our laboratory for detecting bacterial meningitis; broad-range 16S ribosomal DNA PCR detection is indicated when antimicrobial therapy has already started at time of lumbar puncture or when cultures remain negative, although the suspicion of bacterial meningitis remains.

Shopsin, B., B. Mathema, et al. (2003). "Prevalence of agr Specificity Groups among Staphylococcus aureus Strains Colonizing Children and Their Guardians." J. Clin. Microbiol. **41**(1): 456-459.

<http://jcm.asm.org/cgi/content/abstract/41/1/456>

PCR-based assays were used to evaluate agr locus nucleotide polymorphism for the identification of agr autoinducer receptor specificity groups within a population of Staphylococcus aureus isolates colonizing children and their guardians. All isolates could be assigned to one of three major agr groups that had similar prevalences, regardless of whether isolates were implicated in transmission of S. aureus within families. Among healthy carriers, agr groups I to III appear to be equally fit, which may reflect selection for the coexistence of S. aureus strains in a population.

Shukla, S. K., K. A. Bernard, et al. (2003). "Corynebacterium nigricans sp. nov.: Proposed Name for a Black-Pigmented Corynebacterium Species Recovered from the Human Female Urogenital Tract." J. Clin. Microbiol. **41**(9): 4353-4358.

<http://jcm.asm.org/cgi/content/abstract/41/9/4353>

Six independent isolates of an unusual black-pigmented Corynebacterium species (strains CN-1, CN-2, CN-3415, W70124, 91-0032, and 92-0360) were recovered from the human female urogenital tract. Four of the six source patients had complications of pregnancy, including spontaneous abortion, preterm labor, and low amniotic fluid volume at the time of the pathogen isolation. One isolate was recovered from a vaginal ulcer. All six strains yielded black-pigmented colonies on sheep blood agar, chocolate agar, and colistin-nalidixic acid agar after 24 to 48 h of incubation at 35°C. The dry, adherent colonies pitted the agar surface. The cells were coccobacillary to rod-shaped, catalase positive, nonmotile, and nonlipophilic. Only five of six isolates were available for characterization. Biochemical and chemotaxonomic studies revealed that the strains belong to the genus Corynebacterium but differ from known corynebacterial species. Comparative 16S rRNA gene sequence analysis showed that the strains are closely related and form a new subline within the genus Corynebacterium. We propose the name Corynebacterium nigricans sp. nov. for this group of coryneforms. The type strain of Corynebacterium nigricans is CN-1. It is deposited in the American Type Culture Collection (assigned strain number ATCC 700975) and in the Institute Pasteur collection (assigned strain number CIP 107346).

Shutt, C. K., J. I. Pounder, et al. (2005). "Clinical Evaluation of the DiversiLab Microbial Typing System Using Repetitive-Sequence-Based PCR for Characterization of Staphylococcus aureus Strains." J. Clin. Microbiol. **43**(3): 1187-1192.

<http://jcm.asm.org/cgi/content/abstract/43/3/1187>

The DiversiLab System, which includes microfluidics-based detection, reagent kits, and software for data processing and analysis, is an automated method using repetitive sequence-based PCR (rep-PCR) for microbial strain typing. To assess the reliability of the DiversiLab System for strain characterization of Staphylococcus aureus, we tested clinical isolates sent to ARUP Laboratories for typing and compared results to those of pulsed field electrophoresis (PFGE) aided by the cluster analysis provided by BioNumerics software. spa typing was performed when the results of these two methods for an outbreak were not concordant. The study included 89 S. aureus isolates (65 mecA positive, 24 mecA negative) from 19 outbreaks (2 to 11 isolates/outbreak). The DiversiLab and PFGE-BioNumerics results were concordant for 15 of the 19 outbreaks. For the

remaining four outbreaks, there was partial concordance between the two methods. spa typing results were the same as or more similar to rep-PCR results for three of those outbreaks and were more similar to PFGE results for one. With regard to performance, the DiversiLab system was considerably less labor intensive than PFGE and provided results in less than 24 h, compared with 2 to 3 days for PFGE. Additionally, the Web-based DiversiLab software provides standardized comparisons among isolates almost instantaneously and generates user-friendly, customized reports.

Simjee, S., D. G. White, et al. (2002). "Characterization of Tn1546 in Vancomycin-Resistant *Enterococcus faecium* Isolated from Canine Urinary Tract Infections: Evidence of Gene Exchange between Human and Animal Enterococci." *J. Clin. Microbiol.* **40**(12): 4659-4665.

<http://jcm.asm.org/cgi/content/abstract/40/12/4659>

Thirty-five enterococcal isolates were recovered from dogs diagnosed with urinary tract infections at the Michigan State University Veterinary Teaching Hospital over a 2-year period (1996 to 1998). Isolated species included *Enterococcus faecium* (n = 13), *Enterococcus faecalis* (n = 7), *Enterococcus gallinarum* (n = 11), and *Enterococcus casseliflavus* (n = 4). Antimicrobial susceptibility testing revealed several different resistance phenotypes, with the majority of the enterococcal isolates exhibiting resistance to three or more antibiotics. One *E. faecium* isolate, CVM1869, displayed high-level resistance to vancomycin (MIC > 32 {micro}g/ml) and gentamicin (MIC > 2,048 {micro}g/ml). Molecular analysis of this isolate revealed the presence of Tn1546 (vanA), responsible for high-level vancomycin resistance, and Tn5281 carrying aac6'-aph2", conferring high-level aminoglycoside resistance. Pulsed-field gel electrophoresis analysis revealed that CVM1869 was a canine *E. faecium* clone that had acquired Tn1546, perhaps from a human vancomycin-resistant *E. faecium*. Transposons Tn5281 and Tn1546 were located on two different conjugative plasmids. Sequence analysis revealed that in Tn1546, ORF1 had an 889-bp deletion and an IS1216V insertion at the 5' end and an IS1251 insertion between vanS and vanH. To date, this particular form of Tn1546 has only been described in human clinical vancomycin-resistant enterococcus isolates unique to the United States. Additionally, this is the first report of a vancomycin-resistant *E. faecium* isolated from a companion animal in the United States.

Singh, S., V. T. K. Chow, et al. (2002). "Direct Detection of Enterovirus 71 (EV71) in Clinical Specimens from a Hand, Foot, and Mouth Disease Outbreak in Singapore by Reverse Transcription-PCR with Universal Enterovirus and EV71-Specific Primers." *J. Clin. Microbiol.* **40**(8): 2823-2827.

<http://jcm.asm.org/cgi/content/abstract/40/8/2823>

A recent outbreak of hand, foot, and mouth disease in Singapore in 2000 affected several thousand children and resulted in four deaths. The aim of this study was to determine the applicability of reverse transcription-PCR (RT-PCR) with universal pan-enterovirus primers and enterovirus 71 (EV71) type-specific primers for the direct detection of enteroviruses in clinical specimens derived from this outbreak. With the universal primers, EV71 RNA sequences were successfully detected by RT-PCR and direct sequencing in 71% of positive specimens. Three pairs of EV71 type-specific primers were evaluated for rapid detection of EV71 directly from clinical specimens and cell culture isolates. By using a seminested RT-PCR strategy, specific identification of EV71 sequences directly in clinical specimens was achieved, with a detection rate of 53%. In contrast, cell culture could isolate EV71 in only 20% of positive specimens. EV71 was detected directly from brain, heart, and lung specimens of two deceased siblings. Although more than one type of enterovirus was identified in clinical specimens from this outbreak, 90% of the enteroviruses were confirmed as EV71. The data demonstrate the clinical applicability of pan-

enterovirus and seminested RT-PCR for the detection of EV71 RNA directly from clinical specimens in an outbreak situation.

Sinha, S., R. Chakraborty, et al. (2002). "Escalating Association of *Vibrio cholerae* O139 with Cholera Outbreaks in India." J. Clin. Microbiol. **40**(7): 2635-2637.

<http://jcm.asm.org/cgi/content/abstract/40/7/2635>

Between December 1999 and December 2000, teams from the National Institute of Cholera and Enteric Diseases, Calcutta, India, examined eight outbreaks of cholera, which occurred in different parts of the country distant from each other. In two of these outbreaks each, only *V. cholerae* O1 biotype El Tor or *V. cholerae* O139 could be isolated, while in the remaining four outbreaks, both O1 and O139 were isolated. The interesting feature is the escalating association of *V. cholerae* O139 with outbreaks of cholera; two of the most recent outbreaks, one in Calcutta and one in Orissa, were caused exclusively by O139. The O139 strains from the six different outbreaks were genotypically closely related. These trends indicate a shift in the outbreak propensity of *V. cholerae* O139.

Smole, S. C., F. McAleese, et al. (2002). "Clinical and Epidemiological Correlates of Genotypes within the *Mycobacterium avium* Complex Defined by Restriction and Sequence Analysis of hsp65." J. Clin. Microbiol. **40**(9): 3374-3380.

<http://jcm.asm.org/cgi/content/abstract/40/9/3374>

Species identification of isolates of the *Mycobacterium avium* complex (MAC) remains a difficult task. Although *M. avium* and *Mycobacterium intracellulare* can be identified with expensive, commercially available probes, many MAC isolates remain unresolved, including those representing *Mycobacterium lentiflavum* as well as other potentially undefined species. PCR restriction analysis (PRA) of the hsp65 gene has been proposed as a rapid and inexpensive approach. We applied PRA to 278 MAC isolates, including 126 from blood of human immunodeficiency virus (HIV)-infected patients, 59 from sputum of HIV-negative patients with chronic obstructive pulmonary disease, 88 from environmental sources, and 5 pulmonary isolates from a different study. A total of 15 different PRA patterns were observed. For 27 representative isolates, a 441-bp fragment of the hsp65 gene was sequenced; based on 54 polymorphic sites, 18 different alleles were defined, including 12 alleles not previously reported. Species and phylogenetic relationships were more accurately defined by sequencing than by PRA or commercial probe. The distribution of PRA types and, by implication, phylogenetic lineages among blood isolates was significantly different from that for pulmonary and environmental isolates, suggesting that particular lineages have appreciably greater virulence and invasive potential.

Solnick, J. V., K. Chang, et al. (2003). "Natural Acquisition of *Helicobacter pylori* Infection in Newborn Rhesus Macaques." J. Clin. Microbiol. **41**(12): 5511-5516.

<http://jcm.asm.org/cgi/content/abstract/41/12/5511>

Helicobacter pylori infection is usually acquired in childhood, but precise estimates of the age of acquisition are difficult to obtain in young children. Since serial endoscopic biopsies are not feasible in human infants, we examined acquisition of *H. pylori* infection that is known to occur in

socially housed nonhuman primates. By 12 weeks of age, 8 of 20 newborns (40%) were culture positive for *H. pylori*, and prevalence reached 90% by 1 year of age. Newborns from infected dams were more commonly infected than those from uninfected dams, particularly during the peripartum period, suggesting that close contact during this time may facilitate transmission. Transient infection was uncommon and occurred only after the first positive culture. These results suggest that in a high-prevalence environment, persistent *H. pylori* infection may be acquired at an earlier age than was previously thought. Since clean, potable water was readily available, contamination of water supply is not essential for widespread infection at an early age in areas where hygiene is otherwise poor. Furthermore, breastfeeding seems to offer little protection, since newborn macaques breastfeed during the first year of life and typically are fully weaned only when another newborn arrives the following spring.

Sotlar, K., D. Diemer, et al. (2004). "Detection and Typing of Human Papillomavirus by E6 Nested Multiplex PCR." *J. Clin. Microbiol.* **42**(7): 3176-3184.

<http://jcm.asm.org/cgi/content/abstract/42/7/3176>

A nested multiplex PCR (NMPCR) assay that combines degenerate E6/E7 consensus primers and type-specific primers was evaluated for the detection and typing of human papillomavirus (HPV) genotypes 6/11, 16, 18, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 56, 58, 59, 66, and 68 using HPV DNA-containing plasmids and cervical scrapes (n = 1,525). The performance of the NMPCR assay relative to that of conventional PCR with MY09-MY11 and GP5+-GP6+ primers, and nested PCR with these two primer sets (MY/GP) was evaluated in 495 cervical scrapes with corresponding histologic and cytologic findings. HPV prevalence rates determined with the NMPCR assay were 34.7% (102 of 294) in the absence of cervical intraepithelial neoplasia (CIN 0), 94.2% (113 of 120) in the presence of mild or moderate dysplasia (CIN I/II), and 97.8% (44 of 45) in the presence of severe dysplasia (CIN III). The combination of all four HPV detection methods applied in the study was taken as "gold standard": in all three morphological subgroups the NMPCR assay had significantly ($P < 0.0001$) higher sensitivities than the MY09-MY11 and GP5+-GP6+ assays and sensitivities comparable or equal to those of the MY/GP assay. All 18 HPV genotypes investigated were detected among the clinical samples. The ratio of high- to low-risk HPV genotypes increased from 4:1 (80 of 103) in CIN 0 to 19:1 (149 of 157) in CIN I to III. Multiple infections were detected in 47.9% (124 of 259) of the patients. In conclusion, the novel NMPCR method is a sensitive and useful tool for HPV DNA detection, especially when exact HPV genotyping and the identification of multiple HPV infections are required.

Steininger, C., M. Kundi, et al. (2002). "Effectiveness of Reverse Transcription-PCR, Virus Isolation, and Enzyme-Linked Immunosorbent Assay for Diagnosis of Influenza A Virus Infection in Different Age Groups." *J. Clin. Microbiol.* **40**(6): 2051-2056.

<http://jcm.asm.org/cgi/content/abstract/40/6/2051>

The degrees of effectiveness of reverse transcription (RT)-PCR, virus isolation, and antigen enzyme-linked immunosorbent assay (ELISA) for the detection of influenza A virus were evaluated with nasopharyngeal swabs from 150 patients (1 week to 86 years old) with influenza A virus infection. RT-PCR had a sensitivity for influenza A virus in stock virus preparations 103 times higher than virus isolation and 106 to 107 times higher than ELISA. The detection rate achieved by RT-PCR in clinical samples was clearly higher (93%) than that by virus isolation (80%) and ELISA (62%). Despite low overall detection rates achieved by antigen ELISA, samples from patients younger than 5 years old yielded higher-than-average rates in this rapid assay (88%). The likelihood of negative results in the ELISA increased significantly with increasing age of the patient ($P < 0.01$). The degrees of effectiveness of RT-PCR and virus isolation were not

influenced by the age of the patient. Neither influenza immunizations nor the interval between onset of symptoms and laboratory investigation (mean, 4.7 days; standard deviation, 3.3 days) affected results obtained by the three test systems. Our results demonstrate that the ELISA is reliable for rapid laboratory diagnosis of influenza in infants and young children, but for older patients application of RT-PCR or virus isolation is necessary to avoid false negative results.

Stevens, M. P., S. N. Tabrizi, et al. (2004). "Characterization of *Chlamydia trachomatis* omp1 Genotypes Detected in Eye Swab Samples from Remote Australian Communities." J. Clin. Microbiol. **42**(6): 2501-2507.

<http://jcm.asm.org/cgi/content/abstract/42/6/2501>

Chlamydia trachomatis conjunctival samples collected over a 6-month period from individuals with clinical signs of trachoma and located in remote communities in the Australian Northern Territory were differentially characterized according to serovar and variants. The rationale was to gain an understanding of the epidemiology of an apparent increased prevalence of acute trachoma in areas thought to be less conducive to this disease. Characterization was performed through sequencing of a region of the omp1 gene spanning the four variable domains and encoding the major outer membrane protein. Nucleotide and deduced amino acid sequences were genotyped by using a BLAST similarity search and were examined by phylogenetic analyses to illustrate evolutionary relationships between the clinical and GenBank reference strains. The predominant genotype identified corresponded to that of serovar C (87.1%), followed by the genotype corresponding to serovar Ba (12.9%). All nucleotide and amino acid sequences exhibited minor levels of variation with respect to GenBank reference sequences. The omp1 nucleotide sequences of the clinical samples best aligned with those of the conjunctival *C. trachomatis* reference strains C/TW-3/OT and Ba/Apache-2. All clinical samples (of serovar C) exhibited four or five nucleotide changes compared with C/TW-3/OT, while all serovar Ba samples had one or two nucleotide differences from Ba/Apache-2. Phylogenetic analyses revealed close relationships between these Northern Territory chlamydial samples and the respective reference strains, although the high proportion of sequence variants suggests an evolutionarily distinct *C. trachomatis* population causing eye infections in Australia. Given that such genotypic information has gone unreported, these findings provide knowledge and a foundation for trachoma-associated *C. trachomatis* variants circulating in the Northern Territory.

Stevenson, J., W. Hymas, et al. (2005). "Effect of Sequence Polymorphisms on Performance of Two Real-Time PCR Assays for Detection of Herpes Simplex Virus." J. Clin. Microbiol. **43**(5): 2391-2398.

<http://jcm.asm.org/cgi/content/abstract/43/5/2391>

Herpes simplex virus (HSV) is the most common cause of acquired, sporadic encephalitis in the United States. PCR identification of HSV in spinal fluid has become the diagnostic gold standard due to its sensitivity and potential for speed, replacing other methods such as culture. We developed a real-time PCR assay to detect HSV, using a new type of hybridization probe, the Eclipse probe. In this study, we ran 323 samples (171 positives and 152 negatives) with the Eclipse real-time PCR assay and compared these results with another PCR assay using gel detection. The real-time assay agreed with our reference method for 319 out of the 323 samples tested (99%). Using two different real-time PCR platforms, we discovered that SNPs within the amplicon's probe binding region that are used to distinguish HSV-1 from HSV-2 can decrease assay sensitivity. This problem is potentially a general one for assays using fluorescent probes to detect target amplification in a real-time format. While real-time PCR can be a powerful tool in the field of infectious disease, careful sequence evaluation and clinical validation are essential in

creating an effective assay.

Stich, R. W., Y. Rikihisa, et al. (2002). "Detection of Ehrlichia canis in Canine Carrier Blood and in Individual Experimentally Infected Ticks with a p30-Based PCR Assay." J. Clin. Microbiol. **40**(2): 540-546.

<http://jcm.asm.org/cgi/content/abstract/40/2/540>

Detection of vector-borne pathogens is necessary for investigation of their association with vertebrate and invertebrate hosts. The ability to detect Ehrlichia spp. within individual experimentally infected ticks would be valuable for studies to evaluate the relative competence of different vector species and transmission scenarios. The purpose of this study was to develop a sensitive PCR assay based on oligonucleotide sequences from the unique Ehrlichia canis gene, p30, to facilitate studies that require monitoring this pathogen in canine and tick hosts during experimental transmission. Homologous sequences for Ehrlichia chaffeensis p28 were compared to sequences of primers derived from a sequence conserved among E. canis isolates. Criteria for primer selection included annealing scores, identity of the primers to homologous E. chaffeensis sequences, and the availability of similarly optimal primers that were nested within the target template sequence. The p30-based assay was at least 100-fold more sensitive than a previously reported nested 16S ribosomal DNA (rDNA)-based assay and did not amplify the 200-bp target amplicon from E. chaffeensis, the human granulocytic ehrlichiosis agent, or Ehrlichia muris DNA. The assay was used to detect E. canis in canine carrier blood and in experimentally infected Rhipicephalus sanguineus ticks. Optimized procedures for preparing tissues from these hosts for PCR assay are described. Our results indicated that this p30-based PCR assay will be useful for experimental investigations, that it has potential as a routine test, and that this approach to PCR assay design may be applicable to other pathogens that occur at low levels in affected hosts.

Streutker, C. J., C. N. Bernstein, et al. (2004). "Detection of Species-Specific Helicobacter Ribosomal DNA in Intestinal Biopsy Samples from a Population-Based Cohort of Patients with Ulcerative Colitis." J. Clin. Microbiol. **42**(2): 660-664.

<http://jcm.asm.org/cgi/content/abstract/42/2/660>

The inflammatory bowel diseases are considered an abnormal host immune response to an environmental stimulus. Evidence suggests a role for intestinal bacteria in initiating and/or providing an ongoing stimulus for inflammation in inflammatory bowel disease. Helicobacter pylori is the major cause of active chronic gastritis and peptic ulcers in humans and has been linked to gastric carcinoma and lymphoma. Studies in various animal models, particularly mice, have identified enterohepatic Helicobacter species that are capable of causing hepatitis and enterocolitis. We hypothesize that Helicobacter species may have a role in maintaining inflammation in humans with inflammatory bowel disease. In order to investigate this, biopsy specimens were obtained from patients with and without inflammatory bowel disease. DNA was extracted from the tissues and subjected to PCR with primers designed to detect the ribosomal DNA of members of the Helicobacter species. DNA from six biopsy samples from 60 inflammatory bowel disease patients tested positive. This included 5 of 33 ulcerative colitis patients that were positive compared to 0 of 29 age-matched controls ($P < 0.04$). Sequencing of the bands produced by PCR amplification revealed [IMG=" BORDER="0">99% homology with H. pylori. These results indicate that a member of the Helicobacter species may be involved in some cases of ulcerative colitis.

Suchodolski, J. S., C. G. Ruaux, et al. (2004). "Application of Molecular Fingerprinting for Qualitative Assessment of Small-Intestinal Bacterial Diversity in Dogs." J. Clin. Microbiol. **42**(10): 4702-4708.

<http://jcm.asm.org/cgi/content/abstract/42/10/4702>

The aims of this study were to evaluate the use of molecular fingerprinting for assessment of bacterial diversity in canine duodenal juice and to evaluate the variation in the small intestinal microflora at repeated sampling. Two groups of dogs were used. Duodenal juice was collected from eight dogs euthanized for an unrelated project (group 1). Duodenal juice was also collected endoscopically from six dogs at weekly intervals for a total of 3 weeks (group 2). The variable V6-V8 region of bacterial 16S ribosomal DNA was amplified, and PCR amplicons separated by denaturing gradient gel electrophoresis (DGGE). The reproducibility of DGGE profiles and variations in bacterial diversity between dogs were evaluated by comparing similarity indices (Dice's coefficient; 100% represents complete identity) of DGGE profiles from group 1 dogs. Weekly variations in the flora of the small intestine were evaluated by comparison of DGGE profiles from different time points within the same individuals in group 2. The mean ($\pm$ standard deviation) similarity of DGGE profiles of duodenal juice between the dogs in group 1 was 38.3 $\pm$ 15.7% (range, 12.5 to 76.65%). There was a significantly higher variation in DGGE profiles between different dogs than between duplicates obtained from the same dog ($P < 0.0001$). DGGE profiles from samples collected at different time points varied within individuals, possibly due to variation over time or slight variation in sampling location. DGGE profiles indicate that dogs have a highly diverse microflora of the small intestine, with marked differences between individual dogs.

Sugiura, W., K. Shimada, et al. (2003). "Novel Enzyme-Linked Minisequence Assay for Genotypic Analysis of Human Immunodeficiency Virus Type 1 Drug Resistance." J. Clin. Microbiol. **41**(11): 4971-4979.

<http://jcm.asm.org/cgi/content/abstract/41/11/4971>

We constructed a novel tool for genotypic analysis of human immunodeficiency virus type 1 (HIV-1) drug resistance by using an enzyme-linked minisequence assay (ELMA). ELMA is a combination of hybridization and a 1-base extension reaction, and we designed the assay to detect five mutations conferring nucleoside analogue resistance (M41L, D67N, K70R, T215Y, and M184V) and six mutations conferring protease inhibitor resistance (D30N, M46I, G48V, V82A, I84V, and L90M). At all detection points, ELMA demonstrated high sensitivity and specificity, sufficient for clinical use. Compared to that obtained by direct sequencing, the genotypic information obtained by ELMA is limited to the targeted loci for which it was designed. However, ELMA proves advantageous in several respects. The assay does not require expensive equipment, such as an autosequencer, and can be performed in regular clinical diagnostic laboratories. Therefore ELMA can be a candidate for a drug resistance monitoring assay to be introduced in developing countries. In addition, ELMA demonstrated higher sensitivity in the detection of minor resistant populations. We successfully detected a minor virus population (10%) by the assay. The high sensitivity and specificity of the assay recommend it as a first screening assay for drug resistance surveillance.

Sukhnanand, S., B. Dogan, et al. (2005). "Molecular Subtyping and Characterization of Bovine and Human *Streptococcus agalactiae* Isolates." J. Clin. Microbiol. **43**(3): 1177-1186.

<http://jcm.asm.org/cgi/content/abstract/43/3/1177>

Streptococcus agalactiae causes severe invasive disease in humans and mastitis in cattle. Temporally matched bovine milk isolates and clinical human invasive isolates (52 each) collected in New York State over 18 months were characterized by molecular subtyping and phenotypic methods to probe the interspecies transmission potential of this species. *EcoRI* ribotyping differentiated 17 ribotypes, and DNA sequencing of the housekeeping gene *sodA* and the putative virulence gene *hylB* differentiated 7 and 17 allelic types, respectively. Human and bovine isolates were not randomly distributed between ribotypes or *hylB* and *sodA* clusters. The combined analysis of all subtyping data allowed the differentiation of 39 clonal groups; 26 groups contained only bovine isolates, and 2 groups contained both human and bovine isolates. The *EcoRI* ribotype diversity among bovine isolates (Simpson's numerical index of discrimination [mean {+/-} standard deviation], 0.90 {+/-} 0.05) being significantly higher than that among human isolates (0.42 {+/-} 0.15) further supports that these isolates represent distinct populations. Eight human isolates, but no bovine isolates, showed an IS1548 transposon insertion in *hylB*, which encodes a hyaluronidase. Based on data for 43 representative isolates, human isolates, on average, showed lower hyaluronidase activities than bovine isolates. Isolates with the IS1548 insertion in *hylB* showed no hyaluronidase activity. Human and bovine isolates did not differ in their abilities to invade HeLa human epithelial cells. Our data show that (i) *EcoRI* ribotyping, combined with *hylB* and *sodA* sequencing, provides a discriminatory subtype analysis of *S. agalactiae*; (ii) most human invasive and bovine *S. agalactiae* isolates represent distinct subtypes, suggesting limited interspecies transmission; and (iii) hyaluronidase activity is not required for all human infections.

Sum, S. S.-M., D. K.-H. Wong, et al. (2004). "Real-Time PCR Assay Using Molecular Beacon for Quantitation of Hepatitis B Virus DNA." *J. Clin. Microbiol.* **42**(8): 3438-3440.

<http://jcm.asm.org/cgi/content/abstract/42/8/3438>

Levels of hepatitis B virus (HBV) DNA in the blood serve as an important marker in monitoring the disease progression and treatment efficacy of chronic HBV infection. Several commercial assays are available for accurate measurement of HBV genomic DNA, but many of them are hampered by relatively low sensitivity and limited dynamic range. The aim of this study was to develop a sensitive and accurate assay for measuring HBV genomic DNA using real-time PCR with a molecular beacon (HBV beacon assay). The performance of this assay was validated by testing serial dilutions of the two EUROHEP HBV DNA standards (ad and ay subtypes) of known concentrations. The assay showed low intra-assay (<7%) and interassay (<5%) variations for both subtypes. Its dynamic range was found to be 101 to 107 copies per reaction (1.0×10^2 to 1.0×10^9 copies ml⁻¹). The assay was further evaluated clinically using serum samples from 175 individuals with chronic hepatitis B. The HBV DNA level measured by this assay showed good correlation with that measured by the commercially available COBAS AMPLICOR HBV Monitor test ($r = 0.901$; $P < 0.001$). The higher sensitivity and broader dynamic range of this assay compared to the existing commercial assays will provide an ideal tool for monitoring disease progression and treatment efficacy in HBV-infected patients, in particular for those with low levels of HBV viremia.

Sun, Z. F., C. T. Larsen, et al. (2004). "Generation and Infectivity Titration of an Infectious Stock of Avian Hepatitis E Virus (HEV) in Chickens and Cross-Species Infection of Turkeys with Avian HEV." *J. Clin. Microbiol.* **42**(6): 2658-2662.

<http://jcm.asm.org/cgi/content/abstract/42/6/2658>

Avian hepatitis E virus (HEV), a novel virus identified from chickens with hepatitis-splenomegaly syndrome in the United States, is genetically and antigenically related to human HEV. In order to

further characterize avian HEV, an infectious viral stock with a known infectious titer must be generated, as HEV cannot be propagated in vitro. Bile and feces collected from specific-pathogen-free (SPF) chickens experimentally infected with avian HEV were used to prepare an avian HEV infectious stock as a 10% suspension of positive fecal and bile samples in phosphate-buffered saline. The infectivity titer of this infectious stock was determined by inoculating 1-week-old SPF chickens intravenously with 200 μ l of each of serial 10-fold dilutions (10⁻² to 10⁻⁶) of the avian HEV stock (two chickens were inoculated with each dilution). All chickens inoculated with the 10⁻² to 10⁻⁴ dilutions of the infectious stock and one of the two chickens inoculated with the 10⁻⁵ dilution, but neither of the chickens inoculated with the 10⁻⁶ dilution, became seropositive for anti-avian HEV antibody at 4 weeks postinoculation (wpi). Two serologically negative contact control chickens housed together with chickens inoculated with the 10⁻² dilution also seroconverted at 8 wpi. Viremia and shedding of virus in feces were variable in chickens inoculated with the 10⁻² to 10⁻⁵ dilutions but were not detectable in those inoculated with the 10⁻⁶ dilution. The infectivity titer of the infectious avian HEV stock was determined to be 5 x 10⁵ 50% chicken infectious doses (CID₅₀) per ml. Eight 1-week-old turkeys were intravenously inoculated with 10⁵ CID₅₀ of avian HEV, and another group of nine turkeys were not inoculated and were used as controls. The inoculated turkeys seroconverted at 4 to 8 wpi. In the inoculated turkeys, viremia was detected at 2 to 6 wpi and shedding of virus in feces was detected at 4 to 7 wpi. A serologically negative contact control turkey housed together with the inoculated ones also became infected through direct contact. This is the first demonstration of cross-species infection by avian HEV.

Thiem, V. D., O. Sethabutr, et al. (2004). "Detection of Shigella by a PCR Assay Targeting the ipaH Gene Suggests Increased Prevalence of Shigellosis in Nha Trang, Vietnam." J. Clin. Microbiol. **42**(5): 2031-2035.

<http://jcm.asm.org/cgi/content/abstract/42/5/2031>

Shigella spp. are exquisitely fastidious gram-negative organisms which frequently escape detection by traditional culture methods. To get a more complete understanding of the disease burden caused by Shigella in Nha Trang, Vietnam, real-time PCR was used to detect Shigella DNA. Randomly selected rectal swab specimens from 60 Shigella culture-positive patients and 500 Shigella culture-negative patients detected by population-based surveillance of patients seeking care for diarrhea were processed by real-time PCR. The target of the primer pair is the invasion plasmid antigen H gene sequence (ipaH), carried by all four Shigella species and enteroinvasive Escherichia coli. Shigella spp. could be isolated from the rectal swabs of 547 of 19,206 (3%) patients with diarrhea. IpaH was detected in 55 of 60 (93%) Shigella culture-positive specimens, whereas it was detected in 87 of 245 (36%) culture-negative patients free of dysentery ($P < 0.001$). The number of PCR cycles required to detect a PCR product was highest for culture-negative, nonbloody diarrheal specimens (mean number of cycles to detection, 36.6) and was lowest for children with culture-positive, bloody diarrheal specimens (mean number of cycles, 25.3) ($P < 0.001$). The data from real-time PCR amplification indicate that the culture-proven prevalence of Shigella among patients with diarrhea may underestimate the prevalence of Shigella infections. The clinical presentation of shigellosis may be directly related to the bacterial load.

Truman, R., A. B. Fontes, et al. (2004). "Genotypic Variation and Stability of Four Variable-Number Tandem Repeats and Their Suitability for Discriminating Strains of Mycobacterium leprae." J. Clin. Microbiol. **42**(6): 2558-2565.

<http://jcm.asm.org/cgi/content/abstract/42/6/2558>

It has not been possible to distinguish different strains of *Mycobacterium leprae* according to their genetic sequence. However, the genome contains several variable-number tandem repeats (VNTR), which have been used effectively in strain typing of other bacteria. To determine their suitability for differentiating *M. leprae*, we developed PCR systems to amplify 5 different VNTR loci and examined a battery of 12 *M. leprae* strains derived from patients in different regions of the United States, Brazil, Mexico, and the Philippines, as well as from wild armadillos and a sooty mangabey monkey. We found diversity at four VNTR ($D = 0.74$), but one system (C16G8) failed to yield reproducible results. Alleles for the GAA VNTR varied in length from 10 to 16 copies, those for AT17 varied in length from 10 to 15 copies, those for GTA varied in length from 9 to 12 copies, and those for TA18 varied in length from 13 to 20 copies. Relatively little variation was seen with interspecies transfer of bacilli or during short-term passage of strains in nude mice or armadillos. The TA18 locus was more polymorphic than other VNTR, and genotypic variation was more common after long-term expansion in armadillos. Most strain genotypes remained fairly stable in passage, but strain Thai-53 showed remarkable variability. Statistical cluster analysis segregated strains and passage samples appropriately but did not reveal any particular genotype associable with different regions or hosts of origin. VNTR polymorphisms can be used effectively to discriminate *M. leprae* strains. Inclusion of additional loci and other elements will likely lead to a robust typing system that can be used in community-based epidemiological studies and select clinical applications.

Ueti, M. W., G. H. Palmer, et al. (2003). "Expression of Equi Merozoite Antigen 2 during Development of *Babesia equi* in the Midgut and Salivary Gland of the Vector Tick *Boophilus microplus*." J. Clin. Microbiol. **41**(12): 5803-5809.

<http://jcm.asm.org/cgi/content/abstract/41/12/5803>

Equi merozoite antigens 1 and 2 (EMA-1 and EMA-2) are *Babesia equi* proteins expressed on the parasite surface during infection in horses and are orthologues of proteins in *Theileria* spp., which are also tick-transmitted protozoal pathogens. We determined in this study whether EMA-1 and EMA-2 were expressed within the vector tick *Boophilus microplus*. *B. equi* transitions through multiple, morphologically distinct stages, including sexual stages, and these transitions culminate in the formation of infectious sporozoites in the tick salivary gland. EMA-2-positive *B. equi* stages in the midgut lumen and midgut epithelial cells of *Boophilus microplus* nymphs were identified by reactivity with monoclonal antibody 36/253.21. This monoclonal antibody also recognized *B. equi* in salivary glands of adult *Boophilus microplus*. In addition, quantification of *B. equi* in the mammalian host and vector tick indicated that the duration of tick feeding and parasitemia levels affected the percentage of nymphs that contained morphologically distinct *B. equi* organisms in the midgut. In contrast, there was no conclusive evidence that *B. equi* EMA-1 was expressed in either the *Boophilus microplus* midgut or salivary gland when monoclonal antibody 36/18.57 was used. The expression of *B. equi* EMA-2 in *Boophilus microplus* provides a marker for detecting the various development stages and facilitates the identification of novel stage-specific *Babesia* proteins for testing transmission-blocking immunity.

Unemo, M., P. Olcen, et al. (2003). "Comparison of Serologic and Genetic *porB*-Based Typing of *Neisseria gonorrhoeae*: Consequences for Future Characterization." J. Clin. Microbiol. **41**(9): 4141-4147.

<http://jcm.asm.org/cgi/content/abstract/41/9/4141>

Due to temporal changes in the epidemiology of gonorrhea, a precise characterization of *Neisseria gonorrhoeae* is essential. In the present study genetic heterogeneity in the *porB* genes of *N. gonorrhoeae* was examined, and serovar determination was compared to *porB* gene

sequencing. Among 108 *N. gonorrhoeae* isolates, phylogenetic analysis of the entire *porB* alleles (924 to 993 bp) identified 87 unique sequences. By analyzing only the four to six most heterogeneous *porB* gene regions (174 to 363 bp), 86 out of these 87 genetic variants were identified. Consequently, analysis of shorter highly variable regions of the *porB* gene generates high-level discriminatory ability as well as fast, objective, reproducible, and portable data for epidemiological characterization of *N. gonorrhoeae*. Regarding putative antigenic epitopes of PorB for Genetic Systems monoclonal antibodies (MAbs), some of the previous findings were confirmed, but new findings were also observed. For several of the MAbs, however, the precise amino acid residues of PorB critical for single-MAb reactivity were difficult to identify. In addition, repeated serovar determination of 108 *N. gonorrhoeae* isolates revealed discrepancies for 34 isolates, mostly due to nonreproducible reactivity with single MAbs. Thus, the prospects of a genetic typing system with congruent translation of the serovar determination seem to be limited. In conclusion, analysis of short highly variable regions of the *porB* gene could form the basis for a fast molecular epidemiological tool for the examination of emergence and transmission of *N. gonorrhoeae* strains within the community.

Unemo, M., P. Olcen, et al. (2002). "Molecular Epidemiology of *Neisseria gonorrhoeae*: Sequence Analysis of the *porB* Gene Confirms Presence of Two Circulating Strains." J. Clin. Microbiol. **40**(10): 3741-3749.

<http://jcm.asm.org/cgi/content/abstract/40/10/3741>

The phenotypic and genotypic characteristics of *Neisseria gonorrhoeae* strains fluctuate over time both locally and globally, and highly discriminative and precise characterization of the strains is essential. Conventional characterization of *N. gonorrhoeae* strains for epidemiological purposes is mostly based on phenotypic methods, which have some inherent limitations. In the present study sequence analysis of *porB1b* gene sequences was used for examination of the genetic relationships among *N. gonorrhoeae* strains. Substantial genetic heterogeneity was identified in the *porB* genes of serovar IB-2 isolates (8.1% of the nucleotide sites were polymorphic) and serovar IB-3 isolates (5.2% of the nucleotide sites were polymorphic) as well as between isolates of different serovars. The highest degree of diversity was identified in the gene segments encoding the surface-exposed loops of the mature PorB protein. Phylogenetic analysis of the *porB1b* gene sequences confirmed previous findings that have indicated the circulation of one *N. gonorrhoeae* strain each of serovar IB-2 and serovar IB-3 in the Swedish community. These strains caused the majority of the cases in two domestic core groups comprising homosexual men and young heterosexuals, respectively, and were also detected in other patients. The phylogenetic analyses of *porB* gene sequences in the present study showed congruence, but not complete identity, with previous results obtained by pulsed-field gel electrophoresis of the same isolates. In conclusion, *porB* gene sequencing can be used as a molecular epidemiological tool for examination of genetic relationships among emerging and circulating *N. gonorrhoeae* strains, as well as for confirmation or discrimination of clusters of gonorrhea cases.

van der Zee, A., H. Verbakel, et al. (2002). "Novel PCR-Probe Assay for Detection of and Discrimination between *Legionella pneumophila* and Other *Legionella* Species in Clinical Samples." J. Clin. Microbiol. **40**(3): 1124-1125.

<http://jcm.asm.org>

van Doornum, G. J. J., J. Guldemeester, et al. (2003). "Diagnosing Herpesvirus Infections by Real-Time

Amplification and Rapid Culture." *J. Clin. Microbiol.* **41**(2): 576-580.

<http://jcm.asm.org/cgi/content/abstract/41/2/576>

Procedures using real-time technique were developed to demonstrate the presence of herpes simplex virus type 1 (HSV-1) and HSV-2, varicella zoster virus (VZV), and cytomegalovirus (CMV) in miscellaneous clinical specimens. The assays were compared to rapid culture using centrifugation followed by detection with monoclonal antibodies. A total of 711 consecutive samples were collected from different patient groups. Throat swabs were obtained from transplant patients; dermal or oral specimens were collected from patients suspected for VZV or HSV infection. Genital specimens were taken from patients who attended the Clinic for Sexually Transmitted Diseases at the Dijkzigt Hospital Rotterdam presenting with symptoms of a primary genital ulcer. Nucleic acid extraction was carried out using a MagnaPure LC instrument. The amplification steps were performed on the ABI Prism 7700 sequence detection system. To monitor the process of extraction and amplification, a universal control consisting of seal herpesvirus type 1 (PhHV-1) was added to the clinical specimens. By culture 127 of 668 (19%) samples were positive for HSV-1, 72 of 668 (10.8%) specimens were positive for HSV-2, and 17 of 366 (4.6%) were positive for VZV. Using real-time amplification the numbers of positive specimens were 143 of 668 (21.4%), 97 of 668 (14.5%), and 27 of 366 (7.4%), respectively. Eighty-six specimens were tested for CMV, 12 (14.0%) were positive by culture, and 17 (19.8%) were positive by real-time PCR. The clinical data of the patients with discrepant results were reviewed thoroughly. In all cases the patients with only real-time PCR-positive results could be considered as truly infected. We concluded that the real-time amplification technique is suitable for the detection of human herpesvirus infection. It offers a semiquantitative and reliable assay with a quick result that is more sensitive than rapid culture, especially for the diagnosis of HSV-2 and VZV infections.

van Elden, L. J. R., A. M. van Loon, et al. (2003). "Applicability of a Real-Time Quantitative PCR Assay for Diagnosis of Respiratory Syncytial Virus Infection in Immunocompromised Adults." *J. Clin. Microbiol.* **41**(9): 4378-4381.

<http://jcm.asm.org/cgi/content/abstract/41/9/4378>

Respiratory syncytial virus (RSV) accounts for the majority of respiratory virus infections, producing high mortality rates in immunocompromised patients with hematologic malignancies. The available methods for the rapid detection of RSV by antigen detection or PCR either lack sensitivity, require complex laboratory manipulation, or have not been evaluated in this patient population. To assess the applicability of a TaqMan-based real-time PCR technique for the detection of RSV A and B in immunocompromised adults, we developed a rapid, sensitive detection method that simultaneously detects RSV A and B and can be applied in routine diagnostics. The specificity of the assay was assessed using a panel of reference strains of other respiratory viruses and RSV. Electron microscopy-counted stocks of RSV A and B were used to develop a quantitative PCR format. Eleven copies of viral RNA could be detected for RSV A strain Long, and 14 copies could be detected for RSV B strain 9320, corresponding to 50% tissue culture infective doses of 0.86 and 0.34, respectively. The assay was evaluated on 411 combined nose and throat swabs derived from immunocompromised adults with or without signs of respiratory tract infection. The diagnostic efficacy of the TaqMan PCR determined on the clinical samples showed that this real-time PCR technique was substantially more sensitive than the combination of conventional viral culture and shell vial culture. None of the clinical specimens derived from patients without signs of respiratory illness were found to be positive for RSV by real-time TaqMan PCR.

Wadowsky, R. M., E. A. Castilla, et al. (2002). "Evaluation of Chlamydia pneumoniae and Mycoplasma pneumoniae as Etiologic Agents of Persistent Cough in Adolescents and Adults." J. Clin. Microbiol. **40**(2): 637-640.

<http://jcm.asm.org/cgi/content/abstract/40/2/637>

Chlamydia pneumoniae and Mycoplasma pneumoniae were evaluated as agents of persistent cough in adolescents and adults (n = 491). Tests of 473 respiratory specimens by culture or PCR or both identified four episodes (0.8%) of M. pneumoniae-associated illness and no episodes of C. pneumoniae illness, suggesting that these bacteria do not frequently cause persistent cough.

Walker, E. S., F. Levy, et al. (2004). "A Decline in Mupirocin Resistance in Methicillin-Resistant Staphylococcus aureus Accompanied Administrative Control of Prescriptions." J. Clin. Microbiol. **42**(6): 2792-2795.

<http://jcm.asm.org/cgi/content/abstract/42/6/2792>

Susceptibility to mupirocin was assessed in methicillin-resistant Staphylococcus aureus isolates selected from eras corresponding to differences in usage rate and prescription policies at a Veterans Affairs medical center. The eras studied encompassed from the time of introduction of the drug to its widespread use, through recommended judicious use, and finally to subsequent stringent administrative control. Prescriptions declined from 3.0 to 0.1 per 1,000 patient days. Precipitous declines first in the numbers of isolates with high-level resistance (from 31% to 4%) and then in those with low-level resistance (from 26% to 10%) accompanied prescription control.

Waltz, T. L., S. Marras, et al. (2005). "Development of a Molecular-Beacon Assay To Detect the G1896A Precore Mutation in Hepatitis B Virus-Infected Individuals." J. Clin. Microbiol. **43**(1): 254-258.

<http://jcm.asm.org/cgi/content/abstract/43/1/254>

The 1896 precore (PC) mutation is the most frequent cause of hepatitis B virus e-antigen (HBeAg)-negative chronic hepatitis B virus (HBV) infection. Detection of the 1896 PC mutation has application in studies monitoring antiviral therapy and the natural history of the disease. Identification of this mutation is usually performed by direct sequencing, which is both costly and laborious. The aim of this study was to develop a rapid, high-throughput assay to detect the 1896 PC mutation using real-time PCR and molecular-beacon technology. The assay was initially standardized on oligonucleotide targets and plasmids containing the wild-type (WT) and PC mutation and then tested on plasma samples from children with HBV DNA of >10⁶ copies/ml. Nine individuals were HBeAg negative and suspected to harbor HBeAg mutations, while 12 children were HBeAg positive and selected as controls. Ninety percent (19 of 21) of plasma samples tested with molecular beacons were in complete agreement with sequencing results. The remaining 10% (2 of 21) of samples were identified as heterogeneous mixtures of WT and mutant virus by molecular beacons, though sequencing found only a homogeneous mutant in both cases. Overall, the 1896 PC mutation was detected by this assay in 55.5% of the children with HBeAg-negative infection. In summary, this assay is a rapid, sensitive, and specific technique that effectively discriminates WT from 1896 PC mutant HBV and may be useful in clinical and epidemiological studies.

Wang, C.-Y. J., J. J. Giambrone, et al. (2002). "Detection of Duck Hepatitis B Virus DNA on Filter Paper

by PCR and SYBR Green Dye-Based Quantitative PCR." *J. Clin. Microbiol.* **40**(7): 2584-2590.

<http://jcm.asm.org/cgi/content/abstract/40/7/2584>

Duck hepatitis B virus (DHBV) belongs to the Hepadnaviridae family, which includes human Hepatitis B virus (HBV) and Woodchuck hepatitis virus. It is widely distributed in wild and domestic ducks due to congenital transmission. HBV is a worldwide health problem, with carriers at risk of developing cirrhosis and liver cancer. Medical staff and scientists working with HBV must be vaccinated because of its contagious nature. DHBV is a safe surrogate for HBV because of their similarities. Collection of serum and blood samples on filter paper has been used to screen for metabolic disorders, genetic diseases, and viral infection and for evolutionary studies of the genome. In this study, DHBV from serum and blood dried on filters was detected by PCR. A 0.1- μ l sample was sufficient for detection. The immobilization potential of filter papers for DHBV was examined, and the highest yield of PCR products was observed with Whatman paper. Dried serum was stable under different storage temperatures for 4 weeks, but the yields of PCR products decreased when the temperature was $\geq 4^{\circ}\text{C}$. The optimal condition for storage was -70°C . A newly developed quantitative PCR based on monitoring the amplification by measuring the increase in fluorescence caused by the binding of SYBR green dye to double-stranded products was applied herein. DHBV genomic DNA cloned in a plasmid was used for the generation of standard DHBV DNA for quantitative PCR. It validated results from PCR in terms of the copy number of DHBV particles. The specificity of PCR was demonstrated by melting curve analysis, and the differentiation of two DHBV isolates amplified from dried serum was demonstrated based on their melting temperatures determined by GC contents and sequence. It was easier and simpler than other PCR-based DNA techniques. The use of serum dried on filters allows samples from distant field for which cold storage and transportation are a problem to be mailed to the diagnostic laboratory. Samples can be archived for comparison and used as a source of DNA for cloning and sequencing.

Wang, G., C. G. Clark, et al. (2002). "Detection in *Escherichia coli* of the Genes Encoding the Major Virulence Factors, the Genes Defining the O157:H7 Serotype, and Components of the Type 2 Shiga Toxin Family by Multiplex PCR." *J. Clin. Microbiol.* **40**(10): 3613-3619.

<http://jcm.asm.org/cgi/content/abstract/40/10/3613>

Strains of Shiga toxin-producing *Escherichia coli* (STEC) have been associated with outbreaks of diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome in humans. Most clinical signs of disease arise as a consequence of the production of Shiga toxin 1 (Stx1), Stx2 or combinations of these toxins. Other major virulence factors include enterohemorrhagic *E. coli* hemolysin (EHEC hlyA), and intimin, the product of the *eaeA* gene that is involved in the attaching and effacing adherence phenotype. In this study, a series of multiplex-PCR assays were developed to detect the eight most-important *E. coli* genes associated with virulence, two that define the serotype and therefore the identity of the organism, and a built-in gene detection control. Those genes detected were *stx1*, *stx2*, *stx2c*, *stx2d*, *stx2e*, *stx2f*, EHEC hlyA, and *eaeA*, as well as *rfbE*, which encodes the *E. coli* O157 serotype; *fliC*, which encodes the *E. coli* flagellum H7 serotype; and the *E. coli* 16S rRNA, which was included as an internal control. A total of 129 *E. coli* strains, including 81 that were O157:H7, 10 that were O157:non-H7, and 38 that were non-O157 isolates, were investigated. Among the 129 samples, 101 (78.3%) were *stx* positive, while 28 (21.7%) were lacked *stx*. Of these 129 isolates, 92 (71.3%) were EHEC hlyA positive and 96 (74.4%) were *eaeA* positive. All STEC strains were identified by this procedure. In addition, all Stx2 subtypes, which had been initially identified by PCR-restriction fragment length polymorphism, were identified by this method. A particular strength of the assay was the identification of these 11 genes without the need to use restriction enzyme digestion. The proposed method is a simple, reliable, and rapid procedure that can detect the major virulence factors of *E. coli* while differentiating O157:H7 from non-O157 isolates.

Wang, J.-Y., L.-N. Lee, et al. (2004). "Performance Assessment of a Nested-PCR Assay (the RAPID BAP-MTB) and the BD ProbeTec ET System for Detection of Mycobacterium tuberculosis in Clinical Specimens." *J. Clin. Microbiol.* **42**(10): 4599-4603.

<http://jcm.asm.org/cgi/content/abstract/42/10/4599>

The performance of a nested PCR-based assay (the RAPID BAP-MTB; AsiaGen, Taichung, Taiwan) and the BD ProbeTec ET (DTB) system (Becton Dickinson, Sparks, Md.) for detection of Mycobacterium tuberculosis was evaluated with 600 consecutive clinical samples. These samples, including 552 respiratory specimens and 48 nonrespiratory specimens, were collected from 333 patients treated at National Taiwan University Hospital from September to October 2003. The results of both assays were compared to the gold standard of combined culture results and clinical diagnosis. The overall sensitivity and specificity of the RAPID BAP-MTB assay for respiratory specimens were 66.7% and 97.2%, respectively, and for the DTB assay they were 56.7% and 95.3%, respectively. The positive and negative predictive values for the RAPID BAP-MTB were 74.1% and 96.0%, respectively, and for the DTB assay they were 59.6% and 94.7%, respectively. For smear-negative samples, the sensitivity of the RAPID BAP-MTB and DTB assays was 57.1% and 40.5%, respectively. The RAPID BAP-MTB assay produced 14 false-positive results in 14 samples, including one of the six samples yielding Mycobacterium abscessus, one of the six samples yielding Mycobacterium avium intracellulare, one sample from a patient with a history of pulmonary tuberculosis with complete treatment, and three samples from three patients with a previous diagnosis of tuberculosis who were under treatment at the time of specimen collection. Among the 48 nonrespiratory specimens, the RAPID BAP-MTB assay was positive in one biopsy sample from a patient with lumbar tuberculous spondylitis and one pus sample from a patient with tuberculous cervical lymphadenopathy. Our results showed that the RAPID BAP-MTB assay is better than the DTB assay for both respiratory specimens and nonrespiratory specimens. The overall time for processing this assay is only 5 h. In addition, its diagnostic accuracy in smear-negative samples is as high as in smear-positive samples.

Weidmann, M., U. Meyer-König, et al. (2003). "Rapid Detection of Herpes Simplex Virus and Varicella-Zoster Virus Infections by Real-Time PCR." *J. Clin. Microbiol.* **41**(4): 1565-1568.

<http://jcm.asm.org/cgi/content/abstract/41/4/1565>

The herpes simplex viruses types 1 and 2 (HSV-1 and HSV-2) and varicella-zoster virus (VZV) can cause life-threatening infections of the central nervous system and lead to severe infections in immunocompromised subjects and newborns. In these cases, rapid diagnosis is crucial. We developed three different real-time PCR assays based on TaqMan chemistry for the LightCycler instrument to detect HSV-1, HSV-2, and VZV. When the TaqMan assays were compared to our in-house nested PCR assays, the test systems had equal sensitivities of ≥ 10 plasmid copies per assay. When clinical samples were investigated by TaqMan PCR to detect HSV-1, HSV-2, and VZV DNA, 95, 100, and 96% of the samples determined to be positive by nested PCR, respectively, were positive by the real-time PCR assays. The specificities of all PCR assays were almost 100%. Furthermore, the TaqMan PCR assays could be performed within 2.5 h, whereas nested PCR results were available after 9 h. In addition to offering more rapid results, the TaqMan PCR assays appear to be less expensive than nested PCR assays due to less hands-on time. In summary, TaqMan PCR is an excellent alternative to conventional nested PCR assays for the rapid detection of HSV-1, HSV-2, and VZV in clinical samples.

Weidmann, M., P. Schmidt, et al. (2005). "Identification of Genetic Evidence for Dobrava Virus Spillover in Rodents by Nested Reverse Transcription (RT)-PCR and TaqMan RT-PCR." J. Clin. Microbiol. **43**(2): 808-812.

<http://jcm.asm.org/cgi/content/abstract/43/2/808>

A survey of 158 rodents caught in the Czech Republic identified Dobrava virus sequences closely related to that of the Dobrava virus type strain in *Apodemus sylvaticus* and *Mus musculus* rodents. The identity of *A. sylvaticus* was unequivocally confirmed by random amplified polymorphic DNA analysis. The data seem to indicate hantavirus spillover from *Apodemus flavicollis* to other rodents.

Wisselink, H. J., J. J. Joosten, et al. (2002). "Multiplex PCR Assays for Simultaneous Detection of Six Major Serotypes and Two Virulence-Associated Phenotypes of *Streptococcus suis* in Tonsillar Specimens from Pigs." J. Clin. Microbiol. **40**(8): 2922-2929.

<http://jcm.asm.org/cgi/content/abstract/40/8/2922>

Multiplex PCR assays for the detection and identification of various *Streptococcus suis* strains in tonsillar specimens from pigs were developed and evaluated. In two separate reactions, five distinct DNA targets were amplified. Three targets, based on the *S. suis* capsular polysaccharide (*cps*) genes specific for serotypes 1 (and 14), 7, and 9, were amplified in multiplex PCR I. Two other targets, based on the serotype 2- (and 1/2-) specific *cps* gene and the *epf* gene, encoding the EF proteins of virulent serotype 2 and highly virulent serotype 1 strains, were amplified in multiplex PCR II. To identify false-negative results, firefly luciferase (*luc*) DNA and primers based on the *luc* gene were included in the assay. The multiplex PCR assays were evaluated with tonsillar specimens from pigs infected with *S. suis* strains. The results obtained with the PCR assays were compared with the results obtained with a bacteriological examination. Most (94%) of the results obtained with multiplex PCR assays were confirmed by the bacteriological examination. The PCR method seems to be more sensitive compared to the bacteriological method, since the remaining 6% of the samples were positive by PCR and negative by bacteriological examination. These results indicate that the PCR method is highly specific for the detection of *S. suis* strains most frequently involved in clinical disease in infected pig herds. The serotypes found by PCR in tonsillar specimens from diseased pigs were compared with the serotypes of the strains isolated from the affected tissues of the same pigs. The results showed that there is significant association between carriership and clinical illness for *S. suis* serotype 9 and EF-positive serotype 2 strains and not for serotype 7 and EF-negative serotype 2 (or 1/2) strains.

Woo, P. C. Y., K. T. K. Chong, et al. (2003). "AFLMP1 Encodes an Antigenic Cell Wall Protein in *Aspergillus flavus*." J. Clin. Microbiol. **41**(2): 845-850.

<http://jcm.asm.org/cgi/content/abstract/41/2/845>

We have previously reported the cloning and characterization of the MP1 gene in *Penicillium marneffei* and the AFMP1 gene in *Aspergillus fumigatus* and their use for serodiagnosis of penicilliosis and aspergilloma and invasive aspergillosis, respectively. In this study, we describe the cloning of the AFLMP1 gene, which encodes the homologous antigenic cell wall protein in *Aspergillus flavus*, the most common *Aspergillus* species associated with human disease in our locality and in other Asian countries and the second most common *Aspergillus* species associated with human disease in Western countries. AFLMP1 codes for a protein, Aflmp1p, of

273 amino acid residues, with a few sequence features that are present in Mp1p and Afmp1p, the homologous antigenic cell wall proteins in *P. marneffei* and *A. fumigatus*, respectively, as well as several other cell wall proteins of *Saccharomyces cerevisiae* and *Candida albicans*. It contains a serine- and threonine-rich region for O glycosylation, a signal peptide, and a putative glycosylphosphatidylinositol attachment signal sequence. Specific anti-Aflmp1p antibody was generated with recombinant Aflmp1p protein purified from *Escherichia coli* to allow further characterization of Aflmp1p. Indirect immunofluorescence analysis indicated that Aflmp1p is present on the surface of the hyphae of *A. flavus*. Finally, it was observed that patients with aspergilloma and invasive aspergillosis due to *A. flavus* develop a specific antibody response against Aflmp1p. This suggested that the recombinant protein and its antibody may be useful for serodiagnosis in patients with aspergilloma or invasive aspergillosis, and the protein may represent a good cell surface target for host humoral immunity.

Yam, W. C., K. H. Chan, et al. (2003). "Evaluation of Reverse Transcription-PCR Assays for Rapid Diagnosis of Severe Acute Respiratory Syndrome Associated with a Novel Coronavirus." J. Clin. Microbiol. **41**(10): 4521-4524.

<http://jcm.asm.org/cgi/content/abstract/41/10/4521>

The reverse transcription (RT)-PCR protocols of two World Health Organization (WHO) severe acute respiratory syndrome (SARS) network laboratories (WHO SARS network laboratories at The University of Hong Kong [WHO-HKU] and at the Bernhard-Nocht Institute in Hamburg, Germany [WHO-Hamburg]) were evaluated for rapid diagnosis of a novel coronavirus (CoV) associated with SARS in Hong Kong. A total of 303 clinical specimens were collected from 163 patients suspected to have SARS. The end point of both WHO-HKU and WHO-Hamburg RT-PCR assays was determined to be 0.1 50% tissue culture infective dose. Using seroconversion to CoV as the "gold standard" for SARS CoV diagnosis, WHO-HKU and WHO-Hamburg RT-PCR assays exhibited diagnostic sensitivities of 61 and 68% (nasopharyngeal aspirate specimens), 65 and 72% (throat swab specimens), 50 and 54% (urine specimens), and 58 and 63% (stool specimens), respectively, with an overall specificity of 100%. For patients confirmed to have SARS CoV and from whom two or more respiratory specimens were collected, testing the second specimen increased the sensitivity from 64 and 71% to 75 and 79% for the WHO-HKU and WHO-Hamburg RT-PCR assays, respectively. Testing more than one respiratory specimen will maximize the sensitivity of PCR assays for SARS CoV.

Yamasaki, H., M. Nakao, et al. (2002). "DNA Differential Diagnosis of Human Taeniid Cestodes by Base Excision Sequence Scanning Thymine-Base Reader Analysis with Mitochondrial Genes." J. Clin. Microbiol. **40**(10): 3818-3821.

<http://jcm.asm.org/cgi/content/abstract/40/10/3818>

For DNA differential diagnosis of human *Taenia* cestodes, a base excision sequence scanning thymine-base method using the cytochrome c oxidase subunit I and cytochrome b genes as targets was used. The characteristic thymine-base peak profiles provide four distinct types, unique for *T. saginata*, *T. asiatica*, and two genotypes of *T. solium*. This approach provides a useful tool for the identification and diagnosis of human taeniid cestodes without DNA sequencing if nucleotide sequence databases are available.

Yang, S., S. Lin, et al. (2002). "Quantitative Multiprobe PCR Assay for Simultaneous Detection and

Identification to Species Level of Bacterial Pathogens." J. Clin. Microbiol. **40**(9): 3449-3454.

<http://jcm.asm.org/cgi/content/abstract/40/9/3449>

We describe a novel adaptation of the TaqMan PCR assay which potentially allows for highly sensitive detection of any eubacterial species with simultaneous species identification. Our system relies on a unique multiprobe design in which a single set of highly conserved sequences encoded by the 16S rRNA gene serves as the primer pair and is used in combination with both an internal highly conserved sequence, the universal probe, and an internal variable region, the species-specific probe. A pre-PCR ultrafiltration step effectively decontaminates or removes background DNA. The TaqMan system described reliably detected 14 common bacterial species with a detection limit of 50 fg. Further, highly sensitive and specific pathogen detection was demonstrated with a prototype species-specific probe designed to detect *Staphylococcus aureus*. This assay has broad potential in the clinical arena for rapid and specific diagnosis of infectious diseases.

Yera, H., M. E. Bounoux, et al. (2003). "Mycetoma of the Foot Caused by *Fusarium solani*: Identification of the Etiologic Agent by DNA Sequencing." J. Clin. Microbiol. **41**(4): 1805-1808.

<http://jcm.asm.org/cgi/content/abstract/41/4/1805>

We report a case of *Fusarium solani* mycetoma of the foot that could not be diagnosed by culture, but was correctly identified after amplification and sequence analysis of fungal internal transcribed spacers 1 and 2 and 5.8S ribosomal DNA regions.

Yoshida, T., T. Deguchi, et al. (2002). "Quantitative Detection of *Mycoplasma genitalium* from First-Pass Urine of Men with Urethritis and Asymptomatic Men by Real-Time PCR." J. Clin. Microbiol. **40**(4): 1451-1455.

<http://jcm.asm.org/cgi/content/abstract/40/4/1451>

We developed a TaqMan-based real-time PCR assay for quantifying *Mycoplasma genitalium*. This assay is able to specifically quantify concentrations of the *M. genitalium* 16S rRNA gene ranging from 107 to 10 copies/reaction. Using the TaqMan assay, we quantified the *M. genitalium* 16S rRNA gene in first-pass urine of men with urethritis and asymptomatic men who were positive for *M. genitalium* by PCR- and phylogeny-based assay. Of 130 men with gonococcal urethritis (GU), five were positive for *M. genitalium*. The mycoplasma load for each specimen was $<5 \times 10$ copies/ml. Of 84 men with chlamydial non-GU (CNGU), seven were positive for *M. genitalium*. One man had an *M. genitalium* load of $<5 \times 10$ copies/ml, and six men had loads ranging from 1.1×10^7 to 2.7×10^2 copies/ml. Of 86 men with nonchlamydial NGU (NCNGU), 17 were positive for *M. genitalium*. The mycoplasma loads for these men ranged from 3.3×10^6 to 2.3×10^2 copies/ml. Of 76 asymptomatic men, only two were positive for *M. genitalium*. For these men, the loads were 2×10^2 and $<5 \times 10$ copies/ml. The patients with NGU had significantly higher concentrations of *M. genitalium* in their first-pass urine than did men with GU ($P < 0.01$) or asymptomatic men ($P < 0.05$). In addition, *M. genitalium* loads were significantly higher in men with NCNGU than those in asymptomatic men ($P < 0.05$). The quantitative assessment of *M. genitalium* loads by the TaqMan assay will provide useful information for understanding the pathogenicity of this mycoplasma in the urogenital tract.

Yoshida, T., S.-I. Maeda, et al. (2002). "Phylogeny-Based Rapid Identification of Mycoplasmas and Ureaplasmas from Urethritis Patients." J. Clin. Microbiol. **40**(1): 105-110.

<http://jcm.asm.org/cgi/content/abstract/40/1/105>

Some strains of mycoplasmas and ureaplasmas (family Mycoplasmataceae) are associated with nongonococcal urethritis (NGU) or other genitourinary infections. We have developed a rapid and reliable method of identifying the presence and prevalence of mycoplasmas and ureaplasmas in men with NGU. This method is based on the amplification of a part of the 16S rRNA gene by PCR and phylogenetic analysis. A portion of the 16S rRNA gene from 15 prototype strains was amplified with a set of common primers, and their nucleotides were sequenced. The nucleotide sequence of the V4 and V5 regions was analyzed by the neighbor-joining method. The 15 prototype strains were grouped into three distinct clusters, allowing us to clearly segregate the strains into distinct lineages. To determine the prevalence of these pathogens among patients with NGU, this protocol was tested with 148 urine samples. Amplifications were observed for 42 samples, and their nucleotide sequences were analyzed along with those of the 15 prototype strains. The phylogenetic tree thus constructed indicated that 15 of the 42 formed a cluster with *Mycoplasma genitalium*. Among the remaining specimens, 2 formed a cluster with *Mycoplasma hominis*, 19 with *Ureaplasma urealyticum*, and 5 with *Ureaplasma parvum*; the remaining sample contained both *M. genitalium* and *U. urealyticum*. This phylogeny-based identification of mycoplasmas and ureaplasmas provides not only a powerful tool for rapid diagnosis but also the basis for etiological studies of these pathogens.

Zhang, W., M. Bielaszewska, et al. (2002). "Identification, Characterization, and Distribution of a Shiga Toxin 1 Gene Variant (stx1c) in Escherichia coli Strains Isolated from Humans." J. Clin. Microbiol. **40**(4): 1441-1446.

<http://jcm.asm.org/cgi/content/abstract/40/4/1441>

By using sequence analysis of Shiga toxin 1 (Stx 1) genes from human and ovine Stx-producing *Escherichia coli* (STEC) strains, we identified an Stx1 variant in STEC of human origin that was identical to the Stx1 variant from ovine STEC, but demonstrated only 97.1 and 96.6% amino acid sequence identity in its A and B subunits, respectively, to the Stx1 encoded by bacteriophage 933J. We designated this variant "Stx1c" and developed stxB1 restriction fragment length polymorphism and stx1c-specific PCR strategies to determine the frequency and distribution of stx1c among 212 STEC strains isolated from humans. stx1c was identified in 36 (17.0%) of 212 STEC strains, 19 of which originated from asymptomatic subjects and 16 of which were from patients with uncomplicated diarrhea. stx1c was most frequently (in 23 STEC strains [63.9%]) associated with stx2d, but 12 (33.3%) of the 36 STEC strains possessed stx1c only. A single STEC strain possessed stx1c together with stx2 and was isolated from a patient with hemolytic-uremic syndrome. All 36 stx1c-positive STEC strains were eae negative and belonged to 10 different serogroups, none of which was O157, O26, O103, O111, or O145. Stx1c was produced by all stx1c-containing STEC strains, but reacted weakly with a commercial immunoassay. We conclude that STEC strains harboring the stx1c variant account for a significant proportion of human STEC isolates. The procedures developed in this study now allow the determination of the frequency of STEC strains harboring stx1c among clinical STEC isolates and their association with human disease in prospective studies.

Zhou, W., W. Du, et al. (2004). "Detection of gyrA and parC Mutations Associated with Ciprofloxacin Resistance in *Neisseria gonorrhoeae* by Use of Oligonucleotide Biochip Technology." J. Clin. Microbiol. **42**(12): 5819-5824.

<http://jcm.asm.org/cgi/content/abstract/42/12/5819>

An oligonucleotide biochip that specifically detects point mutations in the *gyrA* and *parC* genes of *Neisseria gonorrhoeae* was designed and subsequently evaluated with 87 untreated clinical specimens. The susceptibilities of the *N. gonorrhoeae* strains were tested to determine the prevalence of ciprofloxacin-resistant strains in Anhui Province, People's Republic of China. Conventional DNA sequencing was also performed to identify mutations in *gyrA* and *parC* and to confirm the biochip data. The study demonstrates that all of the point mutations in the *gyrA* and *parC* genes of *N. gonorrhoeae* were easily discriminated by use of the oligonucleotide biochip. Fifteen different alteration patterns involved in the formation of ciprofloxacin resistance were identified by the biochip assay. Double mutations in both Ser91 and Asp95 of the GyrA protein were seen in all nonsensitive isolates. Double mutations in Ser91 and Asp95 of GyrA plus mutation of Glu91 or Ser87 of the ParC protein lead to significant high-level resistance to ciprofloxacin in *N. gonorrhoeae* isolates. The results obtained by use of the oligonucleotide biochip were identical to those obtained by use of DNA sequencing. In conclusion, the oligonucleotide biochip technology has potential utility for the rapid and reliable identification of point mutations in the drug resistance genes of *N. gonorrhoeae*.

Zink, A. R., C. Sola, et al. (2003). "Characterization of Mycobacterium tuberculosis Complex DNAs from Egyptian Mummies by Spoligotyping." *J. Clin. Microbiol.* **41**(1): 359-367.

<http://jcm.asm.org/cgi/content/abstract/41/1/359>

Bone and soft tissue samples from 85 ancient Egyptian mummies were analyzed for the presence of ancient Mycobacterium tuberculosis complex DNA (aDNA) and further characterized by spoligotyping. The specimens were obtained from individuals from different tomb complexes in Thebes West, Upper Egypt, which were used for upper social class burials between the Middle Kingdom (since ca. 2050 BC) and the Late Period (until ca. 500 BC). A total of 25 samples provided a specific positive signal for the amplification of a 123-bp fragment of the repetitive element IS6110, indicating the presence of *M. tuberculosis* DNA. Further PCR-based tests for the identification of subspecies failed due to lack of specific amplification products in the historic tissue samples. Of these 25 positive specimens, 12 could be successfully characterized by spoligotyping. The spoligotyping signatures were compared to those in an international database. They all show either an *M. tuberculosis* or an *M. africanum* pattern, but none revealed an *M. bovis*-specific pattern. The results from a Middle Kingdom tomb (used exclusively between ca. 2050 and 1650 BC) suggest that these samples bear an *M. africanum*-type specific spoligotyping signature. The samples from later periods provided patterns typical for *M. tuberculosis*. This study clearly demonstrates that spoligotyping can be applied to historic tissue samples. In addition, our results do not support the theory that *M. tuberculosis* originated from the *M. bovis* type but, rather, suggest that human *M. tuberculosis* may have originated from a precursor complex probably related to *M. africanum*.

J. Clin. Oncol. (17)

Bull, S. B., H. Ozelik, et al. (2004). "The Combination of p53 Mutation and neu/erbB-2 Amplification Is Associated With Poor Survival in Node-Negative Breast Cancer." *J. Clin. Oncol.* **22**(1): 86-96.

<http://www.jco.org/cgi/content/abstract/22/1/86>

PURPOSE: Increases in neu/erbB-2 have been implicated in breast cancer prognosis, but do not predict all recurrences. On the basis of evidence that p53 mutation is involved in the development of human neoplasia, we examined the prognostic value of p53 alterations in combination with neu/erbB-2 amplification. **PATIENTS AND METHODS:** A consecutive series of women were observed for recurrence and death (median follow-up of 85 months) and tumors from 543 individuals were analyzed for p53 mutation status and neu/erbB-2 amplification. Exons 4 through 10 of the p53 gene were analyzed by single-stranded conformational polymorphism and mutations were confirmed by DNA sequencing. The association of p53 mutation status and neu/erbB-2 amplification with risk of recurrence and death was examined in survival analyses with traditional and histologic markers as prognostic factors. **RESULTS:** p53 mutations occurred in 24.5% of the axillary node-negative breast carcinomas. Mutations were more frequent in carcinomas with neu/erbB-2 amplification: 38.9% compared with only 20.9% in those without neu/erbB-2 amplification. We found elevated risks of disease recurrence and overall mortality in patients with both p53 mutation and neu/erbB-2 amplification in their tumor compared with patients with neither or only one of the alterations. This increase persisted with adjustment for other prognostic factors (relative risk, 2.32; $P = .002$ for recurrence; relative risk, 2.22; $P = .004$ for death). **CONCLUSION:** Evaluation of tumors for p53 mutations may be beneficial to identify women at higher risk of disease recurrence and death when the tumor has neu/erbB-2 amplification, but in the absence of neu/erbB-2 amplification, the presence of p53 mutation may not provide additional independent prognostic information.

Buttitta, F., C. Pellegrini, et al. (2003). "Human Telomerase Reverse Transcriptase mRNA Expression Assessed by Real-Time Reverse Transcription Polymerase Chain Reaction Predicts Chemosensitivity in Patients With Ovarian Carcinoma." *J. Clin. Oncol.* **21**(7): 1320-1325.

<http://www.jco.org/cgi/content/abstract/21/7/1320>

Purpose: To evaluate in vivo whether the expression of the human telomerase reverse transcriptase (hTERT) gene, the catalytic subunit of the telomerase complex, is predictive of response to chemotherapy in ovarian cancer patients. **Patients and Methods:** Fifty-nine advanced-stage ovarian cancer patients who were treated with platinum-based chemotherapy were studied. hTERT levels were evaluated by real-time reverse transcriptase polymerase chain reaction (RT-PCR) on tumor specimens obtained before the treatment. Variables were analyzed by the χ^2 and Fisher's exact tests. Logistic regression analysis was also performed to account for the effects of all the covariates investigated (residual disease, stage, histotype, and grade). **Results:** Twenty-eight (47%) of the 59 tumors showed low hTERT levels, whereas 31 (53%) tumors displayed high hTERT levels. Seventy-five percent of complete responders showed high levels of hTERT expression, whereas 66% of partial responders or nonresponders exhibited low hTERT levels ($P = .002$). Only residual disease and hTERT expression were independent predictors of response (odds ratios, 13.455 and 7.586, respectively). The combination of these two parameters provides powerful predictive information: 18 of the 20 patients with residual disease more than 2 cm and low hTERT levels were partial responders or nonresponders, whereas 11 of the 12 patients with residual disease less than 2 cm and high hTERT levels showed a complete response ($\chi^2 = 21.416$; $P < .00001$). **Conclusion:** Our data indicate that hTERT expression, measured by real-time RT-PCR, is a possible independent marker of response to platinum-based therapy in advanced stage ovarian cancer patients. Prospective validation of this marker will be required to further define its predictive value.

Fiegl, M., M. Haun, et al. (2004). "Combination of Cytology, Fluorescence In Situ Hybridization for Aneuploidy, and Reverse-Transcriptase Polymerase Chain Reaction for Human

Mammaglobin/Mammaglobin B Expression Improves Diagnosis of Malignant Effusions." *J. Clin. Oncol.* **22**(3): 474-483.

<http://www.jco.org/cgi/content/abstract/22/3/474>

PURPOSE: The identification of malignant cells in effusions by conventional cytology is hampered by its limited sensitivity. The aim of this study was to improve tumor cell detection in effusions by molecular approaches. **MATERIALS AND METHODS:** A total of 157 effusions from patients with tumors and 72 effusions from patients without a history or evidence of malignancy were included in this study. All effusion specimens were evaluated in parallel by cytology, fluorescence in situ hybridization (FISH) for aneuploidy, and reverse-transcriptase polymerase chain reaction (RT-PCR) for expression of human mammaglobin (hMAM) and mammaglobin B (hMAM-B). **RESULTS:** In effusions from patients with tumors, the sensitivities of tumor cell detection by cytology, FISH, and hMAM and hMAM-B detection were 46.2%, 53.3%, 36.4%, and 57.7%, respectively. The corresponding specificities were 94.4%, 97.0%, 87.1%, and 88.6%. Notably, a high percentage of effusions containing malignant cells were in fact transudates, indicating the necessity for molecular diagnostic work-up of transudates collected from patients with tumors. Dependent on the tumor type, the use of appropriate marker combinations improved tumor cell detection in effusions significantly. By combining all four diagnostic tests, a positive test result indicating the presence of malignancy was achieved in 81.1%, with a fairly good specificity of 70.1%. **CONCLUSION:** Molecular techniques are definitely useful to detect malignancy in cytologically negative effusions. Tumor cell detection in effusions can be significantly improved by FISH and PCR techniques applying appropriate molecular markers. This finding should help to improve tumor staging, prognostic assessment, and treatment monitoring.

Goetz, M. P., D. Toft, et al. (2005). "Phase I Trial of 17-Allylamino-17-Demethoxygeldanamycin in Patients With Advanced Cancer." *J. Clin. Oncol.* **23**(6): 1078-1087.

<http://www.jco.org/cgi/content/abstract/23/6/1078>

PURPOSE: We determined the maximum-tolerated dose (MTD) and the dose-limiting toxicities (DLT) of 17-allylamino-17-demethoxygeldanamycin (17-AAG) when infused on days 1, 8, and 15 of a 28-day cycle in advanced solid tumor patients. We also characterized the pharmacokinetics of 17-AAG, its effect on chaperone and client proteins, and whether cytochrome P450 (CYP) 3A5 and NAD(P)H:quinone oxidoreductase 1 (NQO1) polymorphisms affected 17-AAG disposition or toxicity. **PATIENTS AND METHODS:** An accelerated titration design was used. Biomarkers were measured in peripheral-blood mononuclear cells (PBMCs) at baseline and on days 1 and 15, and pharmacokinetic analysis was performed on day 1 of cycle 1. CYP3A5*3 and NQO1*2 genotypes were determined and correlated with pharmacokinetics and toxicity. **RESULTS:** Twenty-one patients received 52 courses at 11 dose levels. DLTs at 431 mg/m² were grade 3 bilirubin (n = 1), AST (n = 1), anemia (n = 1), nausea (n = 1), vomiting (n = 1), and myalgias (n = 1). No tumor responses were seen. 17-AAG consistently increased heat shock protein (Hsp) 70 levels in PBMCs. At the MTD, the clearance and half-life (t_{1/2}) of 17-AAG were 11.6 L/h/m² and 4.15 hours, respectively; whereas the active metabolite 17-aminogeldanamycin had a t_{1/2} of 7.63 hours. The CYP3A5*3 and NQO1*2 polymorphisms were not associated with 17-AAG toxicity. The CYP3A5*3 polymorphism was associated with higher 17-AAG clearance. **CONCLUSION:** The MTD of weekly 17-AAG is 308 mg/m². 17-AAG induced Hsp70 in PBMCs, indicating that Hsp90 has been affected. Further evaluation of 17-AAG is ongoing using a twice-weekly regimen, and this schedule of 17-AAG is being tested in combination with chemotherapy.

Gupta, S., T. Hussain, et al. (2003). "Differential Expression of S100A2 and S100A4 During Progression of Human Prostate Adenocarcinoma." *J. Clin. Oncol.* **21**(1): 106-112.

<http://www.jco.org/cgi/content/abstract/21/1/106>

Purpose: To establish the clinical significance of calcium binding proteins S100A2 and S100A4 during progression of human prostate adenocarcinoma. **Patients and Methods:** Expression pattern of S100A2 and S100A4 was determined in normal human prostate epithelial cells (NHPE); virally transformed prostate epithelial cells (PZ-HPV-7); several human prostate carcinoma cells (22Rv1, DU145, LNCaP, and PC3); tissue samples obtained during transurethral prostatic resection from patients with benign prostate hyperplasia (BPH), prostatitis, and adenocarcinoma; and paraffin-embedded sections from pair-matched benign and cancer specimens of different tumor grade. **Results:** High constitutive protein expression of S100A2 was observed in NHPE and PZ-HPV-7 cells, whereas its complete absence was observed in 22Rv1, DU145, LNCaP, and PC3 cells. Tissue samples of BPH and prostatitis exhibited higher mRNA and protein levels of S100A2 than low-grade cancer (Gleason score ≤ 6), whereas a complete loss was observed in high-grade cancer specimens (Gleason score > 6). Immunohistochemical analysis further confirmed high levels of S100A2 in benign tissues and a progressive loss with increasing tumor grade. The protein level of S100A4 was significantly higher in all carcinoma cells compared with NHPE and PZ-HPV-7 cells. The mRNA and protein level of S100A4 was significantly higher in high-grade cancer specimens compared with BPH, prostatitis, and low-grade cancer. The high levels of S100A4 observed in cancer tissue correlated with increasing tumor grade. **Conclusion:** Loss of S100A2 and increased expression of S100A4 may be an important event during progression of prostate cancer in humans.

Howard, O. M., J. G. Gribben, et al. (2002). "Rituximab and CHOP Induction Therapy for Newly Diagnosed Mantle-Cell Lymphoma: Molecular Complete Responses Are Not Predictive of Progression-Free Survival." *J. Clin. Oncol.* **20**(5): 1288-1294.

<http://www.jco.org/cgi/content/abstract/20/5/1288>

PURPOSE: To evaluate the efficacy of rituximab and cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) induction therapy in patients with newly diagnosed mantle-cell lymphoma (MCL). **PATIENTS AND METHODS:** From March 1997 to May 1999, 40 previously untreated patients with stage II through IV MCL were treated with six cycles of rituximab and CHOP chemotherapy in a phase II trial. Pretreatment and interval peripheral-blood (PB) and bone marrow (BM) specimens were also analyzed by polymerase chain reaction (PCR) for tumor-specific BCL-1/immunoglobulin H (IgH) translocations and clonal IgH rearrangements. Study end points included clinical and molecular response rates and long-term progression-free survival (PFS). **RESULTS:** Forty-eight percent of patients achieved a complete response (CR)/CR unconfirmed (CRu), and 48% of patients obtained a partial response (PR). However, 28 of the 40 patients have already relapsed or developed progressive disease with a median PFS of 16.6 months. Twenty-five patients had PCR-detectable BCL-1/IgH or clonal IgH products in PB or BM at diagnosis. Nine of the 25 informative patients had no evidence of PCR-detectable disease in PB or BM after rituximab and CHOP therapy. However, patients who achieved molecular remissions in PB or BM had PFS similar to patients without molecular remissions (16.5 v 18.8 months, $P = .51$). **CONCLUSION:** Favorable clinical and molecular response rates associated with rituximab and CHOP chemotherapy do not translate into prolonged PFS in MCL. Nevertheless, rituximab and combination chemotherapy may transiently clear PB or BM of detectable tumor cells, prompting additional consideration of antibody-based in vivo purging in subsequent clinical trials.

Huguenin, P., K. T. Beer, et al. (2004). "Concomitant Cisplatin Significantly Improves Locoregional Control in Advanced Head and Neck Cancers Treated With Hyperfractionated Radiotherapy." *J. Clin. Oncol.* **22**(23): 4665-4673.

<http://www.jco.org/cgi/content/abstract/22/23/4665>

PURPOSE: To determine whether the application of two courses of cisplatin simultaneously with hyperfractionated radiotherapy improves the outcome in locally advanced and/or node-positive nonmetastatic carcinomas of the head and neck, compared with hyperfractionated radiotherapy alone. **PATIENTS AND METHODS:** From July 1994 to July 2000, 224 patients with squamous cell carcinomas of the head and neck (excluding nasopharynx and paranasal sinus) were randomly assigned to hyperfractionated radiotherapy (median dose, 74.4 Gy; 1.2 Gy twice daily) or the same radiotherapy combined with two cycles of concomitant cisplatin (20 mg/m² on 5 days of weeks 1 and 5). The primary end point was time to any treatment failure; secondary end points were locoregional failure, metastatic relapse, overall survival, and late toxicity. **RESULTS:** There was no difference in radiotherapy between both treatment arms (74.4 Gy in 44 days). The full cisplatin dose was applied in 93% and 71% of patients during the first and second treatment cycles, respectively. Acute toxicity was similar in both arms. Median time to any treatment failure was not significantly different between treatment arms (19 months for combined treatment and 16 months for radiotherapy only, respectively) and the failure-free rate at 2.5 years was 45% and 33%, respectively. Locoregional control and distant disease-free survival were significantly improved with cisplatin (log-rank test, $P = .039$ and $.011$, respectively). The difference in overall survival did not reach significance (log-rank test, $P = .147$). Late toxicity was comparable in both treatment groups. **CONCLUSION:** The therapeutic index of hyperfractionated radiotherapy is improved by concomitant cisplatin.

Kishi, S., J. Griener, et al. (2003). "Homocysteine, Pharmacogenetics, and Neurotoxicity in Children With Leukemia." *J. Clin. Oncol.* **21**(16): 3084-3091.

<http://www.jco.org/cgi/content/abstract/21/16/3084>

Purpose: Despite its clinical success, methotrexate (MTX) therapy is associated with toxicities such as seizures, the pathogenesis of which remains unclear. It has been suggested that hyperhomocysteinemia is caused by MTX and is responsible for its neurotoxic effects. The purposes of this study were to explore whether hyperhomocysteinemia was related to MTX administration and toxicity and whether homocysteine or MTX toxicity differed by methylenetetrahydrofolate reductase (MTHFR) or reduced folate carrier (RFC) genetic polymorphisms. **Patients and Methods:** We studied 53 children with newly diagnosed acute lymphoblastic leukemia who were consecutively treated on a single clinical protocol that included two courses of high-dose MTX (high-dose methotrexate [HDMTX]; 2.5 or 5.0 g/m² per day) as consolidation therapy. **Results:** The study participants' median plasma homocysteine concentrations at 23 and 44 hours after HDMTX (9.00 {micro}mol/L and 10.12 {micro}mol/L, respectively) were greater than the concentrations immediately before HDMTX (5.77 {micro}mol/L, $P < .0001$ for both comparisons). Seven days after HDMTX treatment, their plasma concentration returned to baseline. Nine patients experienced seizures, and five patients experienced thrombosis during the first 15 months of therapy, with a tendency for there to be higher plasma homocysteine in patients with seizures across all time points ($P = .063$) but not in patients with thrombosis ($P = .59$). We observed no significant differences in plasma or cerebrospinal fluid homocysteine levels or in toxicity based on the MTHFR 677C/T or RFC 80G/A genotypes. **Conclusion:** We conclude that homocysteine was transiently elevated after HDMTX and may be related to seizure risk in children with leukemia.

Kohonen-Corish, M. R. J., J. J. Daniel, et al. (2005). "Low Microsatellite Instability Is Associated With Poor Prognosis in Stage C Colon Cancer." *J. Clin. Oncol.* **23**(10): 2318-2324.

<http://www.jco.org/cgi/content/abstract/23/10/2318>

PURPOSE: The significance of low microsatellite instability (MSI-L) in colorectal cancer is poorly understood. No clear biologic distinction has been found between MSI-L and microsatellite stable (MSS) colorectal cancer, and these two phenotypes are usually combined when analyzed against the well-defined high MSI (MSI-H) phenotype. Evidence is emerging that an O6-methylguanine DNA methyltransferase (MGMT) gene defect is associated with MSI-L. Therefore, to further define this phenotype, we undertook a detailed analysis of the prognostic significance of MSI-L and loss of MGMT expression in colon cancer. **PATIENTS AND METHODS:** The study cohort was 183 patients with clinicopathologic stage C colon cancer who had not received adjuvant therapy. We analyzed MSI status, MGMT, and mismatch repair protein expression, as well as MGMT and p16 promoter hypermethylation. **RESULTS:** We showed that MSI-L defines a group of patients with poorer survival ($P = .026$) than MSS patients, and that MSI-L was an independent prognostic indicator ($P = .005$) in stage C colon cancer. Loss of MGMT protein expression was associated with the MSI-L phenotype but was not a prognostic factor for overall survival in colon cancer. p16 methylation was significantly less frequent in MSI-L than in MSI-H and MSS tumors and was not associated with survival. **CONCLUSION:** MSI-L characterizes a distinct subgroup of stage C colon cancer patients, including the MSI-L subset of proximal colon cancer, who have a poorer outcome. Neither the MGMT defect nor p16 methylation are likely to contribute to the worse prognosis of the MSI-L phenotype.

Kuball, J., S. F. Wen, et al. (2002). "Successful Adenovirus-Mediated Wild-Type p53 Gene Transfer in Patients With Bladder Cancer by Intravesical Vector Instillation." *J. Clin. Oncol.* **20**(4): 957-965.

<http://www.jco.org/cgi/content/abstract/20/4/957>

PURPOSE: To study safety, feasibility, and biologic activity of adenovirus-mediated p53 gene transfer in patients with bladder cancer. **PATIENTS AND METHODS:** Twelve patients with histologically confirmed bladder cancer scheduled for cystectomy were treated on day 1 with a single intratumoral injection of SCH 58500 (rAd/p53) at cystoscopy at one dose level (7.5×10^{11} particles) or a single intravesical instillation of SCH 58500 with a transduction-enhancing agent (Big CHAP) at three dose levels (7.5×10^{11} to 7.5×10^{13} particles). Cystectomies were performed in 11 patients on day 3, and transgene expression, vector distribution, and biologic markers of transgene activity were assessed by molecular and immunohistochemical methods in tumors and normal bladder samples. **RESULTS:** Specific transgene expression was detected in tissues from seven of eight assessable patients treated with intravesical instillation of SCH 58500 but in none of three assessable patients treated with intratumoral injection of SCH 58500. Induction of RNA and protein expression of the p53 target gene p21/WAF1 was demonstrated in samples from patients treated with SCH 58500 instillation at higher dose levels. Distribution studies after intravesical instillation of SCH 58500 revealed both high transduction efficacy and vector penetration throughout the whole urothelium and into submucosal tumor cells. No dose-limiting toxicity was observed, and side effects were local and of transient nature. **CONCLUSION:** Intravesical instillation of SCH 58500 combined with a transduction-enhancing agent is safe, feasible, and biologically active in patients with bladder cancer. Studies to evaluate the clinical efficacy of this treatment in patients with localized high-risk bladder cancer are warranted.

Kuo, C. T., D. S. B. Hoon, et al. (2003). "Prediction of Disease Outcome in Melanoma Patients by Molecular Analysis of Paraffin-Embedded Sentinel Lymph Nodes." *J. Clin. Oncol.* **21**(19): 3566-3572.

<http://www.jco.org/cgi/content/abstract/21/19/3566>

Purpose: A significant number of patients who develop recurrence after a histopathologically negative sentinel lymph node (SLN) biopsy will demonstrate occult metastases on re-evaluation

of the SLNs with serial sectioning and immunohistochemistry. Reverse transcriptase polymerase chain reaction (RT-PCR) has been evaluated to improve disease staging and avoid false-negative findings in fresh or frozen-section SLNs. The purpose of this study was to develop a multimarker RT-PCR assay for assessing melanoma patients' archived paraffin-embedded (PE) SLNs. Patients and Methods: Archived PE histopathologically positive (n = 37) and negative (n = 40) SLNs from patients with primary melanoma were analyzed using a semiquantitative multimarker RT-PCR assay. Results: Marker expression in histopathologically positive and negative SLNs were as follows: 89%, 92%, 35%, and 43% (positive) and 40%, 33%, 5%, and 13% (negative) for tyrosinase, melanoma antigen recognized by T cells-1, tyrosinase-related protein-1, and tyrosinase-related protein-2, respectively. Twenty-five percent of histopathologically negative SLN patients were upstaged using at least two markers. Of these, 80% developed a recurrence. Furthermore, at a median follow-up of 55 months, patients with histopathologically negative SLNs who expressed zero or one marker had a significantly improved disease-free (P <.002) and overall (P <.03) survival versus those expressing two or more markers. Conclusion: These findings demonstrate the feasibility of a multimarker RT-PCR assay for evaluating archived PE SLNs. More significantly, identification of molecular risk factors can be detected in histopathologically negative SLNs for distinguishing early-stage melanoma patients with a worse prognosis.

Noura, S., H. Yamamoto, et al. (2002). "Comparative Detection of Lymph Node Micrometastases of Stage II Colorectal Cancer by Reverse Transcriptase Polymerase Chain Reaction and Immunohistochemistry." *J. Clin. Oncol.* **20**(20): 4232-4241.

<http://www.jco.org/cgi/content/abstract/20/20/4232>

PURPOSE: Inconsistent conclusions have been drawn about the clinical significance of micrometastases in lymph nodes (LNs) of node-negative colorectal cancer (CRC) patients. We performed a comparative study of detection of micrometastases using immunohistochemistry (IHC) by anti-cytokeratin antibody and carcinoembryonic antigen (CEA)-specific reverse-transcriptase polymerase chain reaction (RT-PCR) in the same patients, in an attempt to move closer to their clinical application. PATIENTS AND METHODS: Sixty-four CRC patients, with RNA of good quality available from paraffin-embedded LN specimens, were selected from 84 stage II patients who underwent curative surgery between 1988 and 1996. We investigated associations between the presence of micrometastases by each method and prognosis. RESULTS: Micrometastases were detected in 19 (29.6%) of 64 patients by RT-PCR and in 35 (54.7%) of 64 patients by IHC. By RT-PCR analysis, patients exhibiting a positive band for CEA mRNA had a significantly worse prognosis than those who were RT-PCR-negative, with respect to both disease-free and overall survival (P = .027 and .015, respectively). By IHC analysis, the presence of micrometastasis did not predict patient outcome in terms of either disease-free or overall survival. Infiltrating pattern of tumor growth characteristic was significantly associated with shorter disease-free survival among various clinical or pathologic factors. By multivariate Cox regression analysis, micrometastasis detected by RT-PCR and the Crohn's-like lymphoid reaction were both independent prognostic factors. CONCLUSION: Micrometastases detected by RT-PCR, but not IHC, may be of clinical value in identifying patients who may be at high risk for recurrence of CRC and who are therefore likely to benefit from systemic adjuvant therapy.

Ross, J. A., K. C. Oeffinger, et al. (2004). "Genetic Variation in the Leptin Receptor Gene and Obesity in Survivors of Childhood Acute Lymphoblastic Leukemia: A Report From the Childhood Cancer Survivor Study." *J. Clin. Oncol.* **22**(17): 3558-3562.

<http://www.jco.org/cgi/content/abstract/22/17/3558>

PURPOSE: Overweight (body mass index [BMI] 25 to 29 kg/m²) and obesity (BMI $\geq$ 30 kg/m²) frequently follow treatment for childhood acute lymphoblastic leukemia (ALL). Recent studies suggest that risk is most apparent in females treated with cranial radiation at a younger age. Because radiation at a young age may affect the hypothalamus causing leptin receptor insensitivity, we hypothesized that a polymorphism in the leptin receptor (LEPR) gene, Gln223Arg, might influence susceptibility to obesity in survivors of childhood ALL. **PATIENTS AND METHODS:** We genotyped 600 non-Hispanic white adult ALL survivors enrolled onto the Childhood Cancer Survivor Study. BMI was compared between those with two copies of the Arg allele to those who had at least one copy of the Gln allele. **RESULTS:** Female survivors with BMI $\geq$ 25 kg/m² were more likely Arg homozygous than those with BMI less than 25 kg/m² (24% v 12%; $P = .007$). This difference was not observed in males. Moreover, among females treated with $\geq$ 20 Gy cranial radiation, Arg/Arg individuals had six times higher odds of having BMI $\geq$ 25 kg/m² (95% CI, 2.1 to 22.0) than those with a Gln allele ($P = .04$ for interaction). **CONCLUSION:** LEPR polymorphism may influence obesity in female survivors of childhood ALL, particularly those exposed to cranial radiation. Because obesity is associated with increased morbidity and mortality in later life, identification of children at high risk might allow for early targeted interventions.

Schetelig, J., C. Thiede, et al. (2003). "Evidence of a Graft-Versus-Leukemia Effect in Chronic Lymphocytic Leukemia After Reduced-Intensity Conditioning and Allogeneic Stem-Cell Transplantation: The Cooperative German Transplant Study Group." *J. Clin. Oncol.* **21**(14): 2747-2753.

<http://www.jco.org/cgi/content/abstract/21/14/2747>

Purpose: To study whether hematopoietic stem-cell transplantation (HSCT) after reduced-intensity conditioning is effective and tolerable in patients with advanced chronic lymphocytic leukemia (CLL). **Patients and Methods:** Thirty patients with advanced B-cell CLL were included into the study. After reduced-intensity conditioning with fludarabine, busulfan, and antithymocyte globulin, patients received a transplant from related ($n = 15$) or unrelated donors ($n = 15$). Minimal residual disease (MRD) was monitored with a clone-specific polymerase chain reaction. **Results:** After a median follow-up of 2 years, 23 patients are alive (to date). Neutrophil and platelet engraftment occurred after a median of 17.5 and 15 days, respectively. Acute graft-versus-host disease (GVHD) grade 2 to 4 was observed in 17 patients (56%), and chronic GVHD was observed in 21 patients (75%). Twelve patients (40%) achieved a complete remission (CR), and 16 patients (53%) achieved a partial remission. Late CR occurred up to 2 years after transplantation. MRD was monitored in eight patients with CR. All patients achieved a molecular CR. At last follow-up, six patients were in ongoing molecular CR. Causes of death were treatment-related complications in four patients and progressive disease in three patients. The probability of overall survival, progression-free survival, and nonrelapse mortality at 2 years was 72% (95% confidence interval [CI], 54% to 90%), 67% (95% CI, 49% to 85%), and 15% (95% CI, 1% to 29%), respectively. **Conclusion:** Treatment-related mortality after reduced-intensity conditioning followed by allogeneic HSCT was low. The procedure induced molecular remissions in patients with advanced CLL. The observation of late remissions provided evidence of a graft-versus-leukemia effect.

Sihto, H., M. Sarlomo-Rikala, et al. (2005). "KIT and Platelet-Derived Growth Factor Receptor Alpha Tyrosine Kinase Gene Mutations and KIT Amplifications in Human Solid Tumors." *J. Clin. Oncol.* **23**(1): 49-57.

<http://www.jco.org/cgi/content/abstract/23/1/49>

PURPOSE: Mutated KIT and platelet-derived growth factor receptor alpha (PDGFR{alpha}) tyrosine kinases are the principal targets for imatinib mesylate in the treatment of gastrointestinal stromal tumors (GISTs). The frequency of activating KIT and PDGFRA gene mutations in most other histologic types of human cancer is not known. **MATERIALS AND METHODS:** KIT exons 9, 11, 13, and 17 and PDGFRA exons 11 and 17 of 334 human cancers were screened for mutations using sensitive denaturing high-performance liquid chromatography (DHPLC). In addition, all KIT exons from 9 to 21 of 115 tumors were screened. Thirty-two histologic tumor types were examined. Samples with abnormal findings in DHPLC were sequenced. Immunostaining for the KIT protein (CD117) was performed in 322 (96.4%) of the 334 cases. **RESULTS:** Of the 3,039 exons screened, only 17 had mutation. All 17 cases with either mutated KIT (n = 15) or PDGFRA (n = 2) were histologically GIST tumors, whereas none of the other histologic types of cancer (n = 316) harbored KIT or PDGFRA mutation. KIT immunostaining was rarely positive except in GISTs (18 of 18), small-cell lung cancer (10 of 30; 33%), and testicular teratocarcinoma (four of 17; 24%). Wild-type KIT gene amplification or chromosome 4 aneuploidy was common (seven of 12) in non-GIST tumors with strong KIT protein expression when studied with fluorescence in situ hybridization. **CONCLUSION:** Despite frequent KIT protein expression in some tumor types, KIT and PDGFRA gene mutations are uncommon in most human cancers. Cancer KIT expression is frequently associated with multiple copies of the wild-type KIT gene.

Sozzi, G., D. Conte, et al. (2003). "Quantification of Free Circulating DNA As a Diagnostic Marker in Lung Cancer." *J. Clin. Oncol.* **21**(21): 3902-3908.

<http://www.jco.org/cgi/content/abstract/21/21/3902>

Purpose: Analysis of circulating DNA in plasma can provide a useful marker for earlier lung cancer detection. This study was designed to assess the sensitivity and specificity of a quantitative molecular assay of circulating DNA to identify patients with lung cancer and monitor their disease. **Materials and Methods:** The amount of plasma DNA was determined through the use of real-time quantitative polymerase chain reaction (PCR) amplification of the human telomerase reverse transcriptase gene (hTERT) in 100 non-small-cell lung cancer patients and 100 age-, sex-, and smoking-matched controls. Screening performance of the assay was calculated through the receiver operating characteristic (ROC) curve. Odds ratios were calculated using conditional logistic regression analysis. **Results:** Median concentration of circulating plasma DNA in patients was almost eight times the value detected in controls (24.3 v 3.1 ng/mL). The area under the ROC curve was 0.94 (95% CI, 0.907 to 0.973). Plasma DNA was a strong risk factor for lung cancer; concentrations in the upper tertile were associated with an 85-fold higher risk than were those in the lowest tertile. **Conclusion:** This study shows that higher levels of free circulating DNA can be detected in patients with lung cancer compared with disease-free heavy smokers by a PCR assay, and suggests a new, noninvasive approach for early detection of lung cancer. Levels of plasma DNA could also identify higher-risk individuals for lung cancer screening and chemoprevention trials.

Weng, W.-K., D. Czerwinski, et al. (2004). "Clinical Outcome of Lymphoma Patients After Idiotype Vaccination Is Correlated With Humoral Immune Response and Immunoglobulin G Fc Receptor Genotype." *J. Clin. Oncol.* **22**(23): 4717-4724.

<http://www.jco.org/cgi/content/abstract/22/23/4717>

PURPOSE: The unique immunoglobulin idiotype (Id) expressed by each B-cell lymphoma is a target for immunotherapy. Vaccination with Id induces humoral and/or cellular anti-Id immune responses. However, the clinical impact of these anti-Id immune responses is unknown. We and others have previously reported that immunoglobulin G Fc receptor (Fc{gamma}R)

polymorphisms predict the clinical response of lymphoma patients to passive anti-CD20 antibody infusions. In this study, we tested whether anti-Id immune responses or Fc{gamma}R polymorphisms associate with clinical outcome of patients who received Id vaccination. PATIENTS AND METHODS: We analyzed 136 patients with follicular lymphoma who had received Id vaccination. The anti-Id immune responses were measured and Fc{gamma}RIIIa and Fc{gamma}RIIa polymorphisms were determined and correlated with clinical outcome for these patients. RESULTS: Patients who mounted humoral immune responses had a longer progression-free survival (PFS) than those who did not (8.21 v 3.38 years; $P = .018$). Patients with Fc{gamma}RIIIa 158 valine/valine (V/V) genotype also had a longer PFS than those with valine/phenylalanine (V/F) or phenylalanine/phenylalanine (F/F) genotypes (V/V, 8.21 v V/F, 3.38 years; $P = .004$; v F/F, 4.47 years; $P = .035$). Multivariate analysis using the Cox proportional hazards model showed that V/V genotype and humoral immune responses were independent positive predictors for PFS. CONCLUSION: This study is the first to identify the predictive value of Fc{gamma}R polymorphism on clinical outcome in patients who received active immunotherapy with tumor antigen vaccines. Our results imply that the antibodies induced against a tumor antigen are beneficial and that Fc{gamma}R-bearing cells mediate an antitumor effect by killing antibody-coated tumor cells.

J. Clin. Pathol. (11)

Alonso, A., C. Alves, et al. (2005). "Mitochondrial DNA haplotyping revealed the presence of mixed up benign and neoplastic tissue sections from two individuals on the same prostatic biopsy slide." J. Clin. Pathol. **58**(1): 83-86.

<http://jcp.bmjjournals.com/cgi/content/abstract/58/1/83>

DNA typing was requested to investigate a presumptive cancer diagnosis error by confirming whether benign and cancerous prostatic tissue in the same presurgical haematoxylin and eosin stained slide belonged to the same person. After independent histological re-examination of the slide by a pathologist, manual slide dissection was used to guarantee independent and high recovery DNA isolation from each tissue section, avoiding carryover and background contamination. Nuclear DNA quantification performed by real time polymerase chain reaction (PCR) revealed the absence of human DNA for short tandem repeat (STR) typing. Mitochondrial DNA was only obtained by performing PCR of very short fragments (~100 bp), indicating high DNA degradation. Different low frequency hypervariable region I haplotypes were obtained from each tissue section (normal tissue section haplotype: 16224C, 16234T, 16311C, 16356C; cancer tissue section haplotype: 16256T, 16270T, 16293G). Only the normal tissue section haplotype matched that obtained from the patient's blood sample, indicating that the cancer tissue section originated from an unknown patient. These results supported the hypothesis of sample mix up during block processing or slide preparation by a carryover mechanism. Mitochondrial genetic typing is recommended to exclude the possibility of carryover artefacts when low DNA content and high degradation compromise conventional STR typing.

Bohm, J., S. Kock, et al. (2003). "Evidence of clonality in chronic neutrophilic leukaemia." J. Clin. Pathol. **56**(4): 292-295.

<http://jcp.bmjjournals.com/cgi/content/abstract/56/4/292>

Background: Chronic neutrophilic leukaemia (CNL) is a rare myeloproliferative disorder of elderly patients characterised by sustained neutrophilia and splenomegaly. The diagnosis of CNL requires the exclusion of BCR/ABL positive chronic myelogenous leukaemia (CML) and of leukaemoid reactions (LRs). The differentiation between CNL and LR is problematic because both conditions share similar morphological features; it is also important because patients with CNL generally have a poor prognosis. Aims: To determine whether CNL and LR could be distinguished on the basis of different clonality patterns. Methods: Blood samples from 52 women were studied using the human androgen receptor gene assay (HUMARA). Results: Monoclonality was found in the neutrophils in all 17 patients with different myeloproliferative syndromes (MPSs), including those with CNL. In four of the patients with CNL, autologous T cells were also monoclonal, suggesting that they belonged to the neoplastic clone. This finding was in contrast to other MPSs in which T cells were almost always polyclonal. Of nine patients with clinically suspected LR, the neutrophils of five were polyclonal, whereas three patients had monoclonal neutrophils, suggesting that they might be in the process of developing an MPS. Among 26 healthy blood donors, 20 had polyclonal neutrophils and five showed skewed clonality patterns. One case of LR and one normal blood donor were scored "not informative" at the HUMARA locus. Conclusions: Clonality studies of blood neutrophils using HUMARA aid in distinguishing female patients with monoclonal CNL from those with LR. For the diagnosis of CNL, monoclonality of the neutrophils should be demonstrated whenever possible.

Cheng, V. C. C., W. C. Yam, et al. (2004). "Clinical evaluation of the polymerase chain reaction for the rapid diagnosis of tuberculosis." *J. Clin. Pathol.* **57**(3): 281-285.

<http://jcp.bmjjournals.com/cgi/content/abstract/57/3/281>

Aims: Use of the polymerase chain reaction for the detection of Mycobacterium tuberculosis (TB PCR) as a basis for making clinical decisions on the initiation of antituberculosis treatment was studied. Methods: A retrospective study involving a cohort of 155 patients being investigated for tuberculosis in an infectious disease consultation service was undertaken. TB PCR was performed on pulmonary and extrapulmonary specimens from these patients. The sensitivity of TB PCR was analysed. Results: Of the 155 patients, 144 fitted the clinical diagnosis of tuberculosis, and 112 of them were culture positive for M tuberculosis. Sixty (58.3%) patients with clinical features suggestive of tuberculosis received antituberculosis treatment based on positive TB PCR alone. Of 224 clinical specimens (138 pulmonary and 86 extrapulmonary) sent for TB PCR, 148 (99 pulmonary and 49 extrapulmonary) were positive in 117 patients. Of the 690 clinical specimens sent for culture, 279 were positive for M tuberculosis in 112 patients. The diagnostic sensitivity of TB PCR was 75.9% (85 of 112) and 81.3% (117 of 144) in patients with culture confirmed and clinically diagnosed tuberculosis, respectively. Using culture as the gold standard, the overall sensitivity of TB PCR was 78.3%, and for pulmonary and extrapulmonary specimens it was 82.3% and 72.0%, respectively. Conclusions: TB PCR is a rapid and reliable test in the diagnosis and management of tuberculosis.

Choesmel, V., F. Foucault, et al. (2004). "Design of a real time quantitative PCR assay to assess global mRNA amplification of small size specimens for microarray hybridisation." *J. Clin. Pathol.* **57**(12): 1278-1287.

<http://jcp.bmjjournals.com/cgi/content/abstract/57/12/1278>

Background: Low RNA yields from clinical samples are a limiting step for microarray technology. Aims: To design an accurate real time quantitative polymerase chain reaction (PCR) assay to assess the crucial step of global mRNA amplification performed before microarray hybridisation, using less than 1 {micro}g total RNA. Methods: Three RNA extraction procedures were compared

for small size samples. Total RNA was amplified from universal RNA or the BC-H1 breast cancer micrometastatic cell line using three different protocols. Real time quantitative PCR technology was used for accurate measurement of urokinase plasminogen activator receptor and cytokeratin 8 RNA amplification rates and ratios, using primer sets binding at various distances from the 3' end of transcripts. A 50 mer oligomeric array targeting 87 genes potentially involved in breast cancer metastatic progression was built and hybridised with amplified RNA. Results: Eighteen nanograms of total RNA could be purified from 1000 BC-H1 micrometastatic cells. Amplification rates of 25 000 to 100 000 were achieved with as little as 10 ng of starting material. However, results were highly variable, depending on the amount of starting material, gene characteristics, sample quality, and protocols used. Oligomeric array hybridisation with 20 {micro}g reference RNA resulted in specific and reproducible signals for 83% of the genes, whereas mRNA amplification from less than 400 ng of starting material resulted in selective detection of signals from highly expressed genes. Conclusions: Improvements in the design of global mRNA amplification procedures and oligomeric arrays are needed to extract informative gene expression data from clinical samples containing limited cell numbers.

Cruz, I., P. J. F. Snijders, et al. (2002). "Specific p53 immunostaining patterns are associated with smoking habits in patients with oral squamous cell carcinomas." J. Clin. Pathol. **55**(11): 834-840.

<http://jcp.bmjjournals.com/cgi/content/abstract/55/11/834>

Aims: To identify immunostaining patterns that are predictive for p53 mutations and to investigate whether p53 mutations are associated with established risk factors for oral squamous cell carcinoma (OSCC). Methods: Fifty five OSCCs were investigated for p53 protein expression by immunohistochemistry (IHC). Ten of these cases, including five p53 immunopositive and five p53 immunonegative cases, were subjected to microdissection of representative tumour areas followed by sequence analysis for the detection of TP53 mutations. Results: Paired IHC and sequence analysis revealed that p53 immunoeexpression in more than 25% of tumour cells was indicative of TP53 mutations, whereas p53 immunonegativity was not informative. Therefore, for p53 immunohistochemical interpretation, p53 immunonegative cases were excluded from the analysis and the cut off value for p53 immunoeexpression was set at 25%. Of the OSCCs showing any p53 immunoeexpression, 64% revealed staining in more than 25% of the tumour cells. p53 immunoeexpression in more than 25% of the neoplastic cells was significantly associated with smoking but not with alcohol consumption. No significant association with smoking habits was found when OSCCs were dichotomised into p53 immunonegative and p53 immunopositive. Conclusions: In OSCCs the following conclusions can be made: (1) p53 immunonegativity is not informative for TP53 mutations; (2) 25% p53 immunopositive cells appears to be a good cut off value to predict TP53 mutations; (3) p53 immunostaining patterns that appeared to be predictive for TP53 mutations were associated with the smoking habits of the patients.

Jass, J. R., M. Barker, et al. (2003). "APC mutation and tumour budding in colorectal cancer." J. Clin. Pathol. **56**(1): 69-73.

<http://jcp.bmjjournals.com/cgi/content/abstract/56/1/69>

Aim: To determine the frequency of tumour budding and somatic APC mutation in a series of colorectal cancers stratified according to DNA microsatellite instability (MSI) status. Material/Methods: Ninety five colorectal cancers were genotyped for APC mutation in the mutation cluster region (exon 15) and scored for the presence of tumour budding at the invasive margin in haematoxylin and eosin stained sections. A subset was immunostained for {beta} catenin and p16. Results: The frequency of both somatic APC mutation and tumour budding increased *pari passu* in cancers stratified as sporadic MSI high (MSI-H), hereditary non-polyposis

colorectal cancer (HNPCC), MSI low (MSI-L), and microsatellite stable (MSS). Both budding and APC mutation were significantly less frequent in sporadic MSI-H cancers than in MSI-L or MSS cancers. Tumour buds were characterised by increased immunostaining for both {beta} catenin and p16. Conclusion: Tumour budding is associated with an adverse prognosis. The lack of budding in MSI-H colorectal cancer may account for the improved prognosis of this subset and may be explained by an intact WNT signalling pathway and/or inactivated p16INK4a.

Nakamura, S., T. Matsumoto, et al. (2003). "Chromosomal translocation t(11;18)(q21;q21) in gastrointestinal mucosa associated lymphoid tissue lymphoma." J. Clin. Pathol. **56**(1): 36-42.

<http://jcp.bmjjournals.com/cgi/content/abstract/56/1/36>

Aims: To evaluate the chromosomal translocation t(11;18)(q21;q21) in gastrointestinal lymphomas. Methods: A possible API2-MLT fusion transcript specific to t(11;18)(q21;q21) was examined by means of reverse transcription-polymerase chain reaction (RT-PCR) in tumours from 47 cases of primary gastrointestinal lymphoma (28 low grade mucosa associated lymphoid tissue (MALT) lymphomas, four low grade MALT lymphomas with a high grade component, nine secondary diffuse large B cell lymphomas, four primary diffuse large B cell lymphomas, and two T cell lymphomas). Results: API2-MLT fusion was seen in four of 28 cases of low grade MALT lymphoma, but it was not seen in other types of lymphoma. Among the low grade MALT lymphomas, the fusion transcript was seen more frequently in colonic tumours than in gastric tumours (two of three compared with two of 24) and in tumours with submucosal invasion than in those confined to the mucosa (four of 13 compared with 0 of 15). Helicobacter pylori negative tumours tended to show a higher positive rate than H pylori positive tumours (three of six compared with one of 21). None of the gastric tumours that responded to H pylori eradication expressed the API2-MLT fusion transcript. Conclusions: t(11;18)(q21;q21) seems to be one of the genetic alterations related to the development of gastrointestinal low grade MALT lymphoma. Such translocations may be predominantly associated with the development of intestinal MALT lymphoma.

Wong, S. C. C., E. S. F. Lo, et al. (2004). "An optimised protocol for the extraction of non-viral mRNA from human plasma frozen for three years." J. Clin. Pathol. **57**(7): 766-768.

<http://jcp.bmjjournals.com/cgi/content/abstract/57/7/766>

Aims: To detect non-viral mRNA in human plasma that has been frozen for three years using a new protocol. Methods: Plasma from 15 patients with colorectal cancer and 10 normal subjects was separated and frozen with Trizol at -80{degrees}C for three years. As a control measure, plasma from 10 of the 15 patients was separated using the same protocol but no Trizol during storage. After three years, all samples were extracted using Trizol and RNeasy before the reverse transcriptase polymerase chain reaction was performed to detect non-viral {beta} catenin mRNA. In addition, extraction of three plasma samples by Trizol or RNeasy independently was carried out for comparison. Results: {beta} Catenin mRNA was detected in all 15 patient plasma samples and only one of the 10 normal subjects. In contrast, no {beta} catenin mRNA was found in the control and patient samples that were independently extracted by Trizol and RNeasy kit. Conclusions: This new protocol is a reliable method for extracting non-viral mRNA from the plasma of patients with cancer after longterm storage for three years. Extractions using Trizol and RNeasy kits independently could not isolate mRNA with sufficient quantity and quality for detection.

Xu, J., B. C. Millar, et al. (2002). "Comparison of API20C with molecular identification of *Candida* spp isolated from bloodstream infections." J. Clin. Pathol. **55**(10): 774-777.

<http://jcp.bmjjournals.com/cgi/content/abstract/55/10/774>

A study was carried out to compare the API20C technology with polymerase chain reaction amplification and direct sequencing of the short internal transcribed spacer region 2 (ITS2) for the identification of 58 isolates of invasive *Candida* species obtained from patients with bloodstream infections over the seven year period 1994 to 2000. Overall, there was only one disagreement between the phenotypic and genotypic identification, where the API scheme identified the isolate as *C. albicans* but the molecular method identified it as *C. dubliniensis*. This study demonstrated that the API20C method is useful in the identification of *Candida* spp isolated from blood culture and that molecular methods do not enhance identifications made using the API20C scheme. However, for correct reporting of *C. dubliniensis*, an emerging bloodborne pathogen, it is recommended that all isolates identified as *C. albicans* by the API20C scheme are further examined phenotypically and/or genotypically.

Youshya, S., K. Purdie, et al. (2005). "Does human papillomavirus play a role in the development of bladder transitional cell carcinoma? A comparison of PCR and immunohistochemical analysis." J. Clin. Pathol. **58**(2): 207-210.

<http://jcp.bmjjournals.com/cgi/content/abstract/58/2/207>

Aim: To investigate the role of human papillomavirus (HPV) in the development of bladder transitional cell carcinoma (TCC). **Methods:** Seventy eight paraffin wax embedded TCC samples were tested for the presence of HPV by two methods. First, immunohistochemistry was carried out using a polyclonal antibody capable of detecting the capsid protein of all known papillomaviruses. The second method was a consensus GP5+/6+ primer mediated polymerase chain reaction (PCR) technique, with the products analysed by both agarose gel electrophoresis and an enzyme immunoassay using type specific oligonucleotide probes for 10 different mucosal genotypes. To exclude false negative results because of the poor quality of DNA extracted from paraffin wax embedded samples, the series was extended to include 20 further blocks for which the corresponding snap frozen unfixed tissue was available. **Results:** The two methods produced contrasting results, with 47 of the 78 samples positive for HPV antigen and none positive for HPV DNA. HPV DNA was not detected in the 20 additional paraffin wax embedded TCCs or in the 20 paired unfixed samples. In contrast, HPV DNA was amplified by PCR from all six of the paraffin wax embedded cervical carcinoma and anogenital wart control samples. **Conclusion:** The disparity between the two sets of results is probably caused by false positives resulting from the non-specificity of the polyclonal antibody used for immunohistochemistry. These results suggest that HPV is unlikely to play an aetiological role in the development of bladder TCC.

Zhang, S. H., W. M. Cong, et al. (2004). "Focal nodular hyperplasia with concomitant hepatocellular carcinoma: a case report and clonal analysis." J. Clin. Pathol. **57**(5): 556-559.

<http://jcp.bmjjournals.com/cgi/content/abstract/57/5/556>

This report describes a hepatocellular carcinoma (HCC) with concomitant focal nodular hyperplasia (FNH) in a 56 year old Chinese man. There were two well circumscribed tumours measuring 3 x 2.5 x 2 cm and 2 x 1.5 x 1.5 cm. The larger mass was grey and soft with a small area of bleeding and necrosis and an intact capsule. The smaller mass was yellow and had no capsule. Clonal analysis was carried out to clarify the relation between the HCC and the adjacent

FNH. The clonal analysis was based on the methylation pattern of the polymorphic X chromosome linked androgen receptor gene (HUMARA). In FNH, after HpaII digestion, the allelic bands showed two well defined peaks. The intensity of the two peaks in the DNA from cirrhotic tissue did not differ significantly, consistent with a random pattern of X chromosome inactivation. However, in HCC, after HpaII digestion, the allelic bands differed significantly in intensity. Therefore, there was a typical polyclonal pattern of inactivation in FNH but the HCC was interpreted as being monoclonal.

J. Clin. Pharmacol. (1)

Putnam, W. S., J. M. Woo, et al. (2005). "Effect of the MDR1 C3435T Variant and P-Glycoprotein Induction on Dicloxacillin Pharmacokinetics." J. Clin. Pharmacol. **45**(4): 411-421.

<http://jcp.sagepub.com/cgi/content/abstract/45/4/411>

This study investigated 2 hypotheses about genotype-phenotype relationships for the efflux transporter, P-glycoprotein: (1) the presence of a synonymous C3435T variant in exon 26 of the MDR1 gene correlates to higher plasma concentrations of a P-glycoprotein substrate, dicloxacillin, and (2) the effects of genotypic differences decrease under conditions of P-glycoprotein induction by rifampin. Eighteen healthy volunteers received two 1-g doses of dicloxacillin, one on the 1st study day and the other on the 11th day of rifampin dosing (600 mg daily). Dicloxacillin and its 5-hydroxymethyl metabolite were analyzed using liquid chromatography/tandem mass spectrometry. Mean dicloxacillin C_{max} measurements were 30.5 {+/-} 13.5, 33.3 {+/-} 4.7, and 31.1 {+/-} 12.8 {micro}g/mL in individuals with the CC, CT, and TT genotype at position 3435 in exon 26 of the MDR1 gene. Following rifampin dosing, the mean dicloxacillin C_{max} across genotypes decreased from 31.4 {+/-} 10.8 to 22.9 {+/-} 7.0 {micro}g/mL (P < .05), whereas the mean oral clearance increased from 235 {+/-} 82 to 297 {+/-} 71 mL/min (P < .001), and the mean absorption time increased from 0.71 {+/-} 0.55 to 1.34 {+/-} 0.77 h (P < .05). Rifampin treatment increased the formation clearance, C_{max}, and AUC of the 5-hydroxymethyl metabolite by 135%, 119%, and 59%, respectively. The C3435T variant had no effect on dicloxacillin pharmacokinetics. The data suggested that rifampin induced intestinal P-glycoprotein and increased dicloxacillin metabolism.

J. Dent. Res. (10)

Dale, J. B., S. L. Sarich, et al. (2002). "Hormonal Regulation of Androgen Receptor Messenger Ribonucleic Acid Expression in Human Tooth Pulp." J. Dent. Res. **81**(5): 360-365.

<http://jdr.iadrjournals.org/cgi/content/abstract/81/5/360>

Tooth pulp contains steroid receptors and therefore is likely to respond to steroids. Steroids and cytokines together can alter steroid receptor content in many tissues; thus, similar mechanisms may exist in tooth pulp. In this study, reverse-transcription/polymerase chain-reaction was used to

screen human pulp for the mRNAs encoding receptors for androgen (AR), estrogens (ER{beta}), and hepatocyte growth factor (HGF: c-Met). AR mRNA content was greater in male pulp vs. female pulp in all age groups. In both genders, AR mRNA content diminished with age. In pulp cell cultures, androstenedione, estradiol-17{beta}, and HGF each stimulated AR mRNA accumulation. Testosterone inhibited, whereas 5{alpha}-dihydrotestosterone did not affect, AR mRNA content. ER{beta} was not hormonally altered in pulp cell cultures. By showing steroid- and cytokine-orchestrated regulation of AR mRNA in vitro, it is possible that age- and/or pathogen-dependent changes in available steroids and cytokines can affect any androgen-responsiveness of pulp.

Gandhi, M., D. M. Koelle, et al. (2004). "Prevalence of Human Herpesvirus-8 Salivary Shedding in HIV Increases with CD4 Count." J. Dent. Res. **83**(8): 639-643.

<http://jdr.iadrjournals.org/cgi/content/abstract/83/8/639>

Human herpesvirus-8 (HHV-8) is the etiologic agent of Kaposi's sarcoma (KS), which occurs in epidemic form in human immunodeficiency virus(HIV)-infected individuals. Saliva is the only mucosal fluid in which infectious HHV-8 has been identified, although factors associated with HHV-8 salivary shedding remain unclear. Our study performed PCR analysis for HHV-8 DNA in saliva (and other body fluids) in 66 HIV- and HHV-8-co-infected women without KS so that we could examine predictors for HHV-8 DNA detection. CD4 count was the most significant predictor of HHV-8 salivary shedding, with increased prevalence of HHV-8 salivary DNA at higher CD4 counts. The odds of salivary HHV8 shedding at CD4 counts ≥ 350 cells/{micro}L was 63 times the odds of shedding at CD4 < 350 (95%CI, 1.3-3078), with an increase in effect size when the analysis was restricted to those with a CD4 nadir > 200 . Analysis of these data suggests an increased potential for HHV-8 transmission early in HIV infection, with implications for HHV-8 prevention.

Hamlet, S., R. Ellwood, et al. (2004). "Persistent Colonization with *Tannerella forsythensis* and Loss of Attachment in Adolescents." J. Dent. Res. **83**(3): 232-235.

<http://jdr.iadrjournals.org/cgi/content/abstract/83/3/232>

Colonization with *Tannerella forsythensis* may characterize the conversion of periodontally healthy sites into diseased sites. This three-year study describes the prevalence of *T. forsythensis* and its relationship to clinical loss of attachment (LOA) in a group of adolescents considered at risk of developing early chronic periodontitis. Adolescents with (LOA+) and without (LOA-) loss of attachment were examined at baseline and 1.5 and 3 yrs subsequently. On each occasion, attachment loss was measured on selected teeth, and the presence of *T. forsythensis* in their subgingival plaque samples was determined by PCR. *T. forsythensis* prevalence in LOA+ subjects at baseline (64%) increased to 82% and 86% on subsequent examinations. In contrast, prevalence of *T. forsythensis* in LOA- subjects was always significantly lower (25%, 36%, and 32%, respectively). The odds of loss of attachment were 8.16 times greater in subjects infected with *T. forsythensis* at each examination. These results suggest that *T. forsythensis* is strongly associated with loss of attachment in this adolescent population.

Kida, M., T. Ariga, et al. (2002). "Autosomal-dominant Hypoplastic Form of Amelogenesis Imperfecta Caused by an Enamelin Gene Mutation at the Exon-Intron Boundary." J. Dent. Res. **81**(11): 738-742.

<http://jdr.iadrjournals.org/cgi/content/abstract/81/11/738>

Amelogenesis imperfecta (AI) is currently classified into 14 distinct subtypes based on various phenotypic criteria; however, the gene responsible for each phenotype has not been defined. We performed molecular genetic studies on a Japanese family with a possible autosomal-dominant form of AI. Previous studies have mapped an autosomal-dominant human AI locus to chromosome 4q11-q21, where two candidate genes, ameloblastin and enamelin, are located. We studied AI patients in this family, focusing on these genes, and found a mutation in the enamelin gene. The mutation detected was a heterozygous, single-G deletion within a series of 7 G residues at the exon 9-intron 9 boundary of the enamelin gene. The mutation was detected only in AI patients in the family and was not detected in other unaffected family members or control individuals. The male proband and his brother showed hypoplastic enamel in both their deciduous and permanent teeth, and their father showed local hypoplastic defects in the enamel of his permanent teeth. The clinical phenotype of these patients is similar to that of the first report of AI caused by an enamelin gene mutation. Thus, heterogeneous mutations in the enamelin gene are responsible for an autosomal-dominant hypoplastic form of AI.

Klein, M. L., P. Nieminen, et al. (2005). "Novel Mutation of the Initiation Codon of PAX9 Causes Oligodontia." *J. Dent. Res.* **84**(1): 43-47.

<http://jdr.iadrjournals.org/cgi/content/abstract/84/1/43>

Tooth development is under strict genetic control. Oligodontia is defined as the congenital absence of 6 or more permanent teeth, excluding the third molar. The occurrence of non-syndromic oligodontia is poorly understood, but in recent years several cases have been described where a single gene mutation is associated with oligodontia. Several studies have shown that MSX1 and PAX9 play a role in early tooth development. We screened one family with non-syndromic oligodontia for mutations in MSX1 and PAX9. The pedigree showed an autosomal-dominant pattern of inheritance. Direct sequencing and restriction enzyme analysis revealed a novel heterozygous A to G transition mutation in the AUG initiation codon of PAX9 in exon 1 in the affected members of the family. This is the first mutation found in the initiation codon of PAX9, and we suggest that it causes haploinsufficiency.

Murakami, S., N. Yoshimura, et al. (2002). "Activation of Adenosine-receptor-enhanced iNOS mRNA Expression by Gingival Epithelial Cells." *J. Dent. Res.* **81**(4): 236-240.

<http://jdr.iadrjournals.org/cgi/content/abstract/81/4/236>

A series of reports has revealed that adenosine has a plethora of biological actions toward a large variety of cells. In this study, we investigated the influence of adenosine receptor activation on iNOS mRNA expression in human gingival epithelial cells (HGEC) and SV-40-transformed HGEC. HGEC expressed adenosine receptor subtypes A1, A2a, and A2b, but not A3 mRNA. Ligation of adenosine receptors by a receptor agonist, 2-chloroadenosine (2CADO), enhanced iNOS mRNA expression by both HGEC and transformed HGEC. In addition, the adenosine receptor agonist enhanced the production of NO₂-/NO₃-, NO-derived stable end-products. An enhanced expression of iNOS mRNA and NO₂-/NO₃- was also observed when SV40-transformed HGEC were stimulated with CPA or CGS21680, A1- or A2a-selective adenosine receptor agonists, respectively. These results provide new evidence for the possible involvement of adenosine in the regulation of inflammatory responses by HGEC in periodontal tissues.

Nakano, K., M. Matsumura, et al. (2002). "Attenuation of Glucan-binding Protein C Reduces the Cariogenicity of Streptococcus mutans: Analysis of Strains Isolated from Human Blood." J. Dent. Res. **81**(6): 376-379.

<http://jdr.iadrjournals.org/cgi/content/abstract/81/6/376>

A blood isolate of Streptococcus mutans strain TW871 shows relatively low homology with MT8148, a reference oral isolate strain, and lacks the serotype-specific polysaccharide antigen, suggesting that other cell-surface structures correlate with cariogenicity. We compared cariogenicity of TW871 with MT8148 (serotype c) and blood isolate TW964 (serotype f) in rats. Strain TW871 showed significantly lower cariogenicity than MT8148 or TW964 and expressed significantly lower sucrose-independent cellular adhesion to saliva-coated hydroxyapatite and dextran-binding activity than strain MT8148. Strains TW871 and TW964 showed a defect in the gbpA gene by Southern hybridization analysis, while sequencing analysis revealed gbpC variation in TW871. These results suggest that variation in GbpC may alter cellular adherence properties and can be correlated with the cariogenicity of S. mutans in this strain.

Tamesada, M., S. Kawabata, et al. (2004). "Synergistic Effects of Streptococcal Glucosyltransferases on Adhesive Biofilm Formation." J. Dent. Res. **83**(11): 874-879.

<http://jdr.iadrjournals.org/cgi/content/abstract/83/11/874>

Glucosyltransferases (GTF)-I and GTF-SI of Streptococcus mutans synthesize water-insoluble and both water-soluble and -insoluble glucans, respectively, and play essential roles in the sucrose-dependent adhesion of the organism to tooth surfaces. To examine the interactions of different GTFs on artificial biofilm formed by S. mutans and other oral streptococci, we generated GTF-I- and GTF-SI-hyperproducing isogenic mutant strains. Transformant B42-21, which hyperexpressed GTF-SI, exhibited firm adhesion in the presence of sucrose, whereas transformant B42-10, which hyperexpressed GTF-I, failed to exhibit firm adhesion. Furthermore, co-culture of transformant B42-21 with water-soluble glucan-synthesizing Streptococcus sanguinis yielded firm adhesion, while the addition of dextran T10 to B42-21 growing culture had no effect on adhesion. These findings suggest that GTF-SI has a strong effect on sucrose-dependent adhesion and is essential for biofilm formation on smooth surfaces, in cooperation with water-soluble glucans synthesized de novo by oral streptococci that inherently lack cell adhesion ability.

Tanaka, C., K. Uzawa, et al. (2003). "Expression of an Inhibitor of Apoptosis, Survivin, in Oral Carcinogenesis." J. Dent. Res. **82**(8): 607-611.

<http://jdr.iadrjournals.org/cgi/content/abstract/82/8/607>

A novel inhibitor of apoptosis, survivin, plays a role in oncogenesis. To determine the potential involvement of survivin in oral carcinogenesis, we investigated the distribution of survivin protein expression in oral squamous cell carcinomas (OSCCs) and oral pre-malignant lesions. The mRNA expression level and methylation status of the gene also were evaluated in OSCCs and OSCC-derived cell lines. In immunohistochemistry, 58% of tumors and 37% of pre-malignant lesions examined were positive for survivin, while no immunoreaction was observed in corresponding normal tissues. The reverse-transcription/polymerase chain-reaction revealed similar changes in survivin gene expression levels. Furthermore, of the 9 normal oral tissues with no survivin gene expression, 4 showed methylation of the gene, while no methylation was detected in the corresponding tumorous tissues. The results suggest that survivin plays an

important role during oral carcinogenesis, and that the gene expression may be regulated by an epigenetic mechanism.

Wise, G. E., Y. Ren, et al. (2003). "Regulation of Osteoprotegerin Gene Expression in Dental Follicle Cells." *J. Dent. Res.* **82**(4): 298-302.

<http://jdr.iadrjournals.org/cgi/content/abstract/82/4/298>

Colony-stimulating factor-one (CSF-1) and parathyroid-hormone-related protein (PTHrP) down-regulate osteoprotegerin (OPG) gene expression in the dental follicle of the rat first mandibular molar. To examine this regulation at the signal transduction level, we treated cultured dental follicle cells with either phorbolmyristate acetate (PMA) or dibutyryl cyclic AMP (dbcAMP) to activate either protein kinase C (PKC) or protein kinase A (PKA). Our results demonstrate that PMA up-regulates OPG gene expression and down-regulates the expression of CSF-1 and the PTHrP receptor (PTHrP-R). Conversely, dbcAMP down-regulates OPG expression and up-regulates CSF-1 and PTHrP-R expression. Immunostaining shows that PMA also increases the steady-state levels of protein. Thus, treatment with agents that affect protein kinase activity also enhance the steady-state mRNA and protein levels of OPG, as well as decreasing the mRNA levels of CSF-1 and PTHrP-R. The PKC- α isoform may be critical in OPG regulation because PKC- α gene expression is enhanced by PMA and reduced by either CSF-1 or PTHrP.

J. Endocrinol. (2)

Cha, D. R., Y. S. Kang, et al. (2004). "Vascular endothelial growth factor is increased during early stage of diabetic nephropathy in type II diabetic rats." *J. Endocrinol.* **183**(1): 183-194.

<http://joe.endocrinology-journals.org/cgi/content/abstract/183/1/183>

Vascular endothelial growth factor (VEGF) has been implicated in the pathogenesis of diabetic nephropathy. We investigated serial changes of VEGF in the kidney and assessed whether glomerular and urinary VEGF levels are related to the severity of diabetic nephropathy. Furthermore, we examined the relationship between urinary VEGF levels and the urinary albumin excretion (UAE) rate in Otsuka-Long-Evans-Tokushima-Fatty (OLETF) rats. Glomerular VEGF mRNA expression and protein synthesis were evaluated by the reverse transcription-polymerase chain reaction, immunohistochemical staining and in situ hybridization. Urinary levels of VEGF were determined by enzyme-linked immunosorbent assay. UAE was significantly higher in OLETF rats than in control Long-Evans-Tokushima-Fatty (LETO) rats throughout the study period. Urinary VEGF levels were significantly higher from 25 to 37 weeks, and then gradually reduced until 55 weeks, although the levels were still higher than those in control rats. Urinary VEGF levels also showed a significant positive correlation with UAE ($r=0.262$, $P=0.045$) and serum creatinine ($r=0.398$, $P=0.044$), and were found to be independently correlated with UAE by Spearman's rank correlation. By immunohistochemical staining and in situ hybridization, VEGF was mainly detected in the podocytes in the glomeruli. Interestingly, a significant increase in VEGF mRNA expression was observed in the early period of diabetic nephropathy, and this was associated with increased urinary VEGF excretion. Thus, the overproduction of VEGF in the diabetic kidney may participate in the pathogenesis of early-stage diabetic nephropathy.

Jindrichova, S., J. Vcelak, et al. (2004). "Screening of six risk exons of the RET proto-oncogene in families with medullary thyroid carcinoma in the Czech Republic." J. Endocrinol. **183**(2): 257-265.

<http://joe.endocrinology-journals.org/cgi/content/abstract/183/2/257>

Medullary thyroid carcinoma (MTC) occurs as a sporadic form (75%) or as an autosomal dominant inherited familial disorder (25%) called familial MTC (FMTC) or as multiple endocrine neoplasia type 2 (MEN2) syndromes. Germ-line mutations in the rearranged during transfection (RET) proto-oncogene in exons 10, 11, 13, 14, 15 and 16 are known to be a cause of most of the familial forms. In this paper we report molecular genetic testing of 106 families with MTC (358 tested persons) from the Czech Republic in which we directly sequenced these six exons of the RET proto-oncogene. We detected germ-line mutations in 100% of MEN2B families (4/4 families), 90% of MEN2A families (9/10), 40% of FMTC families (4/10) and 7% of apparently sporadic MTC (6/82). Eleven different germ-line mutations were revealed. MEN2B was associated with mutation Met918 Thr in exon 16. In one MEN2B family beside this mutation the Tyr791 Phe was also found, which has not yet been reported. MEN2A was restricted to different mutations in exon 11 (codon 634). In FMTC and sporadic MTC families the mutations in exons 10, 11, 13 and 14 were detected. The genotype/phenotype correlations are given. Genetic testing revealed germ-line mutations in 23 index patients, 24 family members and excluded them in 53 relatives.

J. Exp. Biol. (3)

Lundby, C., H. Pilegaard, et al. (2004). "Acclimatization to 4100 m does not change capillary density or mRNA expression of potential angiogenesis regulatory factors in human skeletal muscle." J. Exp. Biol. **207**(22): 3865-3871.

<http://jeb.biologists.org/cgi/content/abstract/207/22/3865>

Increased skeletal muscle capillary density would be a logical adaptive mechanism to chronic hypoxic exposure. However, animal studies have yielded conflicting results, and human studies are sparse. Neoformation of capillaries is dependent on endothelial growth factors such as vascular endothelial growth factor (VEGF), a known target gene for hypoxia inducible factor 1 (HIF-1). We hypothesised that prolonged exposure to high altitude increases muscle capillary density and that this can be explained by an enhanced HIF-1{alpha} expression inducing an increase in VEGF expression. We measured mRNA levels and capillary density in muscle biopsies from vastus lateralis obtained in sea level residents (SLR; N=8) before and after 2 and 8 weeks of exposure to 4100 m altitude and in Bolivian Aymara high-altitude natives exposed to approximately 4100 m altitude (HAN; N=7). The expression of HIF-1{alpha} or VEGF mRNA was not changed with prolonged hypoxic exposure in SLR, and both genes were similarly expressed in SLR and HAN. In SLR, whole body mass, mean muscle fibre area and capillary to muscle fibre ratio remained unchanged during acclimatization. The capillary to fibre ratio was lower in HAN than in SLR (2.4 ± 0.1 vs 3.6 ± 0.2 ; $P < 0.05$). In conclusion, human muscle VEGF mRNA expression and capillary density are not significantly increased by 8 weeks of exposure to high altitude and are not increased in Aymara high-altitude natives compared with sea level residents.

Seron, T. J., J. Hill, et al. (2004). "A GPI-linked carbonic anhydrase expressed in the larval mosquito midgut." *J. Exp. Biol.* **207**(26): 4559-4572.

<http://jeb.biologists.org/cgi/content/abstract/207/26/4559>

We have previously described the first cloning and partial characterization of carbonic anhydrase from larval *Aedes aegypti* mosquitoes. Larval mosquitoes utilize an alkaline digestive environment in the lumen of their anterior midgut, and we have also demonstrated a critical link between alkalization of the gut and carbonic anhydrase(s). In this report we further examine the nature of the previously described carbonic anhydrase and test the hypothesis that its pattern of expression is consistent with a role in gut alkalization. Additionally we take advantage of the recently published genome of the mosquito *Anopheles gambiae* to assess the complexity of the carbonic anhydrase gene family in these insects. We report here that the previously described carbonic anhydrase from *Aedes aegypti* is similar to mammalian CA IV in that it is a GPI-linked peripheral membrane protein. In situ hybridization analyses identify multiple locations of carbonic anhydrase expression in the larval mosquito. An antibody prepared against a peptide sequence specific to the *Aedes aegypti* GPI-linked carbonic anhydrase labels plasma membranes of a number of cell types including neuronal cells and muscles. A previously undescribed subset of gut muscles is specifically identified by carbonic anhydrase immunohistochemistry. Bioinformatic analyses using the Ensembl automatic analysis pipeline show that there are at least 14 carbonic anhydrase genes in the *Anopheles gambiae* genome, including a homologue to the GPI-linked gene product described herein. Therefore, as in mammals which similarly possess numerous carbonic anhydrase genes, insects require a large family of these genes to handle the complex metabolic pathways influenced by carbonic anhydrases and their substrates.

Zhao, Y., H. Sun, et al. (2005). "Lifespan extension and elevated hsp gene expression in *Drosophila* caused by histone deacetylase inhibitors." *J. Exp. Biol.* **208**(4): 697-705.

<http://jeb.biologists.org/cgi/content/abstract/208/4/697>

The heat shock proteins (Hsps) play a positive role in lifespan determination, and histone acetylation has been shown to be involved in transcription of hsp genes in *Drosophila*. To further determine if hsp22 and hsp70 expression is correlated with lifespan, and if histone acetylation participates in this process, RNA levels for hsp22 and hsp70 were analyzed throughout the lifespan in the long-lived and short-lived iso-female lines. The results showed that hsp22 and hsp70 RNA levels were higher in long-lived line than in short-lived line and that the long-lived flies responded more rapidly to heat but were more tolerant to high temperature. Moreover, we investigated the influences of histone acetylation modification on longevity and on hsp gene expression by using histone deacetylase (HDAC) inhibitors TSA and BuA. The results demonstrated that both inhibitors were able to extend the lifespan and promote hsp22 and hsp70 expression. However, the optimal concentrations of these inhibitors, and probably the mechanisms of their actions, vary with the genetic background. In addition, we showed that HDAC inhibitors caused the hyperacetylation of core histone H3, implicating the involvement of chromatin modulation in hsp gene transcription. These data suggested a close correlation among histone acetylation, hsp gene expression and longevity in *D. melanogaster*.

Akatsuka, Y., T. Nishida, et al. (2003). "Identification of a Polymorphic Gene, BCL2A1, Encoding Two Novel Hematopoietic Lineage-specific Minor Histocompatibility Antigens." *J. Exp. Med.* **197**(11): 1489-1500.

<http://www.jem.org/cgi/content/abstract/197/11/1489>

We report the identification of two novel minor histocompatibility antigens (mHAg), encoded by two separate single nucleotide polymorphisms on a single gene, BCL2A1, and restricted by human histocompatibility leukocyte antigen (HLA)-A*2402 (the most common HLA-A allele in Japanese) and B*4403, respectively. Two cytotoxic T lymphocyte (CTL) clones specific for these mHAg were first isolated from two distinct recipients after hematopoietic cell transplantation. Both clones lyse only normal and malignant cells within the hematopoietic lineage. To localize the gene encoding the mHAg, two-point linkage analysis was performed on the CTL lytic patterns of restricting HLA-transfected B lymphoblastoid cell lines obtained from Centre d'Etude du Polymorphisme Humain. Both CTL clones showed a completely identical lytic pattern for 4 pedigrees and the gene was localized within a 3.6-cM interval of 15q24.3-25.1 region that encodes at least 46 genes. Of those, only BCL2A1 has been reported to be expressed in hematopoietic cells and possess three nonsynonymous nucleotide changes. Minigene transfection and epitope reconstitution assays with synthetic peptides identified both HLA-A*2402- and B*4403-restricted mHAg epitopes to be encoded by distinct polymorphisms within BCL2A1.

Bosma, G. C., J. Kim, et al. (2002). "DNA-dependent Protein Kinase Activity Is Not Required for Immunoglobulin Class Switching." *J. Exp. Med.* **196**(11): 1483-1495.

<http://www.jem.org/cgi/content/abstract/196/11/1483>

Class switch recombination (CSR), similar to V(D)J recombination, is thought to involve DNA double strand breaks and repair by the nonhomologous end-joining pathway. A key component of this pathway is DNA-dependent protein kinase (DNA-PK), consisting of a catalytic subunit (DNA-PKcs) and a DNA-binding heterodimer (Ku70/80). To test whether DNA-PKcs activity is essential for CSR, we examined whether IgM⁺ B cells from scid mice with site-directed H and L chain transgenes were able to undergo CSR. Although B cells from these mice were shown to lack DNA-PKcs activity, they were able to switch from IgM to IgG or IgA with close to the same efficiency as B cells from control transgenic and nontransgenic scid/+ mice, heterozygous for the scid mutation. We conclude that CSR, unlike V(D)J recombination, can readily occur in the absence of DNA-PKcs activity. We suggest nonhomologous end joining may not be the (primary or only) mechanism used to repair DNA breaks during CSR.

Bottino, C., R. Castriconi, et al. (2003). "Identification of PVR (CD155) and Nectin-2 (CD112) as Cell Surface Ligands for the Human DNAM-1 (CD226) Activating Molecule." *J. Exp. Med.* **198**(4): 557-567.

<http://www.jem.org/cgi/content/abstract/198/4/557>

Human natural killer (NK) cells express a series of activating receptors and coreceptors that are involved in recognition and killing of target cells. In this study, in an attempt to identify the cellular ligands for such triggering surface molecules, mice were immunized with NK-susceptible target cells. On the basis of a functional screening, four mAbs were selected that induced a partial down-regulation of the NK-mediated cytotoxicity against the immunizing target cells. As revealed by biochemical analysis, three of such mAbs recognized molecules of [~]70 kD. The other mAb

reacted with two distinct molecules of [~]65 and 60 kD, respectively. Protein purification followed by tryptic digestion and mass spectra analysis, allowed the identification of the 70 kD and the 65/60 kD molecules as PVR (CD155) and Nectin-2 {delta}/{alpha} (CD112), respectively. PVR-Fc and Nectin-2-Fc soluble hybrid molecules brightly stained COS-7 cells transfected with the DNAM-1 (CD226) construct, thus providing direct evidence that both PVR and Nectin-2 represent specific ligands for the DNAM-1 triggering receptor. Finally, the surface expression of PVR or Nectin-2 in cell transfectants resulted in DNAM-1-dependent enhancement of NK-mediated lysis of these target cells. This lysis was inhibited or even virtually abrogated upon mAb-mediated masking of DNAM-1 (on NK cells) or PVR or Nectin-2 ligands (on cell transfectants).

de Stahl, T. D., J. Dahlstrom, et al. (2003). "A Role for Complement in Feedback Enhancement of Antibody Responses by IgG3." J. Exp. Med. **197**(9): 1183-1190.

<http://www.jem.org/cgi/content/abstract/197/9/1183>

IgG1, IgG2a, and IgG2b, passively administered with soluble Ags, enhance specific Ab responses. The effect of IgG3 in this type of feedback regulation has not been studied previously. We immunized mice with trinitrophenyl (TNP)-coupled carrier proteins (bovine serum albumin [BSA] or ovalbumin [OVA]) alone or complexed to monoclonal TNP-specific IgG3. The carrier-specific Ab responses were enhanced by several hundred-fold by IgG3. Enhancement was significantly impaired in mice depleted of complement factor C3 and in mice lacking complement receptors 1 and 2 (Cr2^{-/-}). In contrast, mice lacking the common Fc-receptor gamma chain (FcR{gamma}^{-/-}), resulting in reduced expression of Fc{gamma}RI and lack of Fc{gamma}RIII, and mice lacking Fc{gamma}RIIB (Fc{gamma}RIIB^{-/-}), responded equally well to immunization with IgG3-complexed Ag as wild-type controls. These findings demonstrate that IgG3 can induce feedback enhancement and that IgG3, in analogy with IgM, uses the complement system for this function.

Gonzalez-Aseguinolaza, G., L. Van Kaer, et al. (2002). "Natural Killer T Cell Ligand {alpha}-Galactosylceramide Enhances Protective Immunity Induced by Malaria Vaccines." J. Exp. Med. **195**(5): 617-624.

<http://www.jem.org/cgi/content/abstract/195/5/617>

The important role played by CD8⁺ T lymphocytes in the control of parasitic and viral infections, as well as tumor development, has raised the need for the development of adjuvants capable of enhancing cell-mediated immunity. It is well established that protective immunity against liver stages of malaria parasites is primarily mediated by CD8⁺ T cells in mice. Activation of natural killer T (NKT) cells by the glycolipid ligand, {alpha}-galactosylceramide ({alpha}-GalCer), causes bystander activation of NK, B, CD4⁺, and CD8⁺ T cells. Our study shows that coadministration of {alpha}-GalCer with suboptimal doses of irradiated sporozoites or recombinant viruses expressing a malaria antigen greatly enhances the level of protective anti-malaria immunity in mice. We also show that coadministration of {alpha}-GalCer with various different immunogens strongly enhances antigen-specific CD8⁺ T cell responses, and to a lesser degree, Th1-type responses. The adjuvant effects of {alpha}-GalCer require CD1d molecules, V{alpha}14 NKT cells, and interferon {gamma}. As {alpha}-GalCer stimulates both human and murine NKT cells, these findings should contribute to the design of more effective vaccines against malaria and other intracellular pathogens, as well as tumors.

Goodyear, C. S. and G. J. Silverman (2003). "Death by a B Cell Superantigen: In Vivo VH-targeted Apoptotic Supraclonal B Cell Deletion by a Staphylococcal Toxin." J. Exp. Med. **197**(9): 1125-1139.

<http://www.jem.org/cgi/content/abstract/197/9/1125>

Amongst the many ploys used by microbial pathogens to interfere with host immune responses is the production of proteins with the properties of superantigens. These properties enable superantigens to interact with conserved variable region framework subdomains of the antigen receptors of lymphocytes rather than the complementarity determining region involved in the binding of conventional antigens. To understand how a B cell superantigen affects the host immune system, we infused protein A of *Staphylococcus aureus* (SpA) and followed the fate of peripheral B cells expressing B cell receptors (BCRs) with VH regions capable of binding SpA. Within hours, a sequence of events was initiated in SpA-binding splenic B cells, with rapid down-regulation of BCRs and coreceptors, CD19 and CD21, the induction of an activation phenotype, and limited rounds of proliferation. Apoptosis followed through a process heralded by the dissipation of mitochondrial membrane potential, the induction of the caspase pathway, and DNA fragmentation. After exposure, B cell apoptotic bodies were deposited in the spleen, lymph nodes, and Peyer's patches. Although in vivo apoptosis did not require the Fas death receptor, B cells were protected by interleukin (IL)-4 or CD40L, or overexpression of Bcl-2. These studies define a pathway for BCR-mediated programmed cell death that is VH region targeted by a superantigen.

Guy-Grand, D., O. Azogui, et al. (2003). "Extrathymic T Cell Lymphopoiesis: Ontogeny and Contribution to Gut Intraepithelial Lymphocytes in Athymic and Euthymic Mice." J. Exp. Med. **197**(3): 333-341.

<http://www.jem.org/cgi/content/abstract/197/3/333>

In the absence of thymopoiesis, T lymphocytes are nevertheless present, mainly in the gut epithelium. Ontogeny of the extrathymic pathway and the extent of its involvement in euthymic mice are controversial. These questions have been addressed by assessing the expression of recombinase activating gene (RAG) through the use of green fluorescent protein RAG2 transgenic mouse models. In athymic mice, T lymphopoiesis occurs mainly in the mesenteric lymph node and less in the Peyer's patches. Ontogenic steps of this lymphopoiesis resemble those of thymopoiesis, but with an apparent bias toward $\gamma\delta$ T cell production and with a paucity of oligoclonal $\alpha\beta$ T cells possibly resulting from a deficit in positive selection. Whether in athymic or euthymic mice, neither T intraepithelial lymphocytes (IEL) nor cryptopatch cells (reported to contain precursors of IEL) displayed fluorescence indicating recent RAG protein synthesis. Newly made T cells migrate from the mesenteric node into the thoracic duct lymph to reach the gut mucosa. In euthymic mice, this extrathymic pathway is totally repressed, except in conditions of severe lymphocytic depletion. Thus, in normal animals, all gut T IEL, including CD8 $\alpha\alpha$ cells, are of thymic origin, CD8 $\alpha\alpha$ TCR $\alpha\beta$ IEL being the likely progeny of double negative NK1-1- thymocytes, which show polyclonal V α and V β repertoires.

Hawn, T. R., A. Verbon, et al. (2003). "A Common Dominant TLR5 Stop Codon Polymorphism Abolishes Flagellin Signaling and Is Associated with Susceptibility to Legionnaires' Disease." J. Exp. Med. **198**(10): 1563-1572.

<http://www.jem.org/cgi/content/abstract/198/10/1563>

Although Toll-like receptors (TLRs) are critical mediators of the immune response to pathogens, the influence of polymorphisms in this gene family on human susceptibility to infection is poorly understood. We demonstrated recently that TLR5 recognizes flagellin, a potent inflammatory stimulus present in the flagellar structure of many bacteria. Here, we show that a common stop codon polymorphism in the ligand-binding domain of TLR5 (TLR5392STOP) is unable to mediate flagellin signaling, acts in a dominant fashion, and is associated with susceptibility to pneumonia caused by *Legionella pneumophila*, a flagellated bacterium. We also show that flagellin is a principal stimulant of proinflammatory cytokine production in lung epithelial cells. Together, these observations suggest that TLR5392STOP increases human susceptibility to infection through an unusual dominant mechanism that compromises TLR5's essential role as a regulator of the lung epithelial innate immune response.

Jung, Y.-J., R. LaCourse, et al. (2002). "Virulent but not Avirulent *Mycobacterium tuberculosis* Can Evade the Growth Inhibitory Action of a T Helper 1-dependent, Nitric Oxide Synthase 2-independent Defense in Mice." *J. Exp. Med.* **196**(7): 991-998.

<http://www.jem.org/cgi/content/abstract/196/7/991>

Control of infection with virulent *Mycobacterium tuberculosis* (Mtb) in mice is dependent on the generation of T helper (Th)1-mediated immunity that serves, via secretion of interferon (IFN)- γ and other cytokines, to upregulate the antimycobacterial function of macrophages of which the synthesis of inducible nitric oxide synthase (NOS)2 is an essential event. As a means to understanding the basis of Mtb virulence, the ability of gene-deleted mice incapable of making NOS2 (NOS2^{-/-}), gp91Phox subunit of the respiratory burst NADPH-oxidase complex (Phox^{-/-}), or either enzyme (NOS2/Phox^{-/-}), to control airborne infection with the avirulent R1Rv and H37Ra strains of Mtb was compared with their ability control infection with the virulent H37Rv strain. NOS2^{-/-}, Phox^{-/-}, and NOS2/Phox^{-/-} mice showed no deficiency in ability to control infection with either strain of avirulent Mtb. By contrast, NOS2^{-/-} mice, but not Phox^{-/-} mice, were incapable of controlling H37Rv infection and died early from neutrophil-dominated lung pathology. Control of infection with avirulent, as well as virulent Mtb, depended on the synthesis of IFN- γ , and was associated with a substantial increase in the synthesis in the lungs of mRNA for IFN- γ and NOS2, and with production of NOS2 by macrophages at sites of infection. The results indicate that virulent, but not avirulent, Mtb can overcome the growth inhibitory action of a Th1-dependent, NOS2-independent mechanism of defense.

Khan, I. A., M. Moretto, et al. (2002). "Treatment with Soluble Interleukin-15R α Exacerbates Intracellular Parasitic Infection by Blocking the Development of Memory CD8⁺ T Cell Response." *J. Exp. Med.* **195**(11): 1463-1470.

<http://www.jem.org/cgi/content/abstract/195/11/1463>

Interferon (IFN)- γ -producing CD8⁺ T cells are important for the successful resolution of the obligate intracellular parasite *Toxoplasma gondii* by preventing the reactivation or controlling a repeat infection. Previous reports from our laboratory have shown that exogenous interleukin (IL)-15 treatment augments the CD8⁺ T cell response against the parasite. However, the role of endogenous IL-15 in the proliferation of activated/memory CD8⁺ T cells during toxoplasma or any other infection is unknown. In this study, we treated *T. gondii* immune mice with soluble IL-15 receptor α (sIL-15R α) to block the host endogenous IL-15. The treatment markedly reduced the ability of the immune animals to control a lethal infection. CD8⁺ T cell activities in the sIL-15R α -administered mice were severely reduced as determined by IFN- γ release and target cell lysis assays. The loss of CD8⁺ T cell immunity due to sIL-15R α treatment was further demonstrated by adoptive transfer experiments. Naive recipients transferred with

CD44hi activated/memory CD8+ T cells and treated with sIL-15R{alpha} failed to resist a lethal T. gondii infection. Moreover, sIL-15R{alpha} treatment of the recipients blocked the ability of donor CD44hi activated/memory CD8+ T cells to replicate in response to T. gondii challenge. To our knowledge, this is the first demonstration of the important role of host IL-15 in the development of antigen-specific memory CD8+ T cells against an intracellular infection.

McDonald, J. P., E. G. Frank, et al. (2003). "129-derived Strains of Mice Are Deficient in DNA Polymerase {iota} and Have Normal Immunoglobulin Hypermutation." J. Exp. Med. **198**(4): 635-643.

<http://www.jem.org/cgi/content/abstract/198/4/635>

Recent studies suggest that DNA polymerase {eta} (pol{eta}) and DNA polymerase {iota} (pol{iota}) are involved in somatic hypermutation of immunoglobulin variable genes. To test the role of pol{iota} in generating mutations in an animal model, we first characterized the biochemical properties of murine pol{iota}. Like its human counterpart, murine pol{iota} is extremely error-prone when catalyzing synthesis on a variety of DNA templates in vitro. Interestingly, when filling in a 1 base-pair gap, DNA synthesis and subsequent strand displacement was greatest in the presence of both pols {iota} and {eta}. Genomic sequence analysis of Poli led to the serendipitous discovery that 129-derived strains of mice have a nonsense codon mutation in exon 2 that abrogates production of pol{iota}. Analysis of hypermutation in variable genes from 129/SvJ (Poli-/-) and C57BL/6J (Poli+/+) mice revealed that the overall frequency and spectrum of mutation were normal in pol{iota}-deficient mice. Thus, either pol{iota} does not participate in hypermutation, or its role is nonessential and can be readily assumed by another low-fidelity polymerase.

Migliaccio, A. R., R. A. Rana, et al. (2003). "GATA-1 as a Regulator of Mast Cell Differentiation Revealed by the Phenotype of the GATA-1low Mouse Mutant." J. Exp. Med. **197**(3): 281-296.

<http://www.jem.org/cgi/content/abstract/197/3/281>

Here it is shown that the phenotype of adult mice lacking the first enhancer (DNA hypersensitive site I) and the distal promoter of the GATA-1 gene (neo{Delta}HS or GATA-1low mutants) reveals defects in mast cell development. These include the presence of morphologically abnormal alcian blue+ mast cells and apoptotic metachromatic- mast cell precursors in connective tissues and peritoneal lavage and numerous (60-70% of all the progenitors) "unique" trilineage cells committed to erythroid, megakaryocytic, and mast pathways in the bone marrow and spleen. These abnormalities, which were mirrored by impaired mast differentiation in vitro, were reversed by retroviral-mediated expression of GATA-1 cDNA. These data indicate an essential role for GATA-1 in mast cell differentiation.

Ochsenbein, A. F., S. R. Riddell, et al. (2004). "CD27 Expression Promotes Long-Term Survival of Functional Effector-Memory CD8+ Cytotoxic T Lymphocytes in HIV-infected Patients." J. Exp. Med. **200**(11): 1407-1417.

<http://www.jem.org/cgi/content/abstract/200/11/1407>

Human immunodeficiency virus (HIV)-specific CD8+ T cells persist in high frequencies in HIV-infected patients despite impaired CD4+ T helper response to the virus, but, unlike other differentiated effector cytotoxic T lymphocytes, most continue to express the tumor necrosis

factor receptor family member CD27. Because the ligand for CD27 (CD70) is also overexpressed in HIV-infected hosts, we examined the nature of expression and potential functional consequences of CD27 expression on HIV-specific CD8⁺ T cells. Analysis of CD27⁺ and CD27⁻ T cells derived from the same HIV-specific clone revealed that retention of CD27 did not interfere with acquisition of effector functions, and that after T cell receptor stimulation, CD27⁺ cells that concurrently were triggered via CD27 exhibited more resistance to apoptosis, interleukin 2 production, and proliferation than CD27⁻ T cells. After transfer back into an HIV-infected patient, autologous HIV-specific CD27⁻ T cells rapidly disappeared, but CD27⁺ T cells derived from the same clone persisted at high frequency. Our findings suggest that the CD27-CD70 interaction in HIV infection may provide CD27⁺ CD8⁺ T cells with a survival advantage and compensate for limiting or absent CD4⁺ T help to maintain the CD8 response.

Oh-hora, M., S. Johmura, et al. (2003). "Requirement for Ras Guanine Nucleotide Releasing Protein 3 in Coupling Phospholipase C- γ 2 to Ras in B Cell Receptor Signaling." *J. Exp. Med.* **198**(12): 1841-1851.

<http://www.jem.org/cgi/content/abstract/198/12/1841>

Two important Ras guanine nucleotide exchange factors, Son of sevenless (Sos) and Ras guanine nucleotide releasing protein (RasGRP), have been implicated in controlling Ras activation when cell surface receptors are stimulated. To address the specificity or redundancy of these exchange factors, we have generated Sos1/Sos2 double- or RasGRP3-deficient B cell lines and determined their ability to mediate Ras activation upon B cell receptor (BCR) stimulation. The BCR requires RasGRP3; in contrast, epidermal growth factor receptor is dependent on Sos1 and Sos2. Furthermore, we show that BCR-induced recruitment of RasGRP3 to the membrane and the subsequent Ras activation are significantly attenuated in phospholipase C- γ 2-deficient B cells. This defective Ras activation is suppressed by the expression of RasGRP3 as a membrane-attached form, suggesting that phospholipase C- γ 2 regulates RasGRP3 localization and thereby Ras activation.

Stehlik, C., L. Fiorentino, et al. (2002). "The PAAD/PYRIN-Family Protein ASC Is a Dual Regulator of a Conserved Step in Nuclear Factor κ B Activation Pathways." *J. Exp. Med.* **196**(12): 1605-1615.

<http://www.jem.org/cgi/content/abstract/196/12/1605>

Apoptosis-associated speck-like protein containing a Caspase recruitment domain (ASC) belongs to a large family of proteins that contain a Pyrin, AIM, ASC, and death domain-like (PAAD) domain (also known as PYRIN, DAPIN, Pyk). Recent data have suggested that ASC functions as an adaptor protein linking various PAAD-family proteins to pathways involved in nuclear factor (NF)- κ B and pro-Caspase-1 activation. We present evidence here that the role of ASC in modulating NF- κ B activation pathways is much broader than previously suspected, as it can either inhibit or activate NF- κ B, depending on cellular context. While coexpression of ASC with certain PAAD-family proteins such as Pyrin and Cryopyrin increases NF- κ B activity, ASC has an inhibitory influence on NF- κ B activation by various proinflammatory stimuli, including tumor necrosis factor (TNF) α , interleukin 1 β , and lipopolysaccharide (LPS). Elevations in ASC protein levels or of the PAAD domain of ASC suppressed activation of κ B kinases in cells exposed to pro-inflammatory stimuli. Conversely, reducing endogenous levels of ASC using siRNA enhanced TNF- and LPS-induced degradation of the IKK substrate, κ B α . Our findings suggest that ASC modulates diverse NF- κ B induction pathways by acting upon the IKK complex, implying a broad role for this and similar proteins containing PAAD domains in regulation of inflammatory responses.

Sugita, S. and J. W. Streilein (2003). "Iris Pigment Epithelium Expressing CD86 (B7-2) Directly Suppresses T Cell Activation In Vitro via Binding to Cytotoxic T Lymphocyte-associated Antigen 4." J. Exp. Med. **198**(1): 161-171.

<http://www.jem.org/cgi/content/abstract/198/1/161>

A monolayer of pigment epithelium (PE) lines the iris PE (IPE), ciliary body PE, and retina PE of the inner eye, an immune-privileged site. These neural crest-derived epithelial cells participate in ocular immune privilege through poorly defined molecular mechanisms. Murine PE cells cultured from different ocular tissues suppress T cell activation by differing mechanisms. In particular, IPE cells suppress primarily via direct cell to cell contact. By examining surface expression of numerous candidate molecules (tumor necrosis factor receptor [TNFR]1, TNFR2, CD36, CD40, CD47, CD80, CD86, PD-L1, CD95 ligand, and type I interferon receptor), we report that IPE cells uniquely express on their surface the costimulatory molecule CD86. When IPE were blocked with anti-CD86 or were derived from CD80/CD86 (but not CD80) knockout (KO) mice, the cells displayed reduced capacity to suppress T cell activation. IPE also failed to suppress activation of T cells in the presence of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) immunoglobulin or if the T cells were obtained from CTLA-4 (but not CD28) KO mice. We conclude that iris pigment epithelial cells constitutively express cell surface CD86, which enables the cells to contact inhibit T cells via direct interaction with CTLA-4. Thus, ocular immune privilege is achieved in part by subversion of molecules that are usually used for conventional immune costimulation.

Yu, P., Y. Lee, et al. (2005). "Intratumor depletion of CD4+ cells unmasks tumor immunogenicity leading to the rejection of late-stage tumors." J. Exp. Med. **201**(5): 779-791.

<http://www.jem.org/cgi/content/abstract/201/5/779>

Tumor environment can be critical for preventing the immunological destruction of antigenic tumors. We have observed a selective accumulation of CD4+CD25+ T cells inside tumors. In a murine fibrosarcoma Ld-expressing Ag104, these cells made up the majority of tumor-infiltrating lymphocytes at the late stage of tumor progression, and their depletion during the effector phase, rather than priming phase, successfully enhanced antitumor immunity. We show here that CD4+CD25+ T cells suppressed the proliferation and interferon- γ production of CD8+ T cells in vivo at the local tumor site. Blockade of the effects of IL-10 and TGF- β partially reversed the suppression imposed by the CD4+ cells. Furthermore, local depletion of CD4+ cells inside the tumor resulted in a change of cytokine milieu and led to the eradication of well-established highly aggressive tumors and the development of long-term antitumor memory. Therefore, CD4+CD25+ T cells maintained an environment in the tumor that concealed the immunogenicity of tumor cells to permit progressive growth of antigenic tumors. Our study illustrates that the suppression of antitumor immunity by regulatory T cells occurs predominantly at the tumor site, and that local reversal of suppression, even at a late stage of tumor development, can be an effective treatment for well-established cancers.

Zippelius, A., M. J. Pittet, et al. (2002). "Thymic Selection Generates a Large T Cell Pool Recognizing a Self-Peptide in Humans." J. Exp. Med. **195**(4): 485-494.

<http://www.jem.org/cgi/content/abstract/195/4/485>

The low frequency of self-peptide-specific T cells in the human preimmune repertoire has so far precluded their direct evaluation. Here, we report an unexpected high frequency of T cells specific for the self-antigen Melan-A/MART-1 in CD8 single-positive thymocytes from human histocompatibility leukocyte antigen-A2 healthy individuals, which is maintained in the peripheral blood of newborns and adults. Postthymic replicative history of Melan-A/MART-1-specific CD8 T cells was independently assessed by quantifying T cell receptor excision circles and telomere length *ex vivo*. We provide direct evidence that the large T cell pool specific for the self-antigen Melan-A/MART-1 is mostly generated by thymic output of a high number of precursors. This represents the only known naive self-peptide-specific T cell repertoire directly accessible in humans.

J. Gen. Virol. (28)

Chen, X., W. J. Zhang, et al. (2002). "Comparative analysis of the complete genome sequences of *Helicoverpa zea* and *Helicoverpa armigera* single-nucleocapsid nucleopolyhedroviruses." J. Gen. Virol. **83**(3): 673-684.

<http://vir.sgmjournals.org/cgi/content/abstract/83/3/673>

The complete nucleotide sequence of *Helicoverpa zea* single-nucleocapsid nucleopolyhedrovirus (HzSNPV) has been determined (130869 bp) and compared to the nucleotide sequence of *Helicoverpa armigera* (Ha) SNPV. These two genomes are very similar in their nucleotide (97% identity) and amino acid (99% identity) sequences. The coding regions are much more conserved than the non-coding regions. In HzSNPV/HaSNPV, the 63 open reading frames (ORFs) present in all baculoviruses sequenced so far are much more conserved than other ORFs. HzSNPV has four additional small ORFs compared with HaSNPV, one of these (Hz42) being in a correct transcriptional context. The major differences between HzSNPV and HaSNPV are found in the sequence and organization of the homologous regions (hrs) and the baculovirus repeat ORFs (bro genes). The sequence identity between the HzSNPV and HaSNPV hrs ranges from 90% (hr1) to almost 100% (hr5) and the hrs differ in the presence/absence of one or more type A and/or B repeats. The three HzSNPV bro genes differ significantly from those in HaSNPV and may have been acquired independently in the ancestral past. The sequence data suggest strongly that HzSNPV and HaSNPV are variants of the same virus species, a conclusion that is supported by the physical and biological data.

Chisaka, H., E. Morita, et al. (2002). "A transgenic mouse model for non-immune hydrops fetalis induced by the NS1 gene of human parvovirus B19." J. Gen. Virol. **83**(2): 273-281.

<http://vir.sgmjournals.org/cgi/content/abstract/83/2/273>

Human parvovirus B19 (B19) infection during pregnancy is associated with the adverse foetal outcome known as non-immune hydrops fetalis (NIHF). Although B19 is known to infect erythroid-lineage cells *in vivo* as well as *in vitro*, the mechanism leading to the occurrence of NIHF is not clear. To investigate the possible involvement of the B19 non-structural protein NS1 in NIHF, three independent lines of transgenic mice were generated that expressed NS1 under the control of the Cre-loxP system and the GATA1 promoter. Two of the three lines expressed NS1 in erythroid-lineage cells. Most of the transgenic mice died at the embryonic stage, some of which

developed hydropic changes caused by severe anaemia at embryonic day 15{middle dot}5 (E15{middle dot}5). Histological examination of embryos at E15{middle dot}5 showed significantly fewer erythropoietic islands in the liver parenchyma, whereas their hearts showed no abnormal signs, such as cardiomegaly and apoptotic cells. The NS1-transgenic mouse lines established here provide an animal model for human NIHF and suggest that NS1 plays a crucial role in the adverse outcome associated with intrauterine B19 infection in humans.

Gubser, C. and G. L. Smith (2002). "The sequence of camelpox virus shows it is most closely related to variola virus, the cause of smallpox." J. Gen. Virol. **83**(4): 855-872.

<http://vir.sgmjournals.org/cgi/content/abstract/83/4/855>

Camelpox virus (CMPV) and variola virus (VAR) are orthopoxviruses (OPVs) that share several biological features and cause high mortality and morbidity in their single host species. The sequence of a virulent CMPV strain was determined; it is 202182 bp long, with inverted terminal repeats (ITRs) of 6045 bp and has 206 predicted open reading frames (ORFs). As for other poxviruses, the genes are tightly packed with little non-coding sequence. Most genes within 25 kb of each terminus are transcribed outwards towards the terminus, whereas genes within the centre of the genome are transcribed from either DNA strand. The central region of the genome contains genes that are highly conserved in other OPVs and 87 of these are conserved in all sequenced chordopoxviruses. In contrast, genes towards either terminus are more variable and encode proteins involved in host range, virulence or immunomodulation. In some cases, these are broken versions of genes found in other OPVs. The relationship of CMPV to other OPVs was analysed by comparisons of DNA and predicted protein sequences, repeats within the ITRs and arrangement of ORFs within the terminal regions. Each comparison gave the same conclusion: CMPV is the closest known virus to variola virus, the cause of smallpox.

Hacker, C. V., C. M. Brasier, et al. (2005). "A double-stranded RNA from a Phytophthora species is related to the plant endornaviruses and contains a putative UDP glycosyltransferase gene." J. Gen. Virol. **86**(5): 1561-1570.

<http://vir.sgmjournals.org/cgi/content/abstract/86/5/1561>

A new dsRNA was isolated from a Phytophthora isolate from Douglas fir. Sequence analysis showed the dsRNA to consist of 13 883 bp and to contain a single open reading frame with the potential to encode a polyprotein of 4548 aa. This polyprotein contained amino acid sequence motifs characteristic of virus RNA-dependent RNA polymerases (RdRps) in its C-terminal region and motifs characteristic of RNA helicases in its N-terminal region. These sequence motifs were related to corresponding motifs in plant viruses in the genus Endornavirus. In phylogenetic trees constructed from the RdRp and helicase motifs of a range of ssRNA and dsRNA viruses, the Phytophthora RdRp and helicase sequences clustered with those of the plant endornaviruses with good bootstrap support. The properties of the Phytophthora dsRNA are consistent with its being classified as the first non-plant member of the genus Endornavirus, for which we propose the name phytophthora endornavirus 1 (PEV1). A region between the RdRp and helicase domains of the PEV1 protein had significant amino acid sequence similarity to UDP glycosyltransferases (UGTs). Two sequence motifs were identified, one characteristic of all UGTs and the other characteristic of sterol UGTs. The PEV1 UGT would be the first for an RNA virus, although ecdysteroid UGT genes have been found in many baculoviruses. The PEV1 UGT was only distantly related to baculovirus ecdysteroid UGTs, which belong to a family distinct from the sterol UGTs.

Hatchette, T. F., D. Walker, et al. (2004). "Influenza A viruses in feral Canadian ducks: extensive reassortment in nature." J. Gen. Virol. **85**(8): 2327-2337.

<http://vir.sgmjournals.org/cgi/content/abstract/85/8/2327>

The current dogma of influenza accepts that feral aquatic birds are the reservoir for influenza A viruses. Although the genomic information of human influenza A viruses is increasing, little of this type of data is available for viruses circulating in feral waterfowl. This study presents the genetic characterization of 35 viruses isolated from wild Canadian ducks from 1983 to 2000, as the first attempt at a comprehensive genotypic analysis of influenza viruses isolated from feral ducks. This study demonstrates that influenza virus genes circulating in Canadian ducks have achieved evolutionary stasis. The majority of these duck virus genes are clustered in distinct North American clades; however, some H6 and H9 genes are clustered with those from Eurasian viruses. Genes appeared to reassort in a random fashion. None of the genotypes identified remained present throughout all of the years examined and most PA and PB2 genes that crossed over into swine were clustered in one phylogenetic grouping. Additionally, matrix genes were identified that branch very early in the evolutionary tree. These findings demonstrate the diversity of the influenza virus gene pool in Canadian ducks, and suggest that genes which cluster in specific phylogenetic groupings in the PB2 and PA genes can be used for markers of viruses with the potential for crossing the species barrier. A more comprehensive study of this important reservoir is needed to provide further insight into the genomic composition of viruses that crossover the species barrier, which would be a useful component to pandemic planning.

Huy, T. T.-T., H. Ushijima, et al. (2004). "Genotype C of hepatitis B virus can be classified into at least two subgroups." J. Gen. Virol. **85**(2): 283-292.

<http://vir.sgmjournals.org/cgi/content/abstract/85/2/283>

A genomic characterization of hepatitis B virus (HBV) was done for 56 pre-S1/pre-S2 genes and 10 full-length HBV genotype C isolates from five Asian countries. Phylogenetic analysis of the pre-S1/pre-S2 genes revealed two major groups within genotype C: one for isolates from southeast Asia including Vietnam, Myanmar and Thailand (named HBV/C1) and the other for isolates from Far East Asia including Japan, Korea and China (named HBV/C2). This finding was confirmed by phylogenetic analysis based on the full-length sequence of 32 HBV genotype C isolates, including 22 from database entries. Two isolates from Okinawa, the island off the southern end of Japan, formed a different branch. Specific amino acid sequence changes were identified in the large S protein (amino acids 51, 54, 60, 62 and 73) and P protein (amino acids 231, 233, 236, 248, 252 and 304). Our results indicate that genotype C of HBV can be classified into at least two subgroups.

Imlach, W., C. A. McCaughan, et al. (2002). "Orf virus-encoded interleukin-10 stimulates the proliferation of murine mast cells and inhibits cytokine synthesis in murine peritoneal macrophages." J. Gen. Virol. **83**(5): 1049-1058.

<http://vir.sgmjournals.org/cgi/content/abstract/83/5/1049>

Orf virus (ORFV) is the type species of the parapoxvirus genus and produces cutaneous pustular lesions in sheep, goats and humans. The genome encodes a polypeptide with remarkable homology to interleukin-10 (IL-10), particularly ovine IL-10, and also to IL-10-like proteins

encoded by Epstein-Barr virus (EBV) and equine herpesvirus. IL-10 is a pleiotropic cytokine that can exert either immunostimulatory or immunosuppressive effects on many cell types. We have expressed and purified C-terminal FLAG and His6-tagged versions of ORFV-IL-10 and shown that ORFV-IL-10 costimulates murine mast cells (MC/9) and inhibits tumour necrosis factor- α synthesis in activated mouse peritoneal macrophages. Our results demonstrate that although ORFV-IL-10 is structurally similar to EBV-IL-10 it has evolved a different spectrum of activities. EBV-IL-10 does not stimulate the proliferation of thymocytes or mast cells whereas ORFV-IL-10 has both of these activities. Recent studies show that the critical difference in molecular structure of human IL-10 and EBV-IL-10, which may be the basis of their functional differences, is linked to a single amino acid substitution. Consistent with the activity spectrum reported here for ORFV-IL-10, the viral gene encodes the critical amino acid seen in human IL-10. Although the ORFV-IL-10 gene has clearly undergone significant evolutionary change at the nucleotide level compared with ovine IL-10, it has largely retained the polypeptide structure and functional characteristics of its ovine counterpart, suggesting that mutations of the gene to a potentially more potent immunosuppressive form may compromise the co-existence of host and virus.

Kaverin, N. V., I. A. Rudneva, et al. (2002). "Structure of antigenic sites on the haemagglutinin molecule of H5 avian influenza virus and phenotypic variation of escape mutants." *J. Gen. Virol.* **83**(10): 2497-2505.

<http://vir.sgmjournals.org/cgi/content/abstract/83/10/2497>

To elucidate the structure of the antigenic sites of avian H5 influenza virus haemagglutinin (HA) we analysed escape mutants of a mouse-adapted variant of the H5N2 strain A/Mallard/Pennsylvania/10218/84. A panel of five anti-H5 monoclonal antibodies (mAbs) was used to select 16 escape mutants. The mutants were tested by ELISA and haemagglutination inhibition with this panel of anti-H5 mAbs and the HA genes of the mutants were sequenced. The sequencing demonstrated that the amino acid changes were grouped in two antigenic sites. One corresponded to site A in the H3 HA. The other contained areas that are separated in the amino acid sequence but are topographically close in the three-dimensional structure and partially overlap in the reactions with mAbs. This site corresponds in part to site B in the H3 structure; it also includes a region not involved in site B that partially overlaps site Sa in the H1 HA and an antigenic area in H2 HA. Mutants with the amino acid change K152N, as well as those with the change D126N, showed reduced lethality in mice. The substitution D126N, creating a new glycosylation site, was accompanied by an increase in the sensitivity of the mutants to normal mouse serum inhibitors. Several amino acid changes in the H5 escape mutants occurred at the positions of reported changes in H2 drift variants. This coincidence suggests that the antigenic sites described and analysed here may be important for drift variation if H5 influenza virus ever appears as a pathogen circulating in humans.

Kuntz-Simon, G., G. Le Gall-Recule, et al. (2002). "Muscovy duck reovirus σ C protein is atypically encoded by the smallest genome segment." *J. Gen. Virol.* **83**(5): 1189-1200.

<http://vir.sgmjournals.org/cgi/content/abstract/83/5/1189>

Although muscovy duck reovirus (DRV) shares properties with the reovirus isolated from chicken, commonly named avian reovirus (ARV), the two virus species are antigenically different. Similar to the DRV σ B-encoded gene (1201 bp long) previously identified, the three other double-stranded RNA small genome segments of DRV have been cloned and sequenced. They were 1325, 1191 and 1124 bp long, respectively, and contained conserved terminal sequences common to ARVs. They coded for single expression products, except the smallest (S4), which

contained two overlapping open reading frames (ORF1 and ORF2). BLAST analyses revealed that the proteins encoded by the 1325 and 1191 bp genes shared high identity levels with ARV σ A and σ NS, respectively, and to a lesser extent with other orthoreovirus counterparts. No homology was found for the S4 ORF1-encoded p10 protein. The 29{middle dot}4 kDa product encoded by S4 ORF2 appeared to be 25% identical to ARV S1 ORF3-encoded σ C, a cell-attachment oligomer inducing type-specific neutralizing antibodies. Introduction of large gaps in the N-terminal part of the DRV protein was necessary to improve DRV and ARV σ C amino acid sequence alignments. However, a leucine zipper motif was conserved and secondary structure analyses predicted a three-stranded α -helical coiled-coil feature at this amino portion. Thus, despite extensive sequence divergence, DRV σ C was suggested to be structurally and probably functionally related to ARV σ C. This work provides evidence for the diversity of the polycistronic S class genes of reoviruses isolated from birds and raises the question of the relative classification of DRV in the Orthoreovirus genus.

Lipatov, A. S., S. Andreansky, et al. (2005). "Pathogenesis of Hong Kong H5N1 influenza virus NS gene reassortants in mice: the role of cytokines and B- and T-cell responses." *J. Gen. Virol.* **86**(4): 1121-1130.

<http://vir.sgmjournals.org/cgi/content/abstract/86/4/1121>

The severity of disease caused in humans by H5N1 influenza viruses remains unexplained. The NS gene of Hong Kong H5N1/97 viruses was shown to contribute to high pathogenicity of reassortants in a pig model. However, the molecular pathogenesis and host immune response underlying this phenomenon remain unclear. Here, in a mouse model, H1N1 A/Puerto Rico/8/34 (PR/8) reassortants that contained the H5N1/97 NS gene, the H5N1/01 NS gene, or an altered H5N1/97 NS gene encoding a Glu92[&gt]Asp substitution in NS1 was studied. The pathogenicity of reassortant viruses, the induction of cytokines and chemokine CXCL1 (KC) in the lungs and specific B- and T-cell responses was characterized. In mice infected with reassortant virus containing the H5N1/97 NS gene, the mouse lethal dose (50 %) and lung virus titres were similar to those of PR/8, which is highly pathogenic to mice. This reassortant virus required two more days than PR/8 to be cleared from the lungs of infected mice. Reassortants containing the altered H5N1/97 NS gene or the H5N1/01 NS gene demonstrated attenuated pathogenicity and lower lung titres in mice. Specific B- and T-cell responses were consistent with viral pathogenicity and did not explain the delayed clearance of the H5N1/97 NS reassortant. The reassortant induced elevated pulmonary concentrations of the inflammatory cytokines IL1 α , IL1 β , IL6, IFN- γ and chemokine KC, and decreased concentrations of the anti-inflammatory cytokine IL10. This cytokine imbalance is reminiscent of the clinical findings in two humans who died of H5N1/97 infection and may explain the unusual severity of the disease.

Love, A., V. Molnégren, et al. (2004). "Evolution of hepatitis C virus variants following blood transfusion from one infected donor to several recipients: a long-term follow-up." *J. Gen. Virol.* **85**(2): 441-450.

<http://vir.sgmjournals.org/cgi/content/abstract/85/2/441>

Variants of hepatitis C virus (HCV) from a single infected blood donor and 13 viraemic recipients who were traced were examined by sequencing and cloning to determine the extent of virus diversity in hypervariable region 1. Serum-derived viral isolates were studied from the donor when his HCV infection was discovered in 1993, in his recipients that year (0{middle dot}3-5 years post-transfusion) and 5 years later in the donor and six viraemic recipients who were still alive. Viral variants of broad diversity were readily demonstrated in the baseline samples of the donor (nucleotide p-distance 0{middle dot}130), but significantly less ($P < 0{middle dot}00003$) diversity

was observed in the recipients' first samples (p-distances within recipients 0{middle dot}003-0{middle dot}062). In the first blood samples of the recipients, many of the viral variants identified were closely related to a strain variant from the donor. In follow-up samples drawn 5 years later from the donor and six recipients, the p-distance among donor clones had increased (0{middle dot}172, $P < 0{middle dot}0005$) compared with the recipients, who displayed significantly narrower quasispecies (0{middle dot}011-0{middle dot}086). A common finding was that recipients of blood components processed from the same donation differed substantially in persisting HCV infectious sequence. Markedly few changes leading to changes of amino acids had occurred during follow-up in four of six recipients. These results question the significance of the development of viral variants as a necessary phenomenon in the evolution of HCV and pathogenesis of the disease.

May, J. S., H. M. Coleman, et al. (2004). "Forced lytic replication impairs host colonization by a latency-deficient mutant of murine gammaherpesvirus-68." *J. Gen. Virol.* **85**(1): 137-146.

<http://vir.sgmjournals.org/cgi/content/abstract/85/1/137>

A regulated switch between latent and lytic gene expression is common to all known herpesviruses. However, the effects on host colonization of altering this switch are largely unknown. We deregulated the transcription of the gene encoding the major lytic transactivator of murine gammaherpesvirus-68, ORF50, by inserting a new and powerful promoter element in its 5' untranslated region. In vitro, the mutant virus (M50) transcribed ORF50 at a high level and showed more rapid lytic spread in permissive fibroblast cultures, but in vivo, the M50 virus showed a severe deficit in latency establishment, with no sign of the infectious mononucleosis-like illness normally associated with wild-type infection. Although a low level of M50 viral DNA was detectable by PCR in spleens, replication-competent virus could not be recovered beyond 10 days post-infection. The M50 virus was also attenuated in immunocompromised mice. Thus a gammaherpesvirus unable to shut off lytic cycle gene expression showed severely restricted host colonization.

Mittelholzer, C., K.-O. Hedlund, et al. (2003). "Molecular characterization of a novel astrovirus associated with disease in mink." *J. Gen. Virol.* **84**(11): 3087-3094.

<http://vir.sgmjournals.org/cgi/content/abstract/84/11/3087>

Pre-weaning diarrhoea is a well-known problem in mink farming in Europe, causing morbidity that varies between farms, regions and season. Different causalities for the disease have been proposed, but only most recently has a novel astrovirus been identified as an important risk factor. In this report, the molecular characterization, origin and evolution of this novel astrovirus of mink are discussed. The polyadenylated, positive-stranded RNA genome was sequenced and found to contain 6610 nt, organized into three ORFs and two short UTRs. A ribosomal frameshift sequence links the 5' two ORFs, containing sequence motifs for a serine protease (ORF1a) and an RNA-dependent RNA polymerase (ORF1b). The structural proteins are encoded by ORF2 and, presumably, are expressed as a polyprotein precursor to be cleaved into the mature capsid proteins. These results indicate that mink astrovirus (MiAstV) has all of the features typical of members of the Astroviridae. Phylogenetic analyses revealed that MiAstV is distantly related to established astroviruses, showing less than 67 % similarity at the nucleotide level with its closest relative, ovine astrovirus, and even lower identities at the predicted amino acid level. Nevertheless, sequence analysis of MiAstV isolates from geographically distinct Swedish and Danish farms showed much less diversity. This suggests either the spread in the mink population of a virus that has evolved a long time ago or the recent introduction of an ancient virus into a new host species.

Mork, C., P. Hershberger, et al. (2004). "Isolation and characterization of a rhabdovirus from starry flounder (*Platichthys stellatus*) collected from the northern portion of Puget Sound, Washington, USA." J. Gen. Virol. **85**(2): 495-505.

<http://vir.sgmjournals.org/cgi/content/abstract/85/2/495>

The initial characterization of a rhabdovirus isolated from a single, asymptomatic starry flounder (*Platichthys stellatus*) collected during a viral survey of marine fishes from the northern portion of Puget Sound, Washington, USA, is reported. Virions were bullet-shaped and approximately 100 nm long and 50 nm wide, contained a lipid envelope, remained stable for at least 14 days at temperatures ranging from -80 to 5 {degrees}C and grew optimally at 15 {degrees}C in cultures of epithelioma papulosum cyprini (EPC) cells. The cytopathic effect on EPC cell monolayers was characterized by raised foci containing rounded masses of cells. Pyknotic and dark-staining nuclei that also showed signs of karyorrhexis were observed following haematoxylin and eosin, May-Grunwald Giemsa and acridine orange staining. PAGE of the structural proteins and PCR assays using primers specific for other known fish rhabdoviruses, including Infectious hematopoietic necrosis virus, Viral hemorrhagic septicemia virus, Spring viremia of carp virus, and Hiram rhabdovirus, indicated that the new virus, tentatively termed starry flounder rhabdovirus (SFRV), was previously undescribed in marine fishes from this region. In addition, sequence analysis of 2678 nt of the amino portion of the viral polymerase gene indicated that SFRV was genetically distinct from other members of the family Rhabdoviridae for which sequence data are available. Detection of this virus during a limited viral survey of wild fishes emphasizes the void of knowledge regarding the diversity of viruses that naturally infect marine fish species in the North Pacific Ocean.

Negri, D. R. M., S. Baroncelli, et al. (2004). "Protective efficacy of a multicomponent vector vaccine in cynomolgus monkeys after intrarectal simian immunodeficiency virus challenge." J. Gen. Virol. **85**(5): 1191-1201.

<http://vir.sgmjournals.org/cgi/content/abstract/85/5/1191>

We investigated the protective efficacy of a systemic triple vector (DNA/rSFV/rMVA)-based vaccine against mucosal challenge with pathogenic simian immunodeficiency virus (SIV) in cynomolgus monkeys. Animals were immunized at week 0 with DNA (intradermally), at weeks 8 and 16 with recombinant Semliki Forest virus (rSFV, subcutaneously) and finally, at week 24, with recombinant modified vaccinia virus Ankara strain (rMVA, intramuscularly). Both DNA and recombinant viral vectors expressed a wide range of SIV proteins (Gag, Pol, Tat, Rev, Env and Nef). This immunization strategy elicited cell-mediated rather than humoral responses that were especially increased following the last boost. Upon intrarectal challenge with pathogenic SIVmac251, three of the four vaccinated monkeys dramatically abrogated virus load to undetectable levels up to 41 weeks after challenge. A major contribution to this vaccine effect appeared to be the T-cell-mediated immune response to vaccine antigens (Gag, Rev, Tat, Nef) seen in the early phase of infection in three of the four vaccinated monkeys. Indeed, the frequency of T-cells producing antigen-induced IFN- γ mirrored virus clearance in the vaccinated and protected monkeys. These results, reminiscent of the efficacy of live attenuated virus vaccines, suggest that vaccination with a combination of many viral antigens can induce a robust and stable vaccine-induced immunity able to abrogate virus replication.

Nicot, F., F. Legrand-Abravanel, et al. (2005). "Heterogeneity of hepatitis C virus genotype 4 strains

circulating in south-western France." *J. Gen. Virol.* **86**(1): 107-114.

<http://vir.sgmjournals.org/cgi/content/abstract/86/1/107>

Hepatitis C virus (HCV) is a major cause of liver disease. Knowledge of HCV variability is crucial for clinical and epidemiological analysis. HCV genotype 4 (HCV-4) has become increasingly prevalent in European countries, including France, in recent years. The present study investigates the heterogeneity of HCV-4 in south-western France by phylogenetic analysis of NS5B sequences from 166 patients. The E2 region of 90 strains was also analysed. Genotype 4 accounts for 7{middle dot}4 % of HCV infections in this area. Analysis of the NS5B region revealed 12 subtypes and the NS5B and E2 phylogeny data were congruent, except for one strain. The epidemiological data indicated two main groups of patients. One included intravenous drug users (IVDUs) of French origin, who were infected by homogeneous strains of subtypes 4a or 4d. The second group comprised non-IVDU patients who were infected with heterogeneous strains. This group was subdivided into patients of French origin, who were infected with eight subtypes, and patients from non-European countries (Central Africa or the Middle East), who were mainly infected with 4f, 4k, 4r and other subtypes; they showed the greatest genetic heterogeneity. This study of a large cohort of patients shows the great diversity of HCV-4 strains, and that these subtypes have spread differently.

Ortin, A., C. Cousens, et al. (2003). "Characterization of enzootic nasal tumour virus of goats: complete sequence and tissue distribution." *J. Gen. Virol.* **84**(8): 2245-2252.

<http://vir.sgmjournals.org/cgi/content/abstract/84/8/2245>

The complete genome sequence of a new isolate of enzootic nasal tumour virus (ENTV-2), associated with enzootic nasal adenocarcinoma (ENA) of goats, was determined. The genome exhibits a genetic organization characteristic of{beta}-retroviruses. ENTV-2 is closely related to the retrovirus (ENTV-1) associated with enzootic adenocarcinoma of sheep, and to jaagsiekte retrovirus. The main sequence differences between these viruses reside in orfX, the U3 LTR, two small regions in gag and the transmembrane (TM) region of env. Sequence analysis of the TM region of env from several sheep and goats naturally affected by ENA suggested that ENTV-1 and ENTV-2 are distinct viruses rather than geographical variants. Although both viruses transform secretory epithelial cells of the ethmoid turbinate, the study of their tissue distribution using specific PCRs showed that ENTV-2 establishes a disseminated lymphoid infection whereas ENTV-1 is mainly confined to the tumour.

Ozbun, M. A. (2002). "Infectious human papillomavirus type 31b: purification and infection of an immortalized human keratinocyte cell line." *J. Gen. Virol.* **83**(11): 2753-2763.

<http://vir.sgmjournals.org/cgi/content/abstract/83/11/2753>

Human papillomaviruses (HPVs) are aetiological agents of human malignancies, most notably cervical cancers. The life-cycles of HPVs are dependent on epithelial differentiation, and this has impeded many basic studies of HPV biology. The organotypic (raft) culture system supports epithelial differentiation such that infectious virions are synthesized in raft tissues from epithelial cells that replicate extrachromosomal HPV genomes. The CIN-612 9E cell line maintains episomal copies of HPV type 31b (HPV31b), an HPV type associated with cervical cancers. Many previous studies, including our own, have focused on characterizing the later stages of the HPV31b life-cycle in CIN-612 9E raft tissues. In this study, we have used the raft system to generate large numbers of HPV31b viral DNA (vDNA)-containing particles. We found a

biologically contained homogenization system to be efficient at virion extraction from raft epithelial tissues. We also determined that vDNA-containing particles could be directly quantified from density-gradient fractions. Using an RT-PCR assay, the presence of newly synthesized, spliced HPV31b transcripts was detected following HPV31b infection of the immortalized HaCaT epithelial cell line. Spliced E6 and E1{wedge}E4 RNAs were detected using a single round of RT-PCR from cells infected with a dose as low as 1{middle dot}0 vDNA-containing particle per cell. Spliced E1*1,E2 transcripts were found in cells infected with an HPV31b dose as low as 10 vDNA-containing particles per cell. Infectivity was blocked by HPV31 antiserum, but was not affected by DNase I. This work lays a foundation for a detailed analysis of the early events in HPV infection.

Pignatelli, S., P. Dal Monte, et al. (2003). "Human cytomegalovirus glycoprotein N (gpUL73-gN) genomic variants: identification of a novel subgroup, geographical distribution and evidence of positive selective pressure." *J. Gen. Virol.* **84**(3): 647-655.

<http://vir.sgmjournals.org/cgi/content/abstract/84/3/647>

Human cytomegalovirus (HCMV) ORF UL73 is a polymorphic locus, encoding the viral glycoprotein gpUL73-gN, a component of the gC-II envelope complex. The previously identified gN genomic variants, denoted gN-1, gN-2, gN-3 and gN-4, were further investigated in this work by analysing a large panel of HCMV clinical isolates collected from all over the world (223 samples). Sequencing and phylogenetic analysis confirmed the existence of the four gN genotypes, but also allowed the identification of a novel subgroup belonging to the gN-3 genotype, which was designated gN-3b. The number of non-synonymous (dN) and synonymous (dS) nucleotide substitutions and their ratio (dN/dS) were estimated among the gN genotypes to evaluate the possibility of positive selection. Results showed that the four variants evolved by neutral (random) selection, but that the gN-3 and gN-4 genotypes are maintained by positive selective pressure. The 223 HCMV clinical isolates were subdivided according to their geographical origin, and four main regions of gN prevalence were identified: Europe, China, Australia and Northern America. The gN variants were found to be widespread and represented within the regions analysed without any significant difference, and no new genotype was detected. Finally, for clinical and epidemiological purposes, a rapid and low-cost method for genetic grouping of the HCMV clinical isolates was developed based on the RFLP revealed by SacI, ScaI and Sall digestion of the PCR-amplified UL73 sequence. This technique enabled us to distinguish all four gN genomic variants and also their subtypes.

Sabouri, A. H., M. Saito, et al. (2005). "Differences in viral and host genetic risk factors for development of human T-cell lymphotropic virus type 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis between Iranian and Japanese HTLV-1-infected individuals." *J. Gen. Virol.* **86**(3): 773-781.

<http://vir.sgmjournals.org/cgi/content/abstract/86/3/773>

Human T-cell lymphotropic virus type 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a neurological disease observed only in 1-2 % of infected individuals. HTLV-1 provirus load, certain HLA alleles and HTLV-1 tax subgroups are reported to be associated with different levels of risk for HAM/TSP in Kagoshima, Japan. Here, it was determined whether these risk factors were also valid for HTLV-1-infected individuals in Mashhad in northeastern Iran, another region of endemic HTLV-1 infection. In Iranian HTLV-1-infected individuals (n=132, 58 HAM/TSP patients and 74 seropositive asymptomatic carriers), although HLA-DRB1*0101 was associated with disease susceptibility in the absence of HLA-A*02 (P=0{middle dot}038; odds ratio=2{middle dot}71) as observed in Kagoshima, HLA-A*02 and HLA-Cw*08 had no effect on either the risk of developing HAM/TSP or HTLV-1 provirus load. All

Iranian subjects possessed tax subgroup A sequences, and the protective effects of HLA-A*02 were observed only in Kagoshima subjects with tax subgroup B but not in those with tax subgroup A. Both the prevalence of HTLV-1 subgroups and the host genetic background may explain the different risks levels for HAM/TSP development in these two populations.

Scobie, L., S. Taylor, et al. (2004). "Characterization of germline porcine endogenous retroviruses from Large White pig." J. Gen. Virol. **85**(8): 2421-2428.

<http://vir.sgmjournals.org/cgi/content/abstract/85/8/2421>

Porcine endogenous retroviruses (PERV) are of concern when the microbiological safety aspects of xenotransplantation are considered. Four unique isolates of PERV B have been identified previously from a lambda library constructed from genomic DNA from a Large White pig. This study shows that none of these isolates are replication competent when transfected into permissive human or pig cells in vitro, and the removal of flanking genomic sequences does not confer a human tropic replication competent (HTRC) phenotype on these PERV proviruses. Analysis of the envelope sequences revealed that PERV B demonstrated high similarity to the envelope sequences derived from replication-competent PERV, indicating that lack of replication competence does not appear to be attributable to this region of the provirus. These data complement recent findings that HTRC PERV are recombinants between the PERV A and PERV C subgroups, and that these recombinants are not present in the germline of miniature swine. Together, these results indicate that these individual PERV B proviruses are unlikely to give rise to HTRC PERV.

Simon, S., H. Li, et al. (2003). "The vascular lesions of a cow and bison with sheep-associated malignant catarrhal fever contain ovine herpesvirus 2-infected CD8+ T lymphocytes." J. Gen. Virol. **84**(8): 2009-2013.

<http://vir.sgmjournals.org/cgi/content/abstract/84/8/2009>

Malignant catarrhal fever (MCF) is a herpesvirus disease syndrome of ruminants. The microscopic pathology of MCF is characterized by lymphoid proliferation and infiltration, necrotizing vasculitis and epithelial necrosis. Because previous attempts to detect viral antigen or nucleic acids in lesions have been unsuccessful, the pathogenesis of the lesions in acute MCF has been speculated to involve mechanisms of autoimmunity and lymphocyte dysregulation. In this study, the vascular lesions in the brains of a cow and a bison with acute MCF were evaluated by in situ PCR and immunohistochemistry. The results demonstrated that the predominant infiltrating cell type in these lesions was CD8+ T lymphocytes and that large numbers of these cells were infected with ovine herpesvirus 2. The lesions also contained macrophages, but no detectable CD4+ or B lymphocytes.

Sun, Z. F., C. T. Larsen, et al. (2004). "Genetic identification of avian hepatitis E virus (HEV) from healthy chicken flocks and characterization of the capsid gene of 14 avian HEV isolates from chickens with hepatitis-splenomegaly syndrome in different geographical regions of the United States." J. Gen. Virol. **85**(3): 693-700.

<http://vir.sgmjournals.org/cgi/content/abstract/85/3/693>

Avian hepatitis E virus (HEV), a novel virus identified from chickens with hepatitis-splenomegaly

(HS) syndrome, is genetically and antigenically related to human HEV. Recently, it was found that avian HEV antibody is also prevalent in healthy chickens. A prospective study was done on a known seropositive but healthy chicken farm to identify avian HEV isolates from healthy chickens. Fourteen chickens were randomly selected, tagged and monitored under natural conditions for 19 weeks. All 14 chickens were seronegative at the beginning of the study at 12 weeks of age. By 21 weeks of age, all 14 chickens had seroconverted to avian HEV antibody. None of the chickens had any sign of HS syndrome. Partial helicase gene and capsid gene sequences of avian HEV isolates recovered from a healthy chicken were determined and found to share 75-97 % nucleotide sequence identity with the corresponding regions of avian HEV isolates from chickens with HS syndrome. Thus far, only one strain of avian HEV from a chicken with HS syndrome has been genetically characterized for its capsid gene, therefore the capsid gene region of an additional 14 isolates from chickens with HS syndrome were also characterized. The capsid genes of avian HEV isolates from chickens with HS syndrome were found to be heterogeneous, sharing 76-100 % nucleotide sequence identity with each other. This study indicates that avian HEV is enzootic in chicken flocks and spreads subclinically among chickens in the United States and that the virus is heterogeneous.

Tan, J. and G. A. Tannock (2005). "Role of viral load in the pathogenesis of chicken anemia virus." J. Gen. Virol. **86**(5): 1327-1333.

<http://vir.sgmjournals.org/cgi/content/abstract/86/5/1327>

The pathogenesis of strain 3711 of the chicken anemia virus (CAV), propagated in chickens, and two preparations of strain 3711 that had been adapted to grow to high titre in cells of the MDCC-MSB1 line were studied in chicken embryos and/or chickens. Highest viral loads in infected chickens, as measured by a microplate DNA-hybridization assay, were detected in the thymus, clotted blood and pancreas, and the lowest in the duodenum. The CAV DNA copy number in the organs of chicken embryos was significantly lower than in chickens. Route of infection was an important determinant of the course of disease in chickens, with clinical signs appearing earlier in birds infected by the intramuscular than those infected by the oral route; there was a direct relationship between viral load in particular organs and the extent of clinical signs. No reduction in the pathogenicity for chickens was noted for strain 3711 after 65 or 129 passages in the MDCC-MSB1 cell line.

Tomioka, Y., K. Ochiai, et al. (2004). "Genome sequence analysis of the avian retrovirus causing so-called fowl glioma and the promoter activity of the long terminal repeat." J. Gen. Virol. **85**(3): 647-652.

<http://vir.sgmjournals.org/cgi/content/abstract/85/3/647>

So-called fowl glioma is a retroviral infectious disease caused by avian leukosis virus subgroup A (ALV-A). We determined the complete nucleotide sequence of the virus genome. The full-length sequence was consistent with a genetic organization typical of a replication-competent type C retrovirus lacking viral oncogenes. The coding sequences were well conserved with those of replication-competent viruses, but the 3' noncoding regions including LTR were most related to those of replication-defective sarcoma viruses. The U3 region of the LTR had a few deletions and several point mutations compared to that of other ALVs. The promoter activities of the LTRs of glioma-inducing ALV and ALV-A standard strain, RAV-1, were equivalent in chick embryo fibroblasts (CEF), while that of glioma-inducing ALV was significantly lower than that of RAV-1 in human astrocytic cells. These subtle differences of the promoter activity of the LTR may be related to the induction of glial neoplasm.

van der Wilk, F., A. M. Dulleman, et al. (2002). "Nucleotide sequence and genomic organization of an ophiovirus associated with lettuce big-vein disease." *J. Gen. Virol.* **83**(11): 2869-2877.

<http://vir.sgmjournals.org/cgi/content/abstract/83/11/2869>

The complete nucleotide sequence of an ophiovirus associated with lettuce big-vein disease has been elucidated. The genome consisted of four RNA molecules of approximately 7{middle dot}8, 1{middle dot}7, 1{middle dot}5 and 1{middle dot}4 kb. Virus particles were shown to contain nearly equimolar amounts of RNA molecules of both polarities. The 5'- and 3'-terminal ends of the RNA molecules are largely, but not perfectly, complementary to each other. The virus genome contains seven open reading frames. Database searches with the putative viral products revealed homologies with the RNA-dependent RNA polymerases of rhabdoviruses and Ranunculus white mottle virus, and the capsid protein of Citrus psorosis virus. The gene encoding the viral polymerase appears to be located on the RNA segment 1, while the nucleocapsid protein is encoded by the RNA3. No significant sequence similarities were observed with other viral proteins. In spite of the morphological resemblance with species in the genus Tenuivirus, the ophioviruses appear not to be evolutionary closely related to this genus nor any other viral genus.

van Munster, M., A. M. Dulleman, et al. (2002). "Sequence analysis and genomic organization of Aphid lethal paralysis virus: a new member of the family Dicistroviridae." *J. Gen. Virol.* **83**(12): 3131-3138.

<http://vir.sgmjournals.org/cgi/content/abstract/83/12/3131>

The complete nucleotide sequence of the genomic RNA of an aphid-infecting virus, Aphid lethal paralysis virus (ALPV), has been determined. The genome is 9812 nt in length and contains two long open reading frames (ORFs), which are separated by an intergenic region of 163 nt. The first ORF (5' ORF) is preceded by an untranslated leader sequence of 506 nt, while an untranslated region of 571 nt follows the second ORF (3' ORF). The deduced amino acid sequences of the 5' ORF and 3' ORF products respectively showed similarity to the non-structural and structural proteins of members of the newly recognized genus Cripavirus (family Dicistroviridae). On the basis of the observed sequence similarities and identical genome organization, it is proposed that ALPV belongs to this genus. Phylogenetic analysis showed that ALPV is most closely related to Rhopalosiphum padi virus, and groups in a cluster with Drosophila C virus and Cricket paralysis virus, while the other members of this genus are more distantly related. Infectivity experiments showed that ALPV can not only infect aphid species but is also able to infect the whitefly *Trialeurodes vaporariorum*, extending its host range to another family of the order Hemiptera.

van Munster, M., A. M. Dulleman, et al. (2003). "A new virus infecting Myzus persicae has a genome organization similar to the species of the genus Densovirus." *J. Gen. Virol.* **84**(1): 165-172.

<http://vir.sgmjournals.org/cgi/content/abstract/84/1/165>

The genomic sequence of a new icosahedral DNA virus infecting *Myzus persicae* has been determined. Analysis of 5499 nt of the viral genome revealed five open reading frames (ORFs) evenly distributed in the 5' half of both DNA strands. Three ORFs (ORF1-3) share the same strand, while two other ORFs (ORF4 and ORF5) are detected in the complementary sequence. The overall genomic organization is similar to that of species from the genus Densovirus. ORFs 1-3 most likely encode the non-structural proteins, since their putative products contain

conserved replication motifs, NTP-binding domains and helicase domains similar to those found in the NS-1 protein of parvoviruses. The deduced amino acid sequences from ORFs 4 and 5 show sequence similarities with the structural proteins of the members of the genus Densovirus. These data indicate that this virus is a new species of the genus Densovirus in the family Parvoviridae. The virus was tentatively named Myzus persicae densovirus.

J. Gerontol. A Biol. Sci. Med. Sci. (1)

Neri, S., A. Gardini, et al. (2005). "Mismatch Repair System and Aging: Microsatellite Instability in Peripheral Blood Cells From Differently Aged Participants." J. Gerontol. A Biol. Sci. Med. Sci. **60**(3): 285-292.

<http://biomed.gerontologyjournals.org/cgi/content/abstract/60/3/285>

Age-related alterations of DNA repair could be involved in the accumulation of genetic damage with age. Few data suggest a possible alteration with age of the mismatch repair system, evidenced by the acquisition of microsatellite instability. We aimed to point out a possible implication of this repair system in the accumulation of genetic damage with age. Peripheral blood cell DNA from 226 participants, 110 young (25-35 years), 58 old (85-97 years), and 58 centenarian was analyzed at five polymorphic microsatellite loci (CD4, p53, VWA31, TPOX, and FES/FPS) to point out age-related instabilities or modifications in allele frequencies. FES/FPS microsatellite was the most unstable, showing both the appearance of trizygosity in DNA from old participants and differences in allele patterns among age groups, thus indicating an association between increased microsatellite instability and aging, one of the possible causes of which being an impairment of mismatch repair system capacity with age.

J. Hered. (16)

Deagle, B. E., S. N. Jarman, et al. (2005). "Genetic Screening for Prey in the Gut Contents from a Giant Squid (*Architeuthis* sp.)." J. Hered.: esi036.

<http://jhered.oupjournals.org/cgi/content/abstract/esi036v2>

Giant squids (*Architeuthis* sp.) remain mysterious; they have evaded observation and are rarely taken from their deep sea habitat. Information on the diet of *Architeuthis* is scarce due to the limited number of specimens with morphologically recognizable remains in their digestive tracts. We explored the use of polymerase chain reaction (PCR)-based methods for detection of DNA in the prey remains and amorphous slurry from an *Architeuthis* gut sample. The DNA region amplified varied in size, allowing separation of fish and squid components. Sequence comparisons identified fish prey as *Macruronus novaezelandiae*. Isolation of *Architeuthis* DNA from an ingested tentacle and the presence of chitin fragments indicate cannibalism occurs in giant squid. Denaturing gradient gel electrophoresis was used to screen for less common DNA types, revealing a high frequency of PCR-generated false alleles, but no additional prey species.

Durand, J. D., P. G. Bianco, et al. (2003). "Insight Into the Origin of Endemic Mediterranean Ichthyofauna: Phylogeography of Chondrostoma Genus (Teleostei, Cyprinidae)." *J. Hered.* **94**(4): 315-328.

<http://jhered.oupjournals.org/cgi/content/abstract/94/4/315>

The Chondrostoma genus is widespread in Europe, with numerous endemic species in northern Mediterranean rivers. We reconstructed the phylogenetic relationships of this genus, using the whole cytochrome b sequence and compared the two freshwater fish dispersion hypotheses: (1) dispersion around the Mediterranean Sea during the Lago Mare phase of the Messinian salinity crisis (Bianco's hypothesis) and (2) an older and more gradual colonization of the Mediterranean rivers (Banarescu's hypothesis). All phylogenetic analyses identified two levels of divergences, implying two radiation events in the Chondrostoma genus. The first radiation mainly concerned Mediterranean species, whereas the second one includes Danubian and Mesopotamian species. This phylogeographic pattern was already observed for the genus Squalius, which exhibits a similar geographic range distribution in Europe and probably is shared with several other Mediterranean genera, such as Scardinius, Rutilus, and Telestes. Furthermore, assuming a molecular clock of 1% per million years, the first radiation appears consistent with a Messinian dispersion during the Lago Mare, 5.3 million years ago, whereas the second one may correspond to a Mesopotamian dispersion through the Black Sea to the Danube system. According to our results, the Lago Mare theory is strengthened, and a more recent and pre-Pleistocene colonization of the Danube from Mesopotamian freshwater fishes is suggested.

Green, S. L., R. J. Tolwani, et al. (2002). "Structure, Chromosomal Location, and Analysis of the Canine Cu/Zn Superoxide Dismutase (SOD1) Gene." *J. Hered.* **93**(2): 119-124.

<http://jhered.oupjournals.org/cgi/content/abstract/93/2/119>

Mutations in Cu/Zn superoxide dismutase (SOD1), a major cytosolic antioxidant enzyme in eukaryotic cells, have been reported in approximately 20% of familial amyotrophic lateral sclerosis (FALS) patients. Hereditary canine spinal muscular atrophy (HCSMA), a fatal inherited motor neuron disease in Brittany spaniels, shares many clinical and pathological features with human motor neuron disease, including FALS. The SOD1 coding region has been sequenced and cloned from several animal species, but not from the dog. We have mapped the chromosomal location, sequenced, and characterized the canine SOD1 gene. Extending this analysis, we have evaluated SOD1 as a candidate for HCSMA. The 462 bp SOD1 coding region in the dog encodes 153 amino acid residues and exhibits more than 83% and 79% sequence identity to other mammalian homologues at both the nucleotide and amino acid levels, respectively. The canine SOD1 gene maps to CFA31 close to syntenic group 13 on the radiation hybrid (RH) map in the vicinity of sodium myo/inositol transporter (SMIT) gene. The human orthologous SOD1 and SMIT genes have been localized on HSA 21q22.1 and HSA 21q21, respectively, confirming the conservation of synteny between dog syntenic group 13 and HSA 21. Direct sequencing of SOD1 cDNA from six dogs with HCSMA revealed no mutations. Northern analysis indicated no differences in steady-state levels of SOD1 mRNA.

Haig, S. M., T. R. Mace, et al. (2003). "Parentage and Relatedness in Polyandrous Comb-Crested Jacanas Using ISSRs." *J. Hered.* **94**(4): 302-309.

<http://jhered.oupjournals.org/cgi/content/abstract/94/4/302>

In this article we present the first analysis of parentage and relatedness in a natural vertebrate population, using Intersimple Sequence Repeat (ISSR) markers. Thus, 28 ISSR markers were used in a study of a sex-role reversed, simultaneously polyandrous shorebird from northeastern Australia, the comb-crested jacana (*Irediparra gallinacea*). Assessment of parentage was based on comparison of field observations, novel bands, individual-specific bands found in 7/9 males and 4/6 females, and a 99% CI exclusion criteria. Integrating results from these approaches resulted in confirmation of paternity in all 36 chicks. In only one case (2.8% of chicks) was a co-mate assigned paternity. Thus, comb-crested jacanas appear to be genetically monogamous. These results showed resemblance to sequentially polyandrous birds but differed from the simultaneously polyandrous wattled jacana (*Jacana jacana*; Emlen et al. 1998). A significant relationship between relatedness and ISSR similarity resulted in recognition that 14/15 adults sampled may be related to at least one other adult by 0.25 or more. Lack of dispersal may be explained by physical limitations and adequate regional habitat. ISSRs proved to be simple and helpful in resolving these issues.

Hammond, E. L., A. J. Lymbery, et al. (2002). "Microsatellite Analysis of Genetic Diversity in Wild and Farmed Emus (*Dromaius novaehollandiae*)."
J. Hered. **93**(5): 376-380.

<http://jhered.oupjournals.org/cgi/content/abstract/93/5/376>

The emu (*Dromaius novaehollandiae*) occupies most regions of the Australian continent and in recent times has been farmed for meat, oil, and leather. Very little is known about the genetic structure of natural or farmed populations of these birds. We report a preliminary study of genetic variation in emus undertaken by typing birds from five farms and two natural populations at five polymorphic microsatellite loci. Genetic diversity was high for all populations and there was little evidence of inbreeding, with most populations conforming to Hardy-Weinberg equilibrium for most loci. Significant heterozygote deficiencies at one locus in a number of populations were detected and may indicate the presence of null alleles. Comparisons of allele frequencies showed little evidence of genetic differentiation either among farmed populations or between farmed and natural populations.

Hett, A. K., C. Pitra, et al. (2005). "Characterization of Sox9 in European Atlantic Sturgeon (*Acipenser sturio*)."
J. Hered. **96**(2): 150-154.

<http://jhered.oupjournals.org/cgi/content/abstract/96/2/150>

The Sox9 gene of *Acipenser sturio*, one of the most primitive vertebrates, was analyzed. No sex-specific differences were observed. Sturgeon Sox9 consists of three exons and two introns with completely conserved exon-intron boundaries showing high levels of homology to other vertebrate Sox9 sequences, especially in the N-terminus region containing the HMG box. We found strong evidence for negative (purifying) selection. In contrast to previous studies of other fishes, we observed no evidence for gene duplication in sturgeon. Phylogenetic analyses of Sox9 evolution revealed a basal position for sturgeon Sox9.

Irion, D. N., A. L. Schaffer, et al. (2003). "Analysis of Genetic Variation in 28 Dog Breed Populations With 100 Microsatellite Markers."
J. Hered. **94**(1): 81-87.

<http://jhered.oupjournals.org/cgi/content/abstract/94/1/81>

Dog breeds were created by man choosing for select phenotypic traits such as size, shape, coat color, conformation, and behavior. Rigorous phenotypic selection likely resulted in a loss of genetic information. The present study extends previous dog population observations by assessing the genotypic variation within and across 28 breeds representing the seven recognized breed groups of the American Kennel Club (AKC). One hundred autosomal microsatellite markers distributed across the canine genome were used to examine variation within breeds. Resulting breed-specific allele frequencies were then used in an attempt to elucidate phylogeny and genetic distances between breeds. While the set of autosomal microsatellites was useful in describing genetic variation within breeds, establishing the genetic relatedness between breeds was less conclusive. A more accurate determination of breed phylogeny will likely require the use of single-nucleotide polymorphisms (SNPs).

Ketmaier, V. and C. Bernardini (2005). "Structure of the Mitochondrial Control Region of the Eurasian Otter (*Lutra lutra*; Carnivora, Mustelidae): Patterns of Genetic Heterogeneity and Implications for Conservation of the Species in Italy." J. Hered.: esi037.

<http://jhered.oupjournals.org/cgi/content/abstract/esi037v1>

In this study we determined the complete sequence of the mitochondrial DNA (mtDNA) control region of the Eurasian otter (*Lutra lutra*). We then compared these new sequences with orthologues of nine carnivores belonging to six families (Mustelidae, Mephitidae, Canidae, Hyaenidae, Ursidae, and Felidae). The comparative analyses identified all the conserved regions previously found in mammals. The Eurasian otter and seven other species have a single location with tandem repeats in the right domain, while the spotted hyena (Hyaenidae) and the tiger (Felidae) have repeated sequences in both the right and left domains. To assess the degree of genetic heterogeneity of the Eurasian otter in Italy we sequenced two fragments of the gene and analyzed length polymorphisms of repeated sequences and heteroplasmy in 32 specimens. The study includes 23 museum specimens collected in northern, central, and southern Italy; most of these specimens are from extinct populations, while the southern Italian samples belong to the sole extant Italian population of the Eurasian otter. The study also includes all the captive-reared animals living in the colony "Centro Lontra, Caramanico Terme" (Pescara, central Italy). The colony is maintained for reintroduction of the species. We found a low level of genetic polymorphism; a single haplotype is dominant, but our data indicate the presence in central and southern Italy of two slightly divergent haplotypes. One haplotype belongs to an extinct population, the other is present in the single extant Italian population. Analyses of length polymorphisms and heteroplasmy indicate that the autochthonous Italian samples are characterized by a distinct array of repeated sequences from captive-reared animals.

Lambert, D. M., A. Baker, et al. (2005). "Is a Large-Scale DNA-Based Inventory of Ancient Life Possible?" J. Hered. **96**(3): 279-284.

<http://jhered.oupjournals.org/cgi/content/abstract/96/3/279>

A complete DNA-based inventory of the Earth's present biota using large-scale high-throughput DNA sequencing of signature region(s) (DNA barcoding) is an ambitious proposal rivaling the Human Genome Project. We examine whether this approach will also enable us to assess the past diversity of the earth's biota. To test this, we sequenced the 5' terminus of the mitochondrial cytochrome c oxidase I (COI) gene of individuals belonging to a group of extinct ratite birds, the moa of New Zealand. Moa comprised a large number of taxa that radiated in isolation on this oceanic landmass. Using a phylogenetic approach based on a large data set including protein coding and 12S DNA sequences as well as morphology, we now have precise information about the number of moa species that once existed. We show that each of the moa species detected

using this extensive data set has a unique COI barcode(s) and that they all show low levels of within-species COI variation. Consequently, we conclude that COI sequences accurately identify the species discovered using the larger data set. Hence, more generally, this study suggests that DNA barcoding might also help us detect other extinct animal species and that a large-scale inventory of ancient life is possible.

Menotti-Raymond, M., V. A. David, et al. (2003). "Second-Generation Integrated Genetic Linkage/Radiation Hybrid Maps of the Domestic Cat (*Felis catus*)."
J. Hered. **94**(1): 95-106.

<http://jhered.oupjournals.org/cgi/content/abstract/94/1/95>

We report construction of second-generation integrated genetic linkage and radiation hybrid (RH) maps in the domestic cat (*Felis catus*) that exhibit a high level of marker concordance and provide near-full genome coverage. A total of 864 markers, including 585 coding loci (type I markers) and 279 polymorphic microsatellite loci (type II markers), are now mapped in the cat genome. We generated the genetic linkage map utilizing a multigeneration interspecies backcross pedigree between the domestic cat and the Asian leopard cat (*Prionailurus bengalensis*). Eighty-one type I markers were integrated with 247 type II markers from a first-generation map to generate a map of 328 loci (320 autosomal and 8 X-linked) distributed in 47 linkage groups, with an average intermarker spacing of 8 cM. Genome coverage spans approximately 2,650 cM, allowing an estimate for the genetic length of the sex-averaged map as 3,300 cM. The 834-locus second-generation domestic cat RH map was generated from the incorporation of 579 type I and 255 type II loci. Type I markers were added using targeted selection to cover either genomic regions underrepresented in the first-generation map or to refine breakpoints in human/feline synteny. The integrated linkage and RH maps reveal approximately 110 conserved segments ordered between the human and feline genomes, and provide extensive anchored reference marker homologues that connect to the more gene dense human and mouse sequence maps, suitable for positional cloning applications.

Wallner, B., F. Piumi, et al. (2004). "Isolation of Y Chromosome-specific Microsatellites in the Horse and Cross-species Amplification in the Genus *Equus*."
J. Hered. **95**(2): 158-164.

<http://jhered.oupjournals.org/cgi/content/abstract/95/2/158>

Y chromosome polymorphisms such as microsatellites or single nucleotide polymorphisms represent a paternal counterpart to mitochondrial DNA (mtDNA) for evolutionary and phylogeographic studies. The use of Y chromosome haplotyping in natural populations of species other than humans is still hindered by the lack of sequence information necessary for polymorphism screening. Here we used representational difference analysis (RDA) followed by a screen of a bacterial artificial chromosome (BAC) library for repetitive sequences to obtain polymorphic Y-chromosomal markers. The procedure was performed for the domestic horse (*Equus caballus*) and we report the first six Y-chromosomal microsatellite markers for this species. Three markers were also useful for haplotyping taxa of the zebra/ass lineage. Y-chromosomal microsatellite markers show a single haplotype in the domestic horse, whereas notable variation has been observed in the other members of the genus *Equus*.

Weber, D. S., B. S. Stewart, et al. (2004). "Genetic Consequences of a Severe Population Bottleneck in the Guadalupe Fur Seal (*Arctocephalus townsendi*)."
J. Hered. **95**(2): 144-153.

<http://jhered.oupjournals.org/cgi/content/abstract/95/2/144>

Population bottlenecks may lead to diminished genetic variability and correlative effects on fitness. The Guadalupe fur seal was nearly exterminated by commercial sealers during the late 18th and early 19th centuries. To determine the genetic consequences of this population bottleneck, we compared the variation at a 181 bp section of the mitochondrial DNA (mtDNA) control region from the bones of 26 prebottleneck fur seals versus variation in the extant population. We found 25 different mtDNA genotypes in the prebottleneck fur seals and only 7 genotypes among 32 extant fur seals, including only one of the ancient genotypes. These data demonstrate a substantial loss of genetic variability correlating with the recent population bottleneck. We also found from several genetic measures that the prehistoric population of Guadalupe fur seals was robust and that it had been increasing at some time during the late prehistoric period. Continued recovery of this species may, however, owe more to more immediate nongenetic factors, such as poaching and local availability of food resources during the breeding season and consequent effects on pup survival, than on the reduced genetic variability.

Wehausen, J. D., R. R. Ramey, II, et al. (2004). "Experiments in DNA Extraction and PCR Amplification from Bighorn Sheep Feces: the Importance of DNA Extraction Method." J. Hered. **95**(6): 503-509.

<http://jhered.oupjournals.org/cgi/content/abstract/95/6/503>

Reliability of genotyping is an issue for studies using non-invasive sources of DNA. We emphasize the importance of refining DNA extraction methods to maximize reliability and efficiency of genotyping for such DNA sources. We present a simple and general method to quantitatively compare genotyping reliability of various DNA extraction techniques and sample materials used. For bighorn sheep (*Ovis canadensis*) fecal samples we compare different fecal pellet materials, different amounts of fecal pellet material, and the effects of eliminating two DNA extraction steps for four microsatellite loci and four samples heterozygous at each locus. We evaluated 192 PCR outcomes for each treatment using indices of PCR success and peak height (signal strength) developed from analysis output of sequencer chromatograms. Outermost pellet material produced PCR results almost equivalent to DNA extracted from blood. Where any inner pellet material was used for DNA extraction, PCR results were poorer and inconsistent among samples. PCR success was not sensitive to amount of pellet material used until it was decreased to 15 mg from 60 mg. Our PCR index provides considerably more information relative to potential genotyping errors than simply comparing genotypes derived from paired fecal and blood or tissue samples. Our DNA extraction method probably has wide applicability to herbivores that produce pelleted feces where samples dry rapidly after deposition.

Woeste, K., R. Burns, et al. (2002). "Thirty Polymorphic Nuclear Microsatellite Loci From Black Walnut." J. Hered. **93**(1): 58-60.

<http://jhered.oupjournals.org/cgi/content/abstract/93/1/58>

Black walnut (*Juglans nigra* L) is a large tree, native to the eastern United States, that is prized for its high-quality timber and edible nut. Thirty (GA/CT)_n nuclear microsatellite markers were identified from black walnut for use in population genetic studies, genome mapping, DNA genotyping of important clones, studies of gene flow, and tree breeding. The markers were polymorphic based on a diversity panel of 10 black walnut individuals from eight Midwestern U.S. states.

Wolf, P. G., B. Doche, et al. (2004). "Genetic Structure of *Rhododendron ferrugineum* at a Wide Range of Spatial Scales." *J. Hered.* **95**(4): 301-308.

<http://jhered.oupjournals.org/cgi/content/abstract/95/4/301>

Rhododendron ferrugineum L. (Ericaceae) is a subalpine shrub found throughout the Pyrenees and Alps at elevations of 1600-2200 m. We examined relationships between genetic and geographic distance, using 115 dominant amplified fragment length polymorphism (AFLP) markers to assess genetic structure over a wide range of spatial scales. We sampled 17 sites with distances of 4 km to more than 1000 km between them. At these scales we detected no association between geographic distance and genetic distance between populations. This suggests that genetic drift and gene flow are not in equilibrium for these populations. This pattern could have resulted from recent and rapid postglacial colonization, from more recent human disturbance, or as a function of frequent and random "natural" long-distance colonization. At two of our sites we used transects (two horizontal and two vertical with respect to slope at each site) to sample at distances ranging from 10 m to more than 5000 m. At this scale we observed a positive relationship between genetic and spatial distance along two vertical transects, one at each site. We hypothesize that isolation-by-distance at this smaller scale is a function of restricted gene flow via seed dispersal.

Zufall, R. A. and M. D. Rausher (2003). "The Genetic Basis of a Flower Color Polymorphism in the Common Morning Glory (*Ipomoea purpurea*)."
J. Hered. **94**(6): 442-448.

<http://jhered.oupjournals.org/cgi/content/abstract/94/6/442>

The common morning glory (*Ipomoea purpurea*) is highly polymorphic for flower color. Part of this phenotypic variation is due to allelic variation at the P locus. This locus determines whether flowers will be purple or pink, where purple is dominant to pink. We have determined that the anthocyanin biosynthetic gene flavonoid 3'-hydroxylase (f3'h) corresponds to the P locus. In the pink allele of f3'h there is a large insertion in the third exon, which results in the production of a truncated transcript. This shortened transcript produces a nonfunctional F3'H enzyme, resulting in the production of pink flowers rather than purple. In addition, we describe a polymerase chain reaction (PCR)-based assay that can be used to determine the genotype of a plant at this locus.

J. Histochem. Cytochem. (11)

Beck, K., K. Hayashi, et al. (2003). "The Distribution of Abcc6 in Normal Mouse Tissues Suggests Multiple Functions for this ABC Transporter." *J. Histochem. Cytochem.* **51**(7): 887-902.

<http://www.jhc.org/cgi/content/abstract/51/7/887>

We have studied the tissue distribution of Abcc6, a member of the ABC transmembrane transporter subfamily C, in normal C57BL/6 mice. RNase protection assays revealed that although almost all tissues studied contained detectable levels of the mRNA encoding Abcc6, the highest levels of Abcc6 mRNA were found in the liver. In situ hybridization (ISH) demonstrated

abundant Abcc6 mRNA in epithelial cells from a variety of tissues, including hepatic parenchymal cells, bile duct epithelia, kidney proximal tubules, mucosa and gland cells of the stomach, intestine, and colon, squamous epithelium of the tongue, corneal epithelium of the eye, keratinocytes of the skin, and tracheal and bronchial epithelium. Furthermore, we detected Abcc6 mRNA in arterial endothelial cells, smooth muscle cells of the aorta and myocardium, in circulating leukocytes, lymphocytes in the thymus and lymph nodes, and in neurons of the brain, spinal cord, and the specialized neurons of the retina. Immunohistochemical analysis using a polyclonal Abcc6 rabbit antibody confirmed the tissue distribution of Abcc6 suggested by our ISH studies and revealed the cellular localization of Abcc6 in the basolateral plasma membrane in the epithelial cells of proximal convoluted tubules in the kidney. Although the function of Abcc6 is unknown, mutations in the human ABCC6 gene result in a heritable disorder of connective tissue called pseudoxanthoma elasticum (PXE). Our results demonstrating the presence of Abcc6 in epithelial and endothelial cells in a variety of tissues, including those tissues affected in PXE patients, suggest a possible role for Abcc6 in the normal assembly of extracellular matrix components. However, the presence of Abcc6 in neurons and leukocytes, two cell populations not associated with connective tissue, also suggests a more complex multifunctional role for Abcc6. (J Histochem Cytochem 51:887-902, 2003)

Divjak, M., E. M. Glare, et al. (2002). "Improvement of Non-radioactive In Situ Hybridization in Human Airway Tissues: Use of PCR-generated Templates for Synthesis of Probes and an Antibody Sandwich Technique for Detection of Hybridization." J. Histochem. Cytochem. **50**(4): 541-548.

<http://www.jhc.org/cgi/content/abstract/50/4/541>

We describe the use of non-traditional methods of probe synthesis and quantification and detection of hybridization that appreciably improved non-radioactive in situ hybridization (ISH) in human airway tissue. To avoid the problems of bacterial cloning, plasmid digestion, and probe hydrolysis, we synthesised complementary RNA probes (riboprobes) for ISH from PCR-generated DNA. DNA template was produced by nested PCR incorporation of T7 and SP6 RNA polymerase promoters. We then compared the efficiency of in vitro transcription from PCR-generated template with traditional plasmid template by quantifying the relative probe fluorescence in denaturing gels. Transcription with SP6 or T7 polymerase in either orientation produced TNF riboprobes from a single PCR-generated template more efficiently than from plasmid, providing there were no primer hairpin loops. Fluorescence quantification enabled equal amounts of probe label to be used in ISH, eliminating signals from the sense probe and demonstrating that probes transcribed from PCR templates were as sensitive as hydrolyzed probe transcribed from plasmid. Detection of ISH by a conventional anti-hapten, alkaline phosphatase-based technique was found to cause tissue damage due to extended substrate incubation at high pH. We therefore developed a four-layer, avidin-biotin-peroxidase technique that afforded greater sensitivity, allowing brief substrate incubation and resulting in structural preservation of tissue. (J Histochem Cytochem 50:541-548, 2002)

Hromadnikova, I., L. Vechetova, et al. (2005). "Non-invasive Fetal RHD and RHCE Genotyping Using Real-time PCR Testing of Maternal Plasma in RhD-negative Pregnancies." J. Histochem. Cytochem. **53**(3): 301-305.

<http://www.jhc.org/cgi/content/abstract/53/3/301>

We assessed the feasibility of fetal RHD and RHCE genotyping by analysis of DNA extracted from plasma samples of RhD-negative pregnant women using real-time PCR and primers and probes targeted toward RHD and RHCE genes. We analyzed 45 pregnant women in the 11th to 40th weeks of pregnancy and correlated the results with serological analysis of cord blood after

delivery. Non-invasive prenatal fetal RHD exon 7, RHD exon 10, RHCE exon 2 (C allele), and RHCE exon 5 (E allele) genotyping analysis of maternal plasma samples was correctly performed in 45 out of 45 RhD-negative pregnant women delivering 24 RhD-, 17 RhC-, and 7 RhE-positive newborns. Detection of fetal RHD and the C and E alleles of RHCE gene from maternal plasma is highly accurate and enables implementation into clinical routine. We recommend performing fetal RHD and RHCE genotyping together with fetal sex determination in alloimmunized D-negative pregnancies at risk of hemolytic disease of the newborn. In case of D-negative fetus, amplification of another paternally inherited allele (SRY and/or RhC and/or RhE positivity) proves the presence of fetal DNA in maternal circulation. (J Histochem Cytochem 53:301-305, 2005)

Janes, M. S., B. J. Hanson, et al. (2004). "Rapid Analysis of Mitochondrial DNA Depletion by Fluorescence In Situ Hybridization and Immunocytochemistry: Potential Strategies for HIV Therapeutic Monitoring." J. Histochem. Cytochem. **52**(8): 1011-1018.

<http://www.jhc.org/cgi/content/abstract/52/8/1011>

Nucleoside reverse transcriptase inhibitors (NRTIs) have been a mainstay in the treatment of human immunodeficiency virus since the introduction of azidothymidine (AZT) in 1987. However, none of the current therapies can completely eradicate the virus, necessitating long-term use of anti-retroviral drugs to prevent viral re-growth. One of the side effects associated with long-term use of NRTIs is mitochondrial toxicity stemming from inhibition of the mitochondrial DNA (mtDNA) polymerase {gamma}, which leads to mtDNA depletion and consequently to mitochondrial dysfunction. Here we report the use of fluorescence in situ hybridization (FISH) and immunocytochemistry (ICC) to monitor mtDNA depletion in cultured fibroblasts treated with the NRTI 2',3'-dideoxycytidine (ddC). These techniques are amenable to both microscopy and flow cytometry, allowing analysis of populations of cells on a single-cell basis. We show that, as mtDNA depletion progresses, a mosaic population develops, with some cells being depleted of and others retaining mtDNA. These techniques could be useful as potential therapeutic monitors to indicate when NRTI therapy should be interrupted to prevent mitochondrial toxicity and could aid in the development of less toxic NRTIs by providing an assay suitable for pharmacodynamic evaluation of candidate molecules. (J Histochem Cytochem 52:1011-1018, 2004)

Konig, P., J. Dedio, et al. (2005). "NOSIP and Its Interacting Protein, eNOS, in the Rat Trachea and Lung." J. Histochem. Cytochem. **53**(2): 155-164.

<http://www.jhc.org/cgi/content/abstract/53/2/155>

Endothelial nitric oxide synthase (eNOS), the major nitric oxide (NO)-generating enzyme of the vasculature, is regulated through multiple interactions with proteins, including caveolin-1, Hsp90, Ca²⁺-calmodulin, and the recently discovered eNOS-interacting protein, NOSIP. Previous studies indicate that NOSIP may contribute to the intricate regulation of eNOS activity and availability. Because eNOS has been shown to be abundantly expressed in the airways, we determined the expression and cellular localization of NOSIP in rat trachea and lung by RT-PCR and immunohistochemistry and examined the interaction of NOSIP with eNOS in lung by coimmunoprecipitation. In tracheal epithelium and lung, NOSIP mRNA expression was prevalent, as shown by RT-PCR, and the corresponding protein interacted with eNOS, as demonstrated by coimmunoprecipitation. Using immunohistochemistry, we found both NOSIP and eNOS immunoreactivity in ciliated epithelial cells of trachea and bronchi, while Clara cells showed immunoreactivity for NOSIP only. NOSIP and eNOS were present in vascular and bronchial smooth muscle cells of large arteries and airways, whereas endothelial cells, as well as bronchiolar and arteriolar smooth muscle cells, exclusively stained for NOSIP. Our results point to functional role(s) of NOSIP in the control of airway and vascular diameter, mucosal secretion, NO

synthesis in ciliated epithelium, and, therefore, of mucociliary and bronchial function. (J Histochem Cytochem 53:155-164, 2005)

Lips, K. S., U. Pfeil, et al. (2003). "Expression of the High-affinity Choline Transporter CHT1 in Rat and Human Arteries." J. Histochem. Cytochem. **51**(12): 1645-1654.

<http://www.jhc.org/cgi/content/abstract/51/12/1645>

The arterial vascular wall contains a non-neuronal intrinsic cholinergic system. The rate-limiting step in acetylcholine (ACh) synthesis is choline uptake. A high-affinity choline transporter, CHT1, has recently been cloned from neural tissue and has been identified in epithelial cholinergic cells. Here we investigated its presence in rat and human arteries and in primary cell cultures of rat vascular cells (endothelial cells, smooth muscle cells, fibroblasts). CHT1-mRNA was detected in the arterial wall and in all isolated cell types by RT-PCR using five different CHT1-specific primer pairs. Antisera raised against amino acids 29-40 of the rat sequence labeled a single band (50 kD) in Western blots of rat aorta, and an additional higher molecular weight band appeared in the hippocampus. Immunohistochemistry demonstrated CHT1 immunoreactivity in endothelial and smooth muscle cells in situ and in all cultured cell types. A high-affinity [3H]-choline uptake mechanism sharing characteristics with neuronal high-affinity choline uptake, i.e., sensitivity to hemicholinium-3 and dependence on sodium, was demonstrated in rat thoracic aortic segments by microimager autoradiography. Expression of the high-affinity choline transporter CHT1 is a novel component of the intrinsic non-neuronal cholinergic system of the arterial vascular wall, predominantly in the intimal and medial layers. (J Histochem Cytochem 51:1645-1654, 2003)

Merezhinskaya, N., S. A. Ogunwuyi, et al. (2004). "Presence and Localization of Three Lactic Acid Transporters (MCT1, -2, and -4) in Separated Human Granulocytes, Lymphocytes, and Monocytes." J. Histochem. Cytochem. **52**(11): 1483-1493.

<http://www.jhc.org/cgi/content/abstract/52/11/1483>

We fractionated leukocytes from three donors into >90% pure samples of granulocytes, lymphocytes, and monocytes and tested them for transcriptional and translational expression of three physiologically-proven lactate transporters, monocarboxylate transporter 1(MCT1), MCT2, and MCT4, using RT-PCR and affinity-purified rabbit antibody (Ab) to the C-terminal segment of each human MCT. Transcripts of all three MCTs were identified in each leukocyte fraction by RT-PCR and proven by sequencing of fragments extracted after isolation on agarose gels. Transporter protein of the appropriate size was demonstrated for each of the monocarboxylate transporters MCTs in lymphocytes and monocytes by Western blot, while lower-molecular-weight bands were found in granulocytes and are presumed to be degraded forms, because they were blocked by antibody-antigen (Ab-Ag) preincubation. IHC demonstrated all three MCTs in methanol-fixed droplets of all three leukocyte fractions; stain was abolished on omission of the primary Ab. Plasmalemmal staining occurred with all MCTs in all leukocyte fractions. Because the Km for lactate increases approximately fivefold at each step, with MCT2<1<4, leukocytes must use the full range of lactate binding to survive in acidic and hypoxic environments. Except for MCT4 in lymphocytes, all the MCTs also stained leukocyte cytoplasm, often with distinct granularity. Nuclear membrane staining was also seen with MCT1 and MCT2, while platelet plasmalemma stained only with MCT2. (J Histochem Cytochem 52:1483-1493, 2004)

Mowa, C. N., S. Jesmin, et al. (2004). "Characterization of Vascular Endothelial Growth Factor (VEGF) in

the Uterine Cervix over Pregnancy: Effects of Denervation and Implications for Cervical Ripening." J. Histochem. Cytochem. **52**(12): 1665-1674.

<http://www.jhc.org/cgi/content/abstract/52/12/1665>

Bilateral neurectomy of the pelvic nerve (BLPN) that carries uterine cervix-related sensory nerves induces dystocia, and administration of its vasoactive neuropeptides induces changes in the cervical microvasculature, resembling those that occur in the ripening cervix. This study was designed to test the hypothesis that (a) the cervix of pregnant rats expresses vascular endothelial growth factor (VEGF) and components of the angiogenic signaling pathway [VEGF receptors (Flt-1, KDR), activity of protein kinase B, Akt (phosphorylated Akt), and endothelial nitric oxide synthase (eNOS)] and von Willebrand Factor (vWF) and that these molecules undergo changes with pregnancy, and (b) bilateral pelvic neurectomy (BLPN) alters levels of VEGF concentration in the cervix. Using RT-PCR and sequencing, two VEGF isoforms, 120 and 164, were identified in the rat cervix. VEGF, VEGF receptor-1 (Flt-1), eNOS, and vWF immunoreactivities (ir) were localized in the microvasculature of cervical stroma. Their protein levels increased during pregnancy but decreased to control levels by 2 days postpartum. VEGF receptor-2 (KDR)-ir was confined to the epithelium of the endocervix. BLPN downregulated levels of VEGF by a third. Therefore, the components of the angiogenic signaling pathway are expressed in the cervix and change over pregnancy. Furthermore, angiogenic and sensory neuronal factors may be important in regulating the dynamic microvasculature in the ripening cervix and may subsequently play a role in cervical ripening and the birth process. (*J Histochem Cytochem* 52:1665-1674, 2004)

Ogawa, Y., K. Matsumoto, et al. (2002). "Characterization of Lacrimal Gland Carbonic Anhydrase VI." J. Histochem. Cytochem. **50**(6): 821-828.

<http://www.jhc.org/cgi/content/abstract/50/6/821>

We have previously demonstrated by immunohistochemistry the presence of secreted carbonic anhydrase (CA VI) in the acinar cells of the rat lacrimal glands. In this study we purified the sheep lacrimal gland CA VI to homogeneity and demonstrated by Western analysis that it has the same apparent subunit molecular weight (45 kD) as the enzyme isolated from saliva. RT-PCR analysis showed that CA VI mRNA from the lacrimal gland was identical to that of the parotid gland CA VI mRNA. An RIA specific for sheep CA VI showed the lacrimal gland tissue concentration of the enzyme to be 4.20 $\pm$ 2.60 ng/mg protein, or about 1/7000 of the level found in the parotid gland. Immunohistochemistry (IHC) and in situ hybridization (ISH) showed that lacrimal acinar cells expressed both immunoreactivity and mRNA for CA VI. Moreover, CA VI immunoreactivity was occasionally observed in the lumen of the ducts. Unlike the parotid gland, in which all acinar cells expressed CA VI immunoreactivity and mRNA, only some of the acinar cells of the lacrimal gland showed expression. These results indicate that the lacrimal gland synthesizes and secretes a very small amount of salivary CA VI. In tear fluid, CA VI is presumed to have a role in the maintenance of acid/base balance on the surface of the eye, akin to its role in the oral cavity. (*J Histochem Cytochem* 50:821-827, 2002)

Oshima, Y., N. Watanabe, et al. (2005). "Behavior of Transplanted Bone Marrow-derived GFP Mesenchymal Cells in Osteochondral Defect as a Simulation of Autologous Transplantation." J. Histochem. Cytochem. **53**(2): 207-216.

<http://www.jhc.org/cgi/content/abstract/53/2/207>

To elucidate the behavior of autologously transplanted mesenchymal cells in osteochondral

defects, we followed transplanted cells using green fluorescent protein (GFP) transgenic rats, in which all cells express GFP signals in their cytoplasm and nuclei as transplantation donors. Bone marrow-derived mesenchymal cells, which contain mesenchymal stem cells (MSCs), were obtained from transgenic rats. Then, dense mesenchymal cell masses created by hanging-drop culture were transplanted and fixed with fibrin glue into osteochondral defects of wild-type rats. At 24 weeks after surgery, the defects were repaired with hyaline-like cartilage and subchondral bone. GFP positive cells, indicating transplanted mesenchymal-derived cells, were observed in the regenerated tissues for 24 weeks although GFP positive cells decreased in number with time. Because GFP causes no immunological rejection and requires no chemicals for visualization, transplantation between transgenic and wild-type rats can be regarded as a simulation of autologous transplantation, and the survivability of transplanted cells are able to be followed easily and reliably. Thus, the behavior of transplanted mesenchymal cells was able to be elucidated in vivo by this strategy, and the results could be essential in future tissue engineering for the regeneration of osteochondral defects with original hyaline cartilage and subchondral bone. (J Histochem Cytochem 53:207-216, 2005)

Shi, S.-R., R. J. Cote, et al. (2002). "DNA Extraction from Archival Formalin-fixed, Paraffin-embedded Tissue Sections Based on the Antigen Retrieval Principle: Heating Under the Influence of pH." J. Histochem. Cytochem. **50**(8): 1005-1011.

<http://www.jhc.org/cgi/content/abstract/50/8/1005>

During the course of diagnostic surgical pathology, pathologists have established a large collection of formalin-fixed, paraffin-embedded tissues that form invaluable resources for translational studies of cancer and a variety of other diseases. Accessibility of macromolecules in the fixed tissue specimens is a critical issue as exemplified by heat-induced antigen retrieval (AR) immunohistochemical (IHC) staining. On the basis of observations that heating may also enhance in situ hybridization (ISH) and the similarity of formalin-induced chemical modifications that occur in protein and in DNA, we designed a study to examine the efficiency of DNA extraction from archival formalin-fixed, paraffin-embedded tissues using an adaptation of the basic principles of the AR technique, i.e., heating the tissue under the influence of different pH values. Archival paraffin blocks of lymph nodes, tonsil, and colon were randomly selected. Each paraffin block was prepared in 34 microtubes. For each paraffin block, one tube was used as a control sample, using a non-heating DNA extraction protocol. The other 33 tubes were tested using a heating protocol under 11 variable pH values (pH 2 to 12) under three different heating conditions (80, 100, and 120C). Evaluation of the results of DNA extraction was carried out by measuring yields by photometry and PCR amplification, as well as kinetic thermocycling (KTC)-PCR methods. In general, lower pH (acid) solutions gave inferior results to solutions at higher pH (alkaline). Heating tissues at a higher temperature and at pH 6-9 gave higher yields of DNA. There appeared to be a peak in terms of highest efficiency of extracted DNA at around pH 9. The average ratios 260:280 of extracted DNA also showed better values for samples heated at 120C. PCR products of three primers showed satisfactory results for DNA extracted from archival paraffin-embedded tissues by heating protocols at pH 6-12, with results that were comparable to the control sample subjected to the standard non-heating, enzymatic DNA extraction method. This study is the first to document the use of heating at an alkaline pH for DNA extraction from archival formalin-fixed, paraffin-embedded tissues, a recommendation based on the principles of AR for protein IHC. These findings may lead to a more effective protocol for DNA extraction from archival paraffin-embedded tissues and may also provide enhanced understanding of changes that occur during formalin-induced modification of nucleic acids. (J Histochem Cytochem 50:1005-1011, 2002)

Abraham, E., M. R. Gyetko, et al. (2003). "Urokinase-Type Plasminogen Activator Potentiates Lipopolysaccharide-Induced Neutrophil Activation." J. Immunol. **170**(11): 5644-5651.

<http://www.jimmunol.org/cgi/content/abstract/170/11/5644>

Urokinase plasminogen activator (uPA) is a serine protease that catalyzes the conversion of plasminogen to plasmin. Although increased circulating levels of uPA are present in endotoxemia and sepsis, conditions in which activated neutrophils contribute to the development of acute organ dysfunction, the ability of uPA to participate directly in LPS-induced neutrophil activation has not been examined. In the present experiments, we show that uPA can enhance activation of neutrophils exposed to submaximal stimulatory doses of LPS. In particular, uPA increased LPS-induced activation of intracellular signaling pathways, including Akt and c-Jun N-terminal kinase, nuclear translocation of the transcriptional regulatory factor NF- κ B, and expression of proinflammatory cytokines, including IL-1 β , macrophage-inflammatory protein-2, and TNF- α . There was no effect of uPA on LPS-induced activation of p38 mitogen-activated protein kinase in neutrophils. Transgenic mice unable to produce uPA (uPA $^{-/-}$) were protected from endotoxemia-induced lung injury, as determined by development of lung edema, pulmonary neutrophil accumulation, lung IL-1 β , macrophage-inflammatory protein-2, and TNF- α cytokine levels. These results demonstrate that uPA can potentiate LPS-induced neutrophil responses and also suggest that such effects are sufficiently important in vivo to play a major contributory role in neutrophil-mediated inflammatory responses, such as the development of acute lung injury.

Adriouch, S., C. Dox, et al. (2002). "Cutting Edge: A Natural P451L Mutation in the Cytoplasmic Domain Impairs the Function of the Mouse P2X7 Receptor." J. Immunol. **169**(8): 4108-4112.

<http://www.jimmunol.org/cgi/content/abstract/169/8/4108>

The P2X7 receptor (P2X7R) is an ATP-gated channel that mediates apoptosis of cells of the immune system. The capacity of P2X7R to form large pores depends on its large cytoplasmic tail, which harbors a putative TNFR-related death domain. Previous transfection studies indicated that mouse P2X7R forms pores much less efficiently than its counterparts from humans and rats. In this study, we demonstrate that an allelic mutation (P451L) in the predicted death domain of P2X7R confers a drastically reduced sensitivity to ATP-induced pore formation in cells from some commonly used strains of mice, i.e., C57BL/6 and DBA/2. In contrast, most other strains of mice, including strains derived from wild mice, carry P451 at this position as do rats and humans. The effects of the P451L mutation resemble those of the E496A mutation in human P2X7R. These P2X7R mutants may provide useful tools to decipher the molecular mechanisms leading to pore formation.

Anders, H.-J., M. Frink, et al. (2003). "CC Chemokine Ligand 5/RANTES Chemokine Antagonists Aggravate Glomerulonephritis Despite Reduction of Glomerular Leukocyte Infiltration." J. Immunol. **170**(11): 5658-5666.

<http://www.jimmunol.org/cgi/content/abstract/170/11/5658>

The chemokine CC chemokine ligand (CCL)5/RANTES as well as its respective receptor CCR5

mediate leukocyte infiltration during inflammation and are up-regulated early during the course of glomerulonephritis (GN). We tested the effects of the two CCL5/RANTES blocking analogs, Met-RANTES and amino-oxypentane-RANTES, on the course of horse apoferritin (HAF)-induced GN. HAF-injected control mice had proliferative GN with mesangial immune complex deposits of IgG and HAF. Daily i.p. injections of Met-RANTES or amino-oxypentane-RANTES markedly reduced glomerular cell proliferation and glomerular macrophage infiltration, which is usually associated with less glomerular injury and proteinuria in HAF-GN. Surprisingly, however, HAF-GN mice treated with both analogs showed worse disease with mesangiolysis, capillary obstruction, and nephrotic range albuminuria. These findings were associated with an enhancing effect of the CCL5/RANTES analogs on the macrophage activation state, characterized by a distinct morphology and increased inducible NO synthetase expression in vitro and in vivo, but a reduced uptake of apoptotic cells in vivo. The humoral response and the Th1/Th2 balance in HAF-GN and mesangial cell proliferation in vitro were not affected by the CCL5/RANTES analogs. We conclude that, despite blocking local leukocyte recruitment, chemokine analogs can aggravate some specific disease models, most likely due to interactions with systemic immune reactions, including the removal of apoptotic cells and inducible NO synthetase expression.

Aoyagi, K., J. M. Dijkstra, et al. (2002). "Classical MHC Class I Genes Composed of Highly Divergent Sequence Lineages Share a Single Locus in Rainbow Trout (*Oncorhynchus mykiss*)."
J. Immunol. **168**(1): 260-273.

<http://www.jimmunol.org/cgi/content/abstract/168/1/260>

The classical MHC class I genes have been known to be highly polymorphic in various vertebrates. To date, putative allelic sequences of the classical MHC class I genes in teleost fish have been reported in several studies. However, the establishment of their allelic status has been hampered in most cases by the lack of appropriate genomic information. In the present study, using heterozygous and homozygous fish, we obtained classical-type MHC class I sequences of rainbow trout (*Oncorhynchus mykiss*) and investigated their allelic relationship by gene amplification and Southern and Northern hybridization analyses. The results indicated that all MHC class I sequences we obtained were derived from a single locus. Based on this, a unique polymorphic nature of the MHC class I locus of rainbow trout has been revealed. The mosaic combination of highly divergent ancient sequences in the peptide-binding domains is notable, and the variable nature around the boundary between the {alpha}3 and transmembrane domains is unprecedented.

Attur, M. G., M. N. Dave, et al. (2002). "Functional Genomic Analysis of Type II IL-1{beta} Decoy Receptor: Potential for Gene Therapy in Human Arthritis and Inflammation."
J. Immunol. **168**(4): 2001-2010.

<http://www.jimmunol.org/cgi/content/abstract/168/4/2001>

Gene expression arrays show that human epithelial cells and human arthritis-affected cartilage lack detectable amounts of mRNA for IL-1 antagonizing molecules: IL-1Ra and IL-1RII, but constitutively express IL-1. Functional genomic analysis was performed by reconstituting human IL-1RII expression in various IL-1RII-deficient cell types to examine its antagonist role using gene therapy approaches. Adenovirus-expressing IL-1RII when transduced into human and bovine chondrocytes, human and rabbit synovial cells, human epithelial cells, and rodent fibroblasts expressed membrane IL-1RII and spontaneously released functional soluble IL-1RII. The IL-1RII+ (but not IL-1RII-) cells were resistant to IL-1{beta}-induced, NO, PGE2, IL-6, and IL-8 production or decreased proteoglycan synthesis. IL-1RII inhibited the function of IL-1 in chondrocytes and IL-1- and TNF-{alpha}-induced inflammatory mediators in human synovial and epithelial cells. IL-

1RII⁺ chondrocytes were more resistant to induction of NO and PGE₂ by IL-1{beta} compared with IL-1RII⁻ cells incubated with a 10-fold (weight) excess of soluble type II IL-1R (sIL-1RII) protein. In cocultures, IL-1RII⁺ synovial cells released sIL-1RII, which in a paracrine fashion protected chondrocytes from the effects of IL-1{beta}. Furthermore, IL-1RII⁺ (but not IL-1RII⁻) chondrocytes when transplanted onto human osteoarthritis-affected cartilage in vitro, which showed spontaneous release of sIL-1RII for 20 days, inhibited the spontaneous production of NO and PGE₂ in cartilage in ex vivo. In summary, reconstitution of IL-1RII in IL-1RII⁻ cells using gene therapy approaches significantly protects cells against the autocrine and paracrine effects of IL-1 at the signaling and transcriptional levels.

Austin, A. S., K. M. Haas, et al. (2003). "Identification and Characterization of a Novel Regulatory Factor: IgA-Inducing Protein." *J. Immunol.* **171**(3): 1336-1342.

<http://www.jimmunol.org/cgi/content/abstract/171/3/1336>

IgA is the predominant Ig isotype in mucosal secretions and thus plays a pivotal role in host defense. The mechanisms by which IgA expression is regulated may differ among species and involve multiple pathways. Various cytokines and costimulators have been identified which regulate expression of this isotype, including IL-10, IL-2, vasoactive intestinal peptide, and TGF-β. We have tested a wide array of known factors, but only under very limited conditions do these factors mediate substantial IgA production in vitro from bovine B cells. In response to these findings, we generated a cDNA library in a mammalian expression vector from activated cells derived from bovine gut-associated lymphoid tissues (Peyer's patch and mesenteric lymph node cells) as a source of soluble factor(s) that may regulate IgA production. We have identified a novel factor, IgA-inducing protein, which stimulates relatively high levels of IgA production in vitro following CD40 stimulation in coculture with IL-2. Our data suggest that IgA-inducing protein regulates IgA by acting as a switch or differentiation factor and is expressed in a variety of lymphoid and nonlymphoid tissues.

Bacci, A., C. Montagnoli, et al. (2002). "Dendritic Cells Pulsed with Fungal RNA Induce Protective Immunity to *Candida albicans* in Hematopoietic Transplantation." *J. Immunol.* **168**(6): 2904-2913.

<http://www.jimmunol.org/cgi/content/abstract/168/6/2904>

Immature myeloid dendritic cells (DC) phagocytose yeasts and hyphae of the fungus *Candida albicans* and induce different Th cell responses to the fungus. Ingestion of yeasts activates DC for production of IL-12 and Th1 priming, while ingestion of hyphae induces IL-4 production and Th2 priming. In vivo, generation of antifungal protective immunity is induced upon injection of DC ex vivo pulsed with *Candida* yeasts but not hyphae. In the present study we sought to determine the functional activity of DC transfected with yeast or hyphal RNA. It was found that DC, from either spleens or bone marrow, transfected with yeast, but not hyphal, RNA 1) express fungal mannoproteins on their surface; 2) undergo functional maturation, as revealed by the up-regulated expression of MHC class II Ags and costimulatory molecules; 3) produce IL-12 but no IL-4; 4) are capable of inducing Th1-dependent antifungal resistance when delivered s.c. in vivo in nontransplanted mice; and 5) provide protection against the fungus in allogeneic bone marrow-transplanted mice, by accelerating the functional recovery of *Candida*-specific IFN-γ-producing CD4⁺ donor lymphocytes. These results indicate the efficacy of DC pulsed with *Candida* yeasts or yeast RNA as fungal vaccines and point to the potential use of RNA-transfected DC as anti-infective vaccines in conditions that negate the use of attenuated microorganisms or in the case of poor availability of protective Ags.

Bas, A., S. G. Hammarstrom, et al. (2003). "Extrathymic TCR Gene Rearrangement in Human Small Intestine: Identification of New Splice Forms of Recombination Activating Gene-1 mRNA with Selective Tissue Expression." *J. Immunol.* **171**(7): 3359-3371.

<http://www.jimmunol.org/cgi/content/abstract/171/7/3359>

Two new 5'-untranslated region (5'UTR) exons were identified in the human gene for the lymphocyte-specific endonuclease recombination activating gene-1 (RAG1) required for the somatic recombination yielding functional Ag receptors. These 5'UTR exons were used in three different splice forms by jejunal lymphocytes of the T cell lineage. RAG1 mRNA containing the previously described 5'UTR exon was not expressed in these cells. Conversely, one of the new 5'UTR exons was not expressed in thymus. The new RAG1 mRNA splice forms were all expressed in immature T cells (CD2+CD7+CD3-). This cell population also expressed high levels of mRNA for the pre-T {alpha}-chain. In situ hybridization demonstrated jejunal cells expressing the new splice forms of RAG1 mRNA, both intraepithelially and in lamina propria. Pre-T {alpha}-chain mRNA-expressing cells were detected at the same sites. These results strongly suggest ongoing TCR gene rearrangement in human small intestinal mucosa, yielding T cells specially adapted for this environment. This seems to be achieved by two parallel processes, extrathymic T cell development and peripheral Ag-driven TCR editing.

Bave, U., M. Magnusson, et al. (2003). "Fc{gamma}RIIa Is Expressed on Natural IFN-{alpha}-Producing Cells (Plasmacytoid Dendritic Cells) and Is Required for the IFN-{alpha} Production Induced by Apoptotic Cells Combined with Lupus IgG." *J. Immunol.* **171**(6): 3296-3302.

<http://www.jimmunol.org/cgi/content/abstract/171/6/3296>

An ongoing production of IFN-{alpha} may be of etiopathogenic significance in systemic lupus erythematosus (SLE). It may be due to the natural IFN-producing cells (NIPC), also termed plasmacytoid dendritic cells (PDC), activated by immune complexes that contain nucleic acids derived from apoptotic cells. We here examined the role of Fc{gamma}R in the IFN-{alpha} production in vitro by PBMC induced by the combination of apoptotic U937 cells and autoantibody-containing IgG from SLE patients (SLE-IgG). The Fc portion of the SLE-IgG was essential to induce IFN-{alpha} production, because Fab fragments or F(ab')₂ were ineffective. Normal, especially heat-aggregated, IgG inhibited the IFN-{alpha} production, suggesting a role for Fc{gamma}R on PBMC. Using blocking anti-Fc{gamma}R Abs, the Fc{gamma}RIIa,c (CD32) but not Fc{gamma}RI or Fc{gamma}RIII were shown to be involved in the IFN-{alpha} induction by apoptotic cells combined with SLE-IgG, but not by HSV or CpG DNA. In contrast, the action of all of these inducers was inhibited by the anti-Fc{gamma}RIIa,b,c mAb AT10 or heat-aggregated IgG. Flow cytometric analysis revealed that [~]50% of the BDCA-2-positive PBMC, i.e., NIPC/PDC, expressed low but significant levels of Fc{gamma}RII, as did most of the actual IFN-{alpha} producers activated by HSV. RT-PCR applied to NIPC/PDC purified by FACS demonstrated expression of Fc{gamma}RIIa, but not of Fc{gamma}RIIb or Fc{gamma}RIIc. We conclude that Fc{gamma}RIIa on NIPC/PDC is involved in the activation of IFN-{alpha} production by interferogenic immune complexes, but may also mediate inhibitory signals. The Fc{gamma}RIIa could therefore have a key function in NIPC/PDC and be a potential therapeutic target in SLE.

Bayley, J.-P., J. G. I. van Rietschoten, et al. (2003). "Allele-Specific Expression of the IL-1{alpha} Gene in Human CD4+ T Cell Clones." *J. Immunol.* **171**(5): 2349-2353.

<http://www.jimmunol.org/cgi/content/abstract/171/5/2349>

A number of reports have described the monoallelic expression of murine cytokine genes. Here we describe the monoallelic expression of the human IL-1{alpha} gene in CD4+ T cells. Analysis of peripheral blood T cell clones derived from healthy individuals revealed that the IL-1{alpha} gene shows predominantly monoallelic expression. Monoallelic expression was observed in Th0, Th1, and Th2 cell clones. In addition, we demonstrate monoallelic expression in T cell clones from rheumatoid arthritis patients derived from synovial fluid of the knee joint, suggesting that the occurrence of this phenomenon is not different from that in clones derived from healthy individuals. The finding of monoallelic expression of a cytokine gene in human CD4+ T cell clones provides evidence for allele-specific silencing/activation as another layer of regulation of IL-1{alpha} gene expression.

Bengtén, E., S. M. A. Quiniou, et al. (2002). "The IgH Locus of the Channel Catfish, *Ictalurus punctatus*, Contains Multiple Constant Region Gene Sequences: Different Genes Encode Heavy Chains of Membrane and Secreted IgD." *J. Immunol.* **169**(5): 2488-2497.

<http://www.jimmunol.org/cgi/content/abstract/169/5/2488>

The {delta}-chain of catfish IgD was initially characterized as a unique chimeric molecule containing a rearranged VDJ spliced to C{micro}1, seven C domain-encoding exons ({delta}1-{delta}7), and a transmembrane tail. The presence of cDNA forms showing splicing of {delta}7 to an exon encoding a secretory tail was interpreted to indicate that membrane ({delta}m) and secreted ({delta}s) forms were likely expressed from a single gene by alternative RNA processing. Subsequent cloning and sequence analyses have unexpectedly revealed the presence of three {delta} C region genes, each linked to a {micro} gene or pseudogene. The first (IGHD1) is located 1.6 kb 3' of the functional C{micro} (IGHM1). The second (IGHD3) is positioned immediately downstream of a pseudo C{micro} (IGHM3P), [~]725 kb 5' of IGHM1. These two {delta} genes are highly similar in sequence and each contains a tandem duplication of {delta}2-{delta}3-{delta}4. However, IGHD1 has a terminal exon encoding the transmembrane region, whereas IGHD3 has a single terminal exon encoding a secreted tail. The occurrence of IGHD3 immediately downstream of a {micro} pseudogene indicates that the putative {delta}s product may not be expressed as a chimeric {micro}{delta} molecule. Western blots and protein sequencing data indicate that an IGHD3-encoded protein is expressed in catfish serum. Thus, catfish {delta}m transcripts appear to originate from IGHD1, whereas {delta}s transcripts originate from IGHD3 rather than, as previously inferred, from a single expressed {delta} gene. The third {delta} (IGHD2) is associated with a pseudo C{micro} (IGHM2P); its presence is inferred by Southern blot analyses.

Bengtson, P., A. Lundblad, et al. (2002). "Polymorphonuclear Leukocytes from Individuals Carrying the G329A Mutation in the {alpha}1,3-Fucosyltransferase VII Gene (FUT7) Roll on E- and P-Selectins." *J. Immunol.* **169**(7): 3940-3946.

<http://www.jimmunol.org/cgi/content/abstract/169/7/3940>

We recently identified several individuals carrying a missense mutation (G329A; Arg110-Gln) in the FUT7 gene encoding fucosyltransferase VII. This enzyme is involved in the biosynthesis of the sialyl Lewis x (Lex) epitope on human leukocytes, which has been identified as an important component of leukocyte ligands for E- and P-selectin. No enzyme activity was measurable in expression studies in COS-7 cells using the mutated FUT7 construct. One of the identified individuals carried this mutation homozygously. Flow cytometry analysis of polymorphonuclear

leukocytes (PMN) from this individual showed a nearly complete absence of staining with mAbs directed against sialyl Lex and a diminished staining with an E-selectin IgG chimera. However, staining with P-selectin IgG chimera and Abs directed against P-selectin glycoprotein ligand-1 was not affected by the mutation. PMN from the homozygously mutated individual was further analyzed in an in vitro flow chamber assay. The number of rolling PMN and the rolling velocities on both E- and P-selectin were in the range of PMN from nonmutated individuals. FUT4 and FUT7 mRNA was quantified in PMN isolated from individuals carrying the FUT7 mutation. It was found that PMN from both FUT7 homozygously and heterozygously mutated individuals exhibited an elevated expression of FUT4 mRNA compared with PMN from FUT7 nonmutated individuals. The elevated expression of fucosyltransferase IV was reflected as an increased expression of the Lex and CD65s Ags on PMN from these individuals. The significance of the mutation was supported by transfection of BJAB cells.

Bennett, F., D. Luxenberg, et al. (2003). "Program Death-1 Engagement Upon TCR Activation Has Distinct Effects on Costimulation and Cytokine-Driven Proliferation: Attenuation of ICOS, IL-4, and IL-21, But Not CD28, IL-7, and IL-15 Responses." *J. Immunol.* **170**(2): 711-718.

<http://www.jimmunol.org/cgi/content/abstract/170/2/711>

The program death 1 (PD-1) receptor and its ligands, PD-1 ligand (PD-L)1 and PD-L2, define a novel regulatory pathway with potential inhibitory effects on T, B, and monocyte responses. In the present study, we show that human CD4⁺ T cells express PD-1, PD-L1, and PD-L2 upon activation, and Abs to the receptor can be agonists or antagonists of the pathway. Under optimal conditions of stimulation, ICOS but not CD28 costimulation can be prevented by PD-1 engagement. IL-2 levels induced by costimulation are critical in determining the outcome of the PD-1 engagement. Thus, low to marginal IL-2 levels produced upon ICOS costimulation account for the greater sensitivity of this pathway to PD-1-mediated inhibition. Interestingly, exogenous IL-2, IL-7, and IL-15 but not IL-4 and IL-21 can rescue PD-1 inhibition, suggesting that among these cytokines only those that activate STAT5 can rescue PD-1 inhibition. As STAT5 has been implicated in the maintenance of IL-2R α expression, these results suggest that IL-7 and IL-15 restore proliferation under conditions of PD-1 engagement by enhancing high-affinity IL-2R expression and hence, IL-2 responsiveness.

Biassoni, R., M. Fogli, et al. (2005). "Molecular and Functional Characterization of NKG2D, NKp80, and NKG2C Triggering NK Cell Receptors in Rhesus and Cynomolgus Macaques: Monitoring of NK Cell Function during Simian HIV Infection." *J. Immunol.* **174**(9): 5695-5705.

<http://www.jimmunol.org/cgi/content/abstract/174/9/5695>

An involvement of innate immunity and of NK cells during the priming of adaptive immune responses has been recently suggested in normal and disease conditions such as HIV infection and acute myelogenous leukemia. The analysis of NK cell-triggering receptor expression has been so far restricted to only NKp46 and NKp30 in *Macaca fascicularis*. In this study, we extended the molecular and functional characterization to the various NK cell-triggering receptors using PBMC and to the in vitro-derived NK cell populations by cytofluorometry and by cytolytic activity assays. In addition, RT-PCR strategy, cDNA cloning/sequencing, and transient transfections were used to identify and characterize NKp80, NKG2D, CD94/NKG2C, and CD94/NKG2A in *M. fascicularis* and *Macaca mulatta* as well as in the signal transducing polypeptide DNAX-activating protein DAP-10. Both *M. fascicularis* and *M. mulatta* NK cells express NKp80, NKG2D, and NKG2C molecules, which displayed a high degree of sequence homology with their human counterpart. Analysis of NK cells in simian HIV-infected *M. fascicularis* revealed reduced surface expression of selected NK cell-triggering receptors

associated with a decreased NK cell function only in some animals. Overall surface density of NK cell-triggering receptors on peripheral blood cells and their triggering function on NK cell populations derived in vitro was not decreased compared with uninfected animals. Thus, triggering NK cell receptor monitoring on macaque NK cells is possible and could provide a valuable tool for assessing NK cell function during experimental infections and for exploring possible differences in immune correlates of protection in humans compared with cynomolgus and rhesus macaques undergoing different vaccination strategies.

Blois, S., M. Tometten, et al. (2005). "Intercellular Adhesion Molecule-1/LFA-1 Cross Talk Is a Proximate Mediator Capable of Disrupting Immune Integration and Tolerance Mechanism at the Feto-Maternal Interface in Murine Pregnancies." J. Immunol. **174**(4): 1820-1829.

<http://www.jimmunol.org/cgi/content/abstract/174/4/1820>

Our understanding why a woman's immune system does not reject her histoincompatible fetus is still very limited. Distinct insights into the mechanisms involved in pregnancy maintenance may help us to prevent pregnancy complications, e.g., miscarriages or pre-eclampsia. Immune integration and tolerance at the feto-maternal interface appear to be indispensable for successful pregnancy maintenance. Little is known about the cross talk between ICAM-1, expressed on epithelium, endothelium, and APC, and its ligand, LFA-1, at the feto-maternal interface. However, based on the role of ICAM-1/LFA-1 in allograft acceptance or rejection upon transplantation, adhesion molecules are likely to interfere with successful pregnancy outcome. In this study, we tested the hypothesis that ICAM-1/LFA-1 pathways may be involved in pregnancy rejection in murine models. By blocking ICAM-1/LFA-1-mediated intercellular adhesion events, we show that fetal immune acceptance is restored in challenged pregnancies (e.g., upon exposure to sound stress), and adoptive transfer of LFA-1 cells into pregnant mice induces rejection only in abortion-prone mouse models. ICAM-1/LFA-1 cross talk leads to increased recruitment of proinflammatory cells to the implantation site, promotes dendritic cell maturation in the decidua, and subsequently induces additional local Th1 polarization via mature dendritic cells. Furthermore, our observations clearly point out that mechanisms of fetal tolerance, e.g., indoleamine 2,3-dioxygenase expression, presence of CD4⁺CD25^{bright} regulatory T cells, and synthesis of asymmetric Abs, are ICAM-1/LFA-1 dependent. Hence, our data shed light on a hierarchical network of immune integration at the feto-maternal interface, in which ICAM-1/LFA-1 cross talk is clearly a proximate mediator capable of disrupting successful pregnancy maintenance.

Boniface, K., F.-X. Bernard, et al. (2005). "IL-22 Inhibits Epidermal Differentiation and Induces Proinflammatory Gene Expression and Migration of Human Keratinocytes." J. Immunol. **174**(6): 3695-3702.

<http://www.jimmunol.org/cgi/content/abstract/174/6/3695>

IL-22 belongs to a family of cytokines structurally related to IL-10, including IL-19, IL-20, IL-24, and IL-26. In contrast to IL-10, IL-22 has proinflammatory activities. IL-22 signals through a class II cytokine receptor composed of an IL-22-binding chain, IL-22RA1, and the IL-10RB subunit, which is shared with the IL-10R. In the present study, we show that short-term cultured human epidermal keratinocytes express a functional IL-22R but no IL-10R. Accordingly, IL-22 but not IL-10 induces STAT3 activation in keratinocytes. Using a cDNA array screening approach, real-time RT-PCR, and Western blot analysis, we demonstrate that IL-22 up-regulates, in a dose-dependent manner, the expression of S100A7, S100A8, S100A9, a group of proinflammatory molecules belonging to the S100 family of calcium-binding proteins, as well as the matrix metalloproteinase 3, the platelet-derived growth factor A, and the CXCL5 chemokine. In addition, IL-22 induces keratinocyte migration in an in vitro injury model and down-regulates the

expression of at least seven genes associated with keratinocyte differentiation. Finally, we show that IL-22 strongly induces hyperplasia of reconstituted human epidermis. Taken together, these results suggest that IL-22 plays an important role in skin inflammatory processes and wound healing.

Boyartchuk, V., M. Rojas, et al. (2004). "The Host Resistance Locus *sst1* Controls Innate Immunity to *Listeria monocytogenes* Infection in Immunodeficient Mice." *J. Immunol.* **173**(8): 5112-5120.

<http://www.jimmunol.org/cgi/content/abstract/173/8/5112>

Epidemiological, clinical, and experimental approaches have convincingly demonstrated that host resistance to infection with intracellular pathogens is significantly influenced by genetic polymorphisms. Using a mouse model of infection with virulent *Mycobacterium tuberculosis* (MTB), we have previously identified the *sst1* locus as a genetic determinant of host resistance to tuberculosis. In this study we demonstrate that susceptibility to another intracellular pathogen, *Listeria monocytogenes*, is also influenced by the *sst1* locus. The contribution of *sst1* to anti-listerial immunity is much greater in immunodeficient mice, indicating that this locus controls innate immunity and becomes particularly important when adaptive immunity is significantly depressed. Similar to our previous observations using infection with MTB, the resistant allele of *sst1* prevents formation of necrotic infectious lesions in vivo. We have shown that macrophages obtained from *sst1*-resistant congenic mice possess superior ability to kill *L. monocytogenes* in vitro. The bactericidal effect of *sst1* is dependent on IFN- γ activation and reactive oxygen radical production by activated macrophages after infection, but is independent of NO production. It is possible that there is a single gene that controls common IFN-dependent macrophage function, which is important in the pathogenesis of infections caused by both MTB and *L. monocytogenes*. However, host resistance to the two pathogens may be controlled by two different polymorphic genes encoded within the *sst1* locus. The polymorphic gene(s) encoded within the *sst1* locus that controls macrophage interactions with the two intracellular pathogens remains to be elucidated.

Braun, M., M. Wunderlin, et al. (2002). "Xenopus laevis Stromal Cell-Derived Factor 1: Conservation of Structure and Function During Vertebrate Development." *J. Immunol.* **168**(5): 2340-2347.

<http://www.jimmunol.org/cgi/content/abstract/168/5/2340>

Transmembrane signaling of the CXC chemokine stromal cell-derived factor-1 (SDF-1) is mediated by CXCR4, a G protein-coupled receptor initially identified in leukocytes and shown to serve as a coreceptor for the entry of HIV into lymphocytes. Characterization of SDF-1- and CXCR4-deficient mice has revealed that SDF-1 and CXCR4 are of vital developmental importance. To study the role of the SDF-1/CXCR4-chemokine/receptor system as a regulator of vertebrate development, we isolated and characterized a cDNA encoding SDF-1 of the lower vertebrate *Xenopus laevis* (xSDF-1). Recombinant xSDF-1 was produced in insect cells, purified, and functionally characterized. Although xSDF-1 is only 64-66% identical with its mammalian counterparts, it is indistinguishable from human (h)SDF-1 α in terms of activating both *X. laevis* CXCR4 and hCXCR4. Thus, both xSDF-1 and hSDF-1 α promoted CXCR4-mediated activation of heterotrimeric Gi2 in a cell-free system and induced release of intracellular calcium ions in and chemotaxis of intact lymphoblastic cells. Analysis of the time course of xSDF-1 mRNA expression during *Xenopus* embryogenesis revealed a tightly coordinated regulation of xSDF-1 and *X. laevis* CXCR4. xSDF-1 mRNA was specifically detected in the developing CNS, incipient sensory organs, and the embryonic heart. In *Xenopus*, CXCR4 mRNA appears to be absent from the heart anlage, but present in neural crest cells. This observation suggests that xSDF-1 expressed in the heart anlage may attract cardiac neural crest cells expressing CXCR4 to migrate

to the primordial heart to regulate both septation of the cardiac outflow tract and differentiation of the myocardium during early heart development.

Brooks, S. A., J. E. Connolly, et al. (2004). "The Role of mRNA Turnover in the Regulation of Tristetraprolin Expression: Evidence for an Extracellular Signal-Regulated Kinase-Specific, AU-Rich Element-Dependent, Autoregulatory Pathway." *J. Immunol.* **172**(12): 7263-7271.

<http://www.jimmunol.org/cgi/content/abstract/172/12/7263>

Tristetraprolin (TTP) is a regulator of TNF- α mRNA stability and is the only trans-acting factor shown to be capable of regulating AU-rich element-dependent mRNA turnover at the level of the intact animal. Using the THP-1 myelomonocytic cell line, we demonstrated for the first time that TTP is encoded by an mRNA with a short half-life under resting conditions. Using pharmacologic inhibitors of the mitogen-activated protein kinase pathways, we show that the induction of TTP by LPS activation is mediated through changes in transcription, mRNA stability, and translation. A coordinate increase in both TTP and TNF- α mRNA stability occurs within 15 min of LPS treatment, but is transduced through different mitogen-activated protein kinase pathways. This regulation of TTP and TNF- α mRNA stability is associated with the finding that TTP binds these mRNA under both resting and LPS-activated conditions in vivo. Finally, we demonstrate that TTP can regulate reporter gene expression in a TTP 3' untranslated region-dependent manner and identify three distinct AU-rich elements necessary to mediate this effect. Thus, TTP regulates its own expression in a manner identical to that seen with the TNF- α 3' untranslated region, indicating that this autoregulation is mediated at the level of mRNA stability. In this manner, TTP is able to limit the production of its own proteins as well as that of TNF- α and thus limit the response of the cell to LPS.

Bulanova, E., V. Budagian, et al. (2003). "Mast Cells Express Novel Functional IL-15 Receptor α Isoforms." *J. Immunol.* **170**(10): 5045-5055.

<http://www.jimmunol.org/cgi/content/abstract/170/10/5045>

Mast cells previously have been reported to be regulated by IL-15 and to express a distinct IL-15R, termed IL-15RX. To further examine IL-15 binding and signaling in mast cells, we have studied the nature of the IL-15R and some of its biological activities in these cells. In this study, we report the existence of three novel isoforms of the IL-15R α chain in murine bone marrow-derived mast cells as a result of an alternative exon-splicing mechanism within the IL-15R α gene. These correspond to new mRNA transcripts lacking exon 4; exons 3 and 4; or exons 3, 4, and 5 (IL-15R α Δ 4, IL-15R α Δ 3,4, IL-15R α Δ 3,4,5). After transient transfection in COS-7 cells, all IL-15R α isoforms associate with the Golgi apparatus, the endoplasmic reticulum, the perinuclear space, and the cell membrane. Analysis of glycosylation pattern demonstrates the usage of a single N-glycosylation site, while no O-glycosylation is observed. Importantly, IL-15 binds with high affinity to, and promotes the survival of, murine BA/F3 cells stably transfected with the IL-15R α isoforms. Furthermore, we report that signaling mediated by IL-15 binding to the newly identified IL-15R α isoforms involves the phosphorylation of STAT3, STAT5, STAT6, Janus kinase 2, and Syk kinase. Taken together, our data indicate that murine mast cells express novel, fully functional IL-15R α isoforms, which can explain the selective regulatory effects of IL-15 on these cells.

Carlson, K. A., G. Leisman, et al. (2004). "Molecular Characterization of a Putative Antiretroviral

Transcriptional Factor, OTK18." J. Immunol. **172**(1): 381-391.

<http://www.jimmunol.org/cgi/content/abstract/172/1/381>

Elucidation of the factors involved in host defense against human immunodeficiency viral infection remains pivotal if viral control may be achieved. Toward these ends, we investigated the function of a putative antiretroviral factor, OTK18, isolated by differential display of mRNA from HIV type 1-infected primary human monocyte-derived macrophages. Molecular and immunohistochemical analyses showed that the OTK18 nucleotide sequence contains 13 adjacent C2H2-type zinc finger motifs, a Kruppel-associated box, and is localized to both cytosol and nucleus. Mutational analyses revealed that both the Kruppel-associated box and zinc finger regions of OTK18 are responsible for the transcriptional suppressive activities of this gene. OTK18 was copiously expressed in macrophages following HIV type I infection and diminished progeny virion production. A mechanism for this antiretroviral activity was by suppression of HIV type 1 Tat-induced viral long terminal repeat promoter activity. Our findings suggest that one possible function of OTK18 is as a HIV type 1-inducible transcriptional suppresser.

Chen, Y., S. S. Yan, et al. (2004). "Blockade of Late Stages of Autoimmune Diabetes by Inhibition of the Receptor for Advanced Glycation End Products." J. Immunol. **173**(2): 1399-1405.

<http://www.jimmunol.org/cgi/content/abstract/173/2/1399>

Ligation of the receptor for advanced glycation end products (RAGE) occurs during inflammation. Engagement of RAGE results in enhanced expression of addressins and it is therefore, not surprising that previous studies have shown a role of RAGE/ligand interactions in immune responses including cell/cell contact but the role of RAGE in spontaneous autoimmunity has not been clearly defined. To study the role of RAGE/ligand interactions in autoimmune diabetes, we tested the ability of soluble RAGE, a scavenger of RAGE ligands, in late stages of diabetes development in the NOD mouse-disease transferred with diabetogenic T cells and recurrent disease in NOD/scid recipients of syngeneic islet transplants. RAGE expression was detected on CD4+, CD8+, and B cells from diabetic mice and transferred to NOD/scid recipients. RAGE and its ligand, S100B, were found in the islets of NOD/scid mice that developed diabetes. Treatment of recipient NOD/scid mice with soluble RAGE prevented transfer of diabetes and delayed recurrent disease in syngeneic islet transplants. RAGE blockade was associated with increased expression of IL-10 and TGF- β in the islets from protected mice. RAGE blockade reduced the transfer of disease with enriched T cells, but had no effect when diabetes was transferred with the activated CD4+ T cell clone, BDC2.5. We conclude that RAGE/ligand interactions are involved in the differentiation of T cells to a mature pathogenic phenotype during the late stages of the development of diabetes.

Christiansen, S. C., J. Eddleston, et al. (2002). "Up-Regulation of Functional Kinin B1 Receptors in Allergic Airway Inflammation." J. Immunol. **169**(4): 2054-2060.

<http://www.jimmunol.org/cgi/content/abstract/169/4/2054>

B1 receptors are known to be induced during allergic airway inflammation in animal models. However, little is known regarding in vivo B1 receptor expression in humans. We examined B1 receptor mRNA expression in nasal tissue samples from allergic rhinitis and normal subjects. Allergic rhinitis subjects displayed significantly higher expression of B1 receptor mRNA than did the normal subjects, and nasal allergen challenge increased B1 receptor mRNA expression at 8 to 24 h time points in allergic rhinitis subjects. No significant difference was found in B2 receptor

expression. To confirm B2 and B1 receptor functional activity, subjects were challenged with kinin agonists. Nasal challenge with the B1 receptor ligand, Lys-des-Arg-bradykinin (BK), activated extracellular signal-regulated kinase in allergic rhinitis, but not normal, subjects. Nasal challenge with the B2 receptor ligand, BK, activated extracellular signal-regulated kinase in both allergic rhinitis and normal subjects. The consequences of B1 receptor activation were investigated using the human airway epithelial cell lines A549 and BEAS-2B. We demonstrated that Lys-des-Arg-BK activates the transcription factor AP-1. Taken together, these results show that functional B1 receptors are induced in the airway during allergic inflammation and suggest that they participate in the regulation of gene expression.

Clancy, R. M., C. B. Backer, et al. (2003). "Cytokine Polymorphisms and Histologic Expression in Autopsy Studies: Contribution of TNF- α and TGF- β 1 to the Pathogenesis of Autoimmune-Associated Congenital Heart Block." *J. Immunol.* **171**(6): 3253-3261.

<http://www.jimmunol.org/cgi/content/abstract/171/6/3253>

Although Abs to SSA/Ro-SSB/La are necessary for the development of congenital heart block (CHB), the low frequency suggests that fetal factors are contributory. Because CHB involves a cascade from inflammation to scarring, polymorphisms of the TNF- α promoter region and codons 10 and 25 of the TGF- β gene were evaluated in 88 children (40 CHB, 17 rash, 31 unaffected siblings) and 74 mothers from the Research Registry for Neonatal Lupus (NL). Cytokine expression was assessed in autopsy material from two fetuses with CHB. Significantly increased frequency of the -308A (high-producer) allele of TNF- α was observed in all NL groups compared with controls. In contrast, the TGF- β polymorphism Leu10 (associated with increased fibrosis) was significantly higher in CHB children (genotypic frequency 60%, allelic frequency 78%) than unaffected offspring (genotypic frequency 29%, $p = 0.016$; allelic frequency 56%, $p = 0.011$) and controls, while there were no significant differences between controls and other NL groups. For the TGF- β polymorphism, Arg25, there were no significant differences between NL groups and controls. In fetal CHB hearts, protein expression of TGF- β , but not TNF- α , was demonstrated in septal regions, extracellularly in the fibrous matrix, and intracellularly in macrophage infiltrates. Age-matched fetal hearts from voluntary terminations expressed neither cytokine. TNF- α may be one of several factors that amplify susceptibility; however, the genetic studies, backed by the histological data, more convincingly link TGF- β to the pathogenesis of CHB. This profibrosing cytokine and its secretion/activation circuitry may provide a novel direction for evaluating fetal factors in the development of a robust animal model of CHB as well as therapeutic strategies in humans.

Combriato, G. and H. G. Klobeck (2002). "Regulation of Human Ig λ Light Chain Gene Expression by NF- κ B." *J. Immunol.* **168**(3): 1259-1266.

<http://www.jimmunol.org/cgi/content/abstract/168/3/1259>

The human Ig λ enhancer consists of three separated sequence elements that we identified previously by mapping DNase I-hypersensitive regions (HSS) downstream of the C region of the Ig λ L chain genes (HSS-1, HSS-2, and HSS-3). It has been shown by several laboratories that expression of the H chain genes as well as the κ genes, but not the λ genes, is dependent on constitutive NF- κ B proteins present in the nucleus. In this study we show by band-shift experiments, in vivo footprinting, and transient transfection assays that all three hypersensitive sites of the human Ig λ enhancer contain functional NF- κ B sites that act synergistically on expression. We further show that the chicken λ enhancer also contains a functional NF- κ B site but the mouse λ enhancer contains a mutated, nonfunctional NF- κ B site that is responsible for its low enhancer

activity. It is possible that the inactivating mutation in the mouse Ig{lambda} enhancer was compensated for by an expansion of the Ig{kappa} L chain locus, followed by a contraction of the Ig{lambda} locus in this species.

Corbaz, A., T. ten Hove, et al. (2002). "IL-18-Binding Protein Expression by Endothelial Cells and Macrophages Is Up-Regulated During Active Crohn's Disease." J. Immunol. **168**(7): 3608-3616.

<http://www.jimmunol.org/cgi/content/abstract/168/7/3608>

The pathogenesis of Crohn's disease (CD) remains under intense investigation. Increasing evidence suggests a role for mature IL-18 in the induction of proinflammatory cytokines and Th1 polarization in CD lesions. The aim of this study was to investigate the contribution of the IL-18-neutralizing (a and c) and non-neutralizing (b and d) isoforms of IL-18-binding protein (IL-18BP) during active CD. Intestinal endothelial cells and macrophages were the major source of IL-18BP within the submucosa, and this IL-18BP production was also found to be relevant to other types of endothelial cells (HUVEC) and macrophages (peripheral monocytes). IL-18BP messenger transcript and protein were significantly increased in surgically resected specimens from active CD compared with control patients, correlating with an up-regulation of IL-18. Analysis of the expression of the four IL-18BP isoforms as well as being free or bound to IL-18 was reported and revealed that unbound IL-18BP isoforms a and c and inactive isoform d were present in specimens from active CD and control patients while isoform b was not detected. IL-18/IL-18BP complex was also detected. Interestingly, although most was complexed, free mature IL-18 could still be detected in active CD specimens even in the presence of the IL-18BP isoform a/c. These results demonstrate that the appropriate neutralizing isoforms are present in the intestinal tissue of patients with active CD and highlights the complexity of IL-18/IL-18BP biology.

Corcoran, L., I. Ferrero, et al. (2003). "The Lymphoid Past of Mouse Plasmacytoid Cells and Thymic Dendritic Cells." J. Immunol. **170**(10): 4926-4932.

<http://www.jimmunol.org/cgi/content/abstract/170/10/4926>

There has been controversy over the possible lymphoid origin of certain dendritic cell (DC) subtypes. To resolve this issue, DC and plasmacytoid pre-DC isolated from normal mouse tissues were analyzed for transient (mRNA) and permanent (DNA rearrangement) markers of early stages of lymphoid development. About 27% of the DNA of CD8⁺ DC from thymus, and 22-35% of the DNA of plasmacytoid pre-DC from spleen and thymus, was found to contain IgH gene D-J rearrangements, compared with 40% for T cells. However, the DC DNA did not contain IgH gene V-D-J rearrangements nor T cell Ag receptor {beta} gene D-J rearrangements. The same DC lineage populations containing IgH D-J rearrangements expressed mRNA for CD3 chains, and for pre-T{alpha}. In contrast, little of the DNA of the conventional DC derived from spleen, lymph nodes, or skin, whether CD8⁺ or CD8⁻, contained IgH D-J rearrangements and splenic conventional DC expressed very little CD3{epsilon} or pre-T{alpha} mRNA. Therefore, many plasmacytoid pre-DC and thymic CD8⁺ DC have shared early steps of development with the lymphoid lineages, and differ in origin from conventional peripheral DC.

De Creus, A., K. Van Beneden, et al. (2002). "Developmental and Functional Defects of Thymic and Epidermal V{gamma}3 Cells in IL-15-Deficient and IFN Regulatory Factor-1-Deficient Mice." J. Immunol. **168**(12): 6486-6493.

<http://www.jimmunol.org/cgi/content/abstract/168/12/6486>

In this study, the role of IL-15 and its regulation by the transcription factor IFN regulatory factor-1 (IRF-1) in murine V γ 3 T cell development and activity is assessed. Compared with wild-type (WT) mice, reduced numbers of mature V γ 3 cells were found in the fetal thymus of IL-15 $^{-/-}$ mice, while IRF-1 $^{-/-}$ mice displayed normal frequencies. V γ 3 $^{+}$ dendritic epidermal T cells (DETCs) were absent in IL-15 $^{-/-}$ mice but present in IRF-1 $^{-/-}$ mice. DETCs from IRF-1 $^{-/-}$ mice displayed morphologically a less mature phenotype and showed different emergence kinetics during ontogeny. This corresponded with lower IL-15 mRNA levels in the skin epidermis. Comparable levels of IL-7 were found in the skin of WT and IL-15 $^{-/-}$ mice. Adoptive transfer experiments of WT fetal thymocytes into IL-15 $^{-/-}$ mice did not result in the development of V γ 3 $^{+}$ DETCs, confirming the nonredundant role of IL-15 in the skin during DETC development. In vitro, cytolytic activity of IL-15 $^{-/-}$ V γ 3 cells was normal after stimulation with IL-15 and was further enhanced by addition of IL-12. In contrast, cytolytic activity of IRF-1 $^{-/-}$ V γ 3 cells remained defective after stimulation with IL-15 in combination with IL-12. These data suggest that IL-15 is redundant for the development and/or survival of mature V γ 3 cells in the fetal thymus, whereas it is essential for the localization of V γ 3 cells in the skin. Furthermore, a possible role for IRF-1 in inducing morphological maturation of DETCs and cytolytic capacity of V γ 3 cells is suggested.

Diaz, L. S., H. Foster, et al. (2005). "VCAM-1 Expression on CD8 $^{+}$ Cells Correlates with Enhanced Anti-HIV Suppressing Activity." *J. Immunol.* **174**(3): 1574-1579.

<http://www.jimmunol.org/cgi/content/abstract/174/3/1574>

CD8 $^{+}$ cells from HIV-infected individuals showing the CD8 $^{+}$ cell noncytotoxic antiviral response unexpectedly revealed mRNA for VCAM-1, a cell surface molecule found on endothelial cells. Uninfected subjects had undetectable levels of VCAM-1 mRNA in their CD8 $^{+}$ cells. Flow cytometry analysis showed that up to 12% of the CD8 $^{+}$ cells from HIV-positive individuals expressed VCAM-1 compared with 0.8% of the CD8 $^{+}$ cells of HIV-negative individuals. Enrichment of the CD8 $^{+}$ VCAM-1 $^{+}$ cell population and subsequent coculture with CD4 $^{+}$ cells acutely infected with HIV-1 showed that the VCAM-1 $^{+}$ CD8 $^{+}$ cells were able to suppress viral replication with 50% less input cells than the unseparated CD8 $^{+}$ cell population. This study demonstrates, for the first time, the expression of VCAM-1 on CD8 $^{+}$ cells. Moreover, the CD8 $^{+}$ VCAM-1 $^{+}$ cells show enhanced CD8 $^{+}$ cell noncytotoxic antiviral response activity that could have clinical importance in HIV infection.

Dogusan, Z., E. Montecino-Rodriguez, et al. (2004). "Macrophages and Stromal Cells Phagocytose Apoptotic Bone Marrow-Derived B Lineage Cells." *J. Immunol.* **172**(8): 4717-4723.

<http://www.jimmunol.org/cgi/content/abstract/172/8/4717>

It has been hypothesized that B cell precursors that undergo programmed cell death due to nonproductive Ig gene rearrangements are cleared from the bone marrow by macrophages. However, a role for macrophages in this process is supported only by micrographs showing their association with apoptotic-appearing, B lineage cells. Functional data demonstrating phagocytosis of apoptotic, bone marrow lymphocytes by macrophages have not been presented, nor have receptors potentially involved in that process been identified. The data in this report demonstrate that macrophages isolated from murine bone marrow efficiently phagocytose apoptotic murine B lineage cells using multiple receptors that include CD14, integrins, class A scavenger receptor, and CD31 (PECAM-1). In addition, the results further reveal a new role for

the hemopoietic microenvironment in B cell development in view of data demonstrating that murine bone marrow stromal cells are also capable of clearing apoptotic cells via an integrin-dependent mechanism.

Du, C. and S. Sriram (2002). "Increased Severity of Experimental Allergic Encephalomyelitis in lyn^{-/-} Mice in the Absence of Elevated Proinflammatory Cytokine Response in the Central Nervous System." J. Immunol. **168**(6): 3105-3112.

<http://www.jimmunol.org/cgi/content/abstract/168/6/3105>

lyn, a member of the src kinase family, is an important signaling molecule in B cells. lyn^{-/-} mice display hyperactive B-1 cells and IgM hyperglobulinemia. The role of lyn on T cell function and development of Th1-mediated inflammatory disease is not known. Therefore, we examined the effect of disruption of the lyn gene on the development of experimental allergic encephalomyelitis (EAE), a well-established Th1-mediated autoimmune disease. Following immunization with myelin oligodendrocyte protein (MOG) p35-55, lyn^{-/-} mice had higher clinical and pathological severity scores of EAE when compared with wild type (WT). The increase in the severity of EAE in lyn^{-/-} mice was not associated with a commensurate increase in the production of proinflammatory cytokines in the CNS. lyn^{-/-} mice with EAE showed elevation in serum anti-IgM MOG Ab levels over that seen in WT mice, along with a modest increase in the mRNA levels of complement C5 and its receptor, C5aR, in the spinal cord. Transfer of serum from MOG-immunized lyn^{-/-} mice worsened EAE in WT mice, suggesting a pathogenic role for anti-MOG IgM Abs in EAE. These observations underscore the potential role of lyn in regulation of Th1-mediated disease and the role of autoantibodies and complement in the development of EAE.

Eddleston, J., S. C. Christiansen, et al. (2002). "Functional Expression of the C-X-C Chemokine Receptor CXCR4 by Human Bronchial Epithelial Cells: Regulation by Proinflammatory Mediators." J. Immunol. **169**(11): 6445-6451.

<http://www.jimmunol.org/cgi/content/abstract/169/11/6445>

CXCR4 and its ligand stromal cell-derived factor 1{alpha} (SDF-1{alpha}) have recently been implicated in the development of airway inflammation in a mouse model of allergic airway disease. Here we report, for the first time, the expression of a functional CXCR4 in primary human normal bronchial epithelial cells and the regulation of CXCR4 gene expression by proinflammatory mediators. Both bradykinin (BK) and IL-1{beta} induced an accumulation of CXCR4 mRNA in normal bronchial epithelial cells in a time-dependent manner, with peak levels of CXCR4 mRNA reached between 4 and 24 h after stimulation. Ligand activation of CXCR4 in airway epithelial cells resulted in the activation of the extracellular signal-regulated kinase and stress-activated protein kinase/c-Jun amino-terminal kinase signaling pathways and calcium mobilization. Pretreatment of airway epithelial cells with BK or IL-1{beta} enhanced SDF-1{alpha} induced phospho-extracellular signal-regulated kinase and calcium mobilization, in addition to increasing the level of CXCR4 protein. Finally, we describe the expression of CXCR4 mRNA and its regulation by BK in vivo in human nasal tissue. CXCR4 mRNA levels are significantly higher in the nasal tissue of symptomatic allergic rhinitis subjects compared with normal subjects. Moreover, BK challenge significantly increased CXCR4 mRNA levels in nasal tissue of mild allergic rhinitis subjects in vivo, but not normal controls. In conclusion, this study demonstrates that human airway epithelial cells respond to proinflammatory mediators by up-regulating the chemokine receptor CXCR4, thus enabling the cells to respond more effectively to constitutively expressed SDF-1{alpha}. This may lead to enhanced activation of intracellular signaling pathways resulting in the release of mediators involved in inflammatory allergic airway disease.

Egwuagu, C. E., C.-R. Yu, et al. (2002). "Suppressors of Cytokine Signaling Proteins Are Differentially Expressed in Th1 and Th2 Cells: Implications for Th Cell Lineage Commitment and Maintenance." J. Immunol. **168**(7): 3181-3187.

<http://www.jimmunol.org/cgi/content/abstract/168/7/3181>

Positive regulatory factors induced by IL-12/STAT4 and IL-4/STAT6 signaling during T cell development contribute to polarized patterns of cytokine expression manifested by differentiated Th cells. These two critical and antagonistic signaling pathways are under negative feedback regulation by a multimember family of intracellular proteins called suppressor of cytokine signaling (SOCS). However, it is not known whether these negative regulatory factors also modulate Th1/Th2 lineage commitment and maintenance. We show here that CD4⁺ naive T cells constitutively express low levels of SOCS1, SOCS2, and SOCS3 mRNAs. These mRNAs and their proteins increase significantly in nonpolarized Th cells after activation by TCR signaling. We further show that differentiation into Th1 or Th2 phenotype is accompanied by preferential expression of distinct SOCS mRNA transcripts and proteins. SOCS1 expression is 5-fold higher in Th1 than in Th2 cells, whereas Th2 cells contain 23-fold higher levels of SOCS3. We also demonstrate that IL-12-induced STAT4 activation is inhibited in Th2 cells that express high levels of SOCS3 whereas IL-4/STAT6 signaling is constitutively activated in Th2 cells, but not Th1 cells, with high SOCS1 expression. These results suggest that mutually exclusive use of STAT4 and STAT6 signaling pathways by differentiated Th cells may derive in part, from SOCS3- or SOCS1-mediated repression of IL-12/STAT4- or IL-4/STAT6 signaling in Th2 and Th1 cells, respectively. Given the strong correlation between distinct patterns of SOCS expression and differentiation into the Th1 or Th2 phenotype, SOCS1 and SOCS3 proteins are therefore Th lineage markers that can serve as therapeutic targets for immune modulation therapy.

Estes, J. D., B. F. Keele, et al. (2002). "Follicular Dendritic Cell-Mediated Up-Regulation of CXCR4 Expression on CD4 T Cells and HIV Pathogenesis." J. Immunol. **169**(5): 2313-2322.

<http://www.jimmunol.org/cgi/content/abstract/169/5/2313>

Follicular dendritic cells (FDCs) represent a major reservoir of HIV, and active infection occurs surrounding these cells, suggesting that this microenvironment is highly conducive to virus transmission. Because CD4 T cells around FDCs in germinal centers express the HIV coreceptor, CXCR4, whereas CD4 lymphocytes in many other sites do not, it prompted the hypothesis that FDCs may increase CXCR4 expression on CD4 T cells, thereby facilitating infection. To test this, HIV receptor/coreceptor expression was determined on CD4 T cells cultured with or without FDCs, and its consequence to infection was assessed by measuring virus binding and entry. FDCs had little effect on CCR5 or CD4 expression but increased CXCR4 expression on CD4 T cells. FDC-mediated up-regulation of CXCR4 on CD4 T cells occurred by 24 h and was sustained for at least 96 h in vitro, and FDC-CD4 T cell contact was necessary. Importantly, increased CXCR4 expression directly correlated with increased binding and entry of HIV-1 X4 isolates. Furthermore, CD4⁺CD57⁺ germinal center T cells expressed high levels of CXCR4 and supported enhanced entry of X4 HIV compared with other CD4 T cells from the same tissue. Thus, in addition to serving as a reservoir of infectious virus, FDCs render surrounding germinal center T cells highly susceptible to infection with X4 isolates of HIV-1.

Fontenot, A. P., L. A. Maier, et al. (2002). "Beryllium Skin Patch Testing to Analyze T Cell Stimulation and Granulomatous Inflammation in the Lung." J. Immunol. **168**(7): 3627-3634.

<http://www.jimmunol.org/cgi/content/abstract/168/7/3627>

Chronic beryllium disease (CBD) is characterized by granulomatous inflammation and the accumulation of CD4⁺ T cells in the lung. Patch testing of CBD patients with beryllium sulfate results in granulomatous inflammation in the skin. We investigated whether the T cell clonal populations present in the lung of CBD patients would also be present in the involved skin of a positive beryllium patch test and thus mirror the granulomatous process in the lung. CBD patients with clonal TCR expansions in bronchoalveolar lavage (BAL) were selected for study. All three CBD patients studied had a positive response to beryllium sulfate application and a negative patch test to normal saline. Immunohistochemistry showed extensive infiltration with CD4⁺ T cells and few, if any, CD8⁺ T cells both at 3 days and at later times when granulomas were apparent. T cell infiltration early after skin testing appeared to be nonspecific with the TCR repertoire of infiltrating T cells being distinct from that present in BAL. At later times when granulomas were present, T cell clones in skin overlapped with those in BAL in all patients tested. Total TCR matches in skin and BAL were as high as 40% in selected V β T cell subsets. Studies of peripheral blood T cells before and after patch testing provided evidence for mobilization of large numbers of pathogenic beryllium-reactive T cells into the circulating pool. These studies using skin patch testing provide new insight into the dynamics of T cell influx and mobilization during granulomatous inflammation.

Galiotta, L. J. V., P. Pagesy, et al. (2002). "IL-4 Is a Potent Modulator of Ion Transport in the Human Bronchial Epithelium In Vitro." *J. Immunol.* **168**(2): 839-845.

<http://www.jimmunol.org/cgi/content/abstract/168/2/839>

Recent data show that proinflammatory stimuli may modify significantly ion transport in the airway epithelium and therefore the properties of the airway surface fluid. We have studied the effect of IL-4, a cytokine involved in the pathogenesis of asthma, on transepithelial ion transport in the human bronchial epithelium in vitro. Incubation of polarized bronchial epithelial cells with IL-4 for 6-48 h causes a marked inhibition of the amiloride-sensitive Na⁺ channel as measured in short circuit current experiments. On the other hand, IL-4 evokes a 2-fold increase in the current activated by a cAMP analog, which reflects the activity of the cystic fibrosis transmembrane conductance regulator (CFTR). Similarly, IL-4 enhances the response to apical UTP, an agonist that activates Ca²⁺-dependent Cl⁻ channels. These effects are mimicked by IL-13 and blocked by an antagonist of IL-4R α . RT-PCR experiments show that IL-4 elicits a 7-fold decrease in the level of the γ amiloride-sensitive Na⁺ channel mRNA, one of the subunits of the amiloride-sensitive Na⁺ channel, and an increase in CFTR mRNA. Our data suggest that IL-4 may favor the hydration of the airway surface by decreasing Na⁺ absorption and increasing Cl⁻ secretion. This could be required to fluidify the mucus, which is hypersecreted during inflammatory conditions. On the other hand, the modifications of ion transport could also affect the ion composition of airway surface fluid.

Garcia-Martinez, L. F., M. W. Appleby, et al. (2004). "A Novel Mutation in CD83 Results in the Development of a Unique Population of CD4⁺ T Cells." *J. Immunol.* **173**(5): 2995-3001.

<http://www.jimmunol.org/cgi/content/abstract/173/5/2995>

Using a mouse mutagenesis screen, we have identified CD83 as being critical for the development of CD4⁺ T cells and for their function postactivation. CD11c⁺ dendritic cells develop and function normally in mice with a mutated CD83 gene but CD4⁺ T cell development is substantially reduced. Additionally, we now show that those CD4⁺ cells that develop in a CD83

mutant animal fail to respond normally following allogeneic stimulation. This is at least in part due to an altered cytokine expression pattern characterized by an increased production of IL-4 and IL-10 and diminished IL-2 production. Thus, in addition to its role in selection of CD4⁺ T cells, absence of CD83 results in the generation of cells with an altered activation and cytokine profile.

Gasche, C., P. Grundtner, et al. (2003). "Novel Variants of the IL-10 Receptor 1 Affect Inhibition of Monocyte TNF- α Production." *J. Immunol.* **170**(11): 5578-5582.

<http://www.jimmunol.org/cgi/content/abstract/170/11/5578>

IL-10-deficient mice exhibit spontaneous enterocolitis and other symptoms akin to Crohn's disease, indicating that IL-10 might regulate normal physiology in the gut. However, clinical trials with IL-10 in Crohn's disease were disappointing, although some patients showed healing of intestinal mucosa. This study searched for genetic polymorphisms within the IL-10 pathway. We decided to screen for mutations of the IL-10R1 cDNA in healthy volunteers and Crohn's disease patients and identified two novel variants: a serine 138-to-glycine (S138G) and a glycine 330-to-arginine (G330R) substitution. The allelic frequency in a European cohort was relatively high (16% for the S138G and 33% for the G330R), and S138G was in strong linkage disequilibrium with G330R. A similar allele frequency was found in a group of Crohn's patients. In IL-10R1 G330R-expressing monocytes, the inhibitory effect of IL-10 on TNF- α production was diminished, indicating that this variant may be a loss-of-function allele. No such difference was observed between haplotypes 4 (G330R only) and 7 (S138G and G330R). In addition, these IL-10R1 variants had no influence on the IL-10R1 expression density. Structural analysis of the S138G variant revealed that the substitution of S138G may interfere with binding of IL-10 to IL-10R1.

Ge, B., O. Li, et al. (2003). "NF- κ B Regulates BCL3 Transcription in T Lymphocytes Through an Intronic Enhancer." *J. Immunol.* **171**(8): 4210-4218.

<http://www.jimmunol.org/cgi/content/abstract/171/8/4210>

Exposure to soluble protein Ags in vivo leads to abortive proliferation of responding T cells. In the absence of a danger signal, artificially provided by adjuvants, most responding cells die, and the remainder typically become anergic. The adjuvant-derived signals provided to T cells are poorly understood, but recent work has identified BCL3 as the gene, of those tested, with the greatest differential transcriptional response to adjuvant administration in vivo. As an initial step in analyzing transcriptional responses of BCL3 in T cells, we have identified candidate regulatory regions within the locus through their evolutionary conservation and by analysis of DNase hypersensitivity. An evolutionarily conserved DNase hypersensitive site (HS3) within intron 2 was found to act as a transcriptional enhancer in response to stimuli that mimic TCR activation, namely, PHA and PMA. In luciferase reporter gene constructs transiently transfected into the Jurkat T cell line, the HS3 enhancer can cooperate not only with the BCL3 promoter, but also with an exogenous promoter from herpes simplex thymidine kinase. Deletional analysis revealed that a minimal sequence of $\sim$ 81 bp is required for full enhancer activity. At the 5' end of this minimal sequence is a κ B site, as confirmed by EMSAs. Mutation of this site in the context of the full-length HS3 abolished enhancer activity. Cotransfection with NF- κ B p65 expression constructs dramatically increased luciferase activity, even without stimulation. Conversely, cotransfection with the NF- κ B inhibitor I κ B α reduced activation. Together, these results demonstrate a critical role for NF- κ B in BCL3 transcriptional up-regulation by TCR-mimetic signals.

Gonzalez-Munoz, A. L., A.-F. Le Rolle, et al. (2003). "A Novel Instance of Class I Modification (cim) Affecting Two of Three Rat Class I RT1-A Molecules Within One MHC Haplotype." J. Immunol. **171**(1): 274-284.

<http://www.jimmunol.org/cgi/content/abstract/171/1/274>

MHC class I expression by rats of the RT1o, RT1d, and RT1m MHC haplotypes was investigated. Identical, functional cDNAs were obtained from RT1o and BDIX (RT1dv1) rats for three MHC class I molecules. RT1-A1o/d and -A2o/d are closely related in sequence to other cloned rat class Ia genes that have been shown to map to the RT1-A region, while RT1-A3{degrees} is highly homologous to a class I gene identified by sequencing an RT1-An genomic contig and is named A3n. Detailed analysis of the three molecules was undertaken using serology with mAbs, two-dimensional gel analysis of immunoprecipitates, and killing assays using cytotoxic T cells. Arguments are presented suggesting that A1{degrees} is the principal MHC class Ia (classical) restricting element of this haplotype. A2{degrees}, which is highly cross-reactive with A1{degrees}, and A3{degrees} probably play more minor or distinct roles in Ag presentation. Unexpectedly, cDNAs encoding exactly the same three molecules were cloned from rats of the RT1m haplotype, an MHC that until now was thought to possess unique class Ia genes. RT1m contains the TAP-B allele of the TAP transporter, and we present evidence that functional polymorphism in rat TAP has an even greater impact on the expression of RT1-A1{degrees} and -A2{degrees} than it does on RT1-Aa in the established case of class I modification (cim). Historically, this led to the misclassification of RT1m class Ia molecules as separate and distinct.

Gough, M., M. Crittenden, et al. (2005). "Gene Therapy to Manipulate Effector T Cell Trafficking to Tumors for Immunotherapy." J. Immunol. **174**(9): 5766-5773.

<http://www.jimmunol.org/cgi/content/abstract/174/9/5766>

Strategies that generate tumor Ag-specific effector cells do not necessarily cure established tumors. We hypothesized that the relative efficiency with which tumor-specific effector cells reach the tumor is critical for therapy. We demonstrate in this study that activated T cells respond to the chemokine CCL3, both in vitro and in vivo, and we further demonstrate that expression of CCL3 within tumors increases the effector T cell infiltrate in those tumors. Importantly, we show that adenoviral gene transfer to cause expression of CCL3 within B16ova tumors in vivo increases the efficacy of adoptive transfer of tumor-specific effector OT1 T cells. We additionally demonstrate that such therapies result in endogenous immune responses to tumor Ags that are capable of protecting animals against subsequent tumor challenge. Strategies that modify the "visibility" of tumors have the potential to significantly enhance the efficacy of both vaccine and adoptive transfer therapies currently in development.

Gounni, A. S., Q. Hamid, et al. (2004). "IL-9-Mediated Induction of Eotaxin1/CCL11 in Human Airway Smooth Muscle Cells." J. Immunol. **173**(4): 2771-2779.

<http://www.jimmunol.org/cgi/content/abstract/173/4/2771>

Recent work has shown the potential importance of IL-9 in allergic diseases. The development of transgenic mice overexpressing IL-9 has suggested a key role for this cytokine in the development of the asthmatic phenotype including airway eosinophilia. In this study, we evaluated the expression of the IL-9R and the effects of IL-9 on human ASM cells by examining

the release of Th2-associated chemokines (eotaxin1/CCL11 and thymus- and activation-regulated chemokine (TARC)/CCL17). IL-9R {alpha}-chain mRNA and surface expression were detected in cultured human airway smooth muscle (ASM) cells. In addition, primary cultured ASM cells, as well as bronchial smooth muscle cells within biopsies of asthmatics and not control subjects, revealed IL-9R protein expression. IL-9 stimulation of human ASM cells resulted in release of eotaxin1/CCL11, but had no effect on the release of TARC/CCL17, in time- and dose-dependent manner. Moreover, in vitro chemotaxis assay demonstrated that conditioned medium from IL-9-stimulated ASM cells attracted human eosinophils. Neutralizing Abs to IL-9, but not to IL-4 or IL-13, reduced significantly IL-9-induced production of eotaxin1/CCL11 from ASM cells. Interestingly, real-time RT-PCR showed that IL-9 up-regulated eotaxin1/CCL11 mRNA expression, but had no effect on TARC/CCL17. Treatment with Act D abrogates IL-9-induced eotaxin1/CCL11 mRNA and protein release by ASM cells. Finally, transfection study using eotaxin1/CCL11 promoter luciferase construct confirmed that IL-9 induced eotaxin1/CCL11 at the transcriptional level. Taken together, these data provide new evidence demonstrating that IL-9-dependent activation of ASM cells contributes to eosinophilic inflammation observed in asthma.

Gray Parkin, K., R. P. Stephan, et al. (2002). "Expression of CD28 by Bone Marrow Stromal Cells and Its Involvement in B Lymphopoiesis." *J. Immunol.* **169**(5): 2292-2302.

<http://www.jimmunol.org/cgi/content/abstract/169/5/2292>

Young mice lacking CD28 have normal numbers of peripheral B cells; however, abnormalities exist in the humoral immune response that may result from an intrinsic defect in the B cells. The goal of this study was to assess whether CD28 could be involved in the development of B cells. CD28 mRNA was detected preferentially in the fraction of bone marrow enriched for stromal cells. Flow cytometry and RT-PCR analysis demonstrated that CD28 was also expressed by primary-cultured stromal cells that supported B lymphopoiesis. Confocal microscopy revealed that in the presence of B-lineage cells, CD28 was localized at the contact interface between B cell precursors and stromal cells. In addition, CD80 was detected on 2-6% of freshly isolated pro- and pre-B cells, and IL-7 stimulation led to induction of CD86 on 15-20% of pro- and pre-B cells. We also observed that stromal cell-dependent production of B-lineage cells in vitro was greater on stromal cells that lacked CD28. Finally, the frequencies of B-lineage precursors in the marrow from young (4- to 8-wk-old) CD28^{-/-} mice were similar to those in wild-type mice; however, older CD28^{-/-} mice (15-19 mo old) exhibited a 30% decrease in pro-B cells and a 50% decrease in pre-B cells vs age-matched controls. Our results suggest that CD28 on bone marrow stromal cells participates in stromal-dependent regulation of B-lineage cells in the bone marrow. The localization of CD28 at the stromal cell:B cell precursor interface suggests that molecules important for T cell:B cell interactions in the periphery may also participate in stromal cell:B cell precursor interactions in the bone marrow.

Guillonneau, C., C. Louvet, et al. (2004). "The Role of TNF-Related Activation-Induced Cytokine-Receptor Activating NF- κ B Interaction in Acute Allograft Rejection and CD40L-Independent Chronic Allograft Rejection." *J. Immunol.* **172**(3): 1619-1629.

<http://www.jimmunol.org/cgi/content/abstract/172/3/1619>

We analyzed the role of TNF-related activation-induced cytokine (TRANCE), a member of the TNF family expressed on activated T cells that shares functional properties with CD40L, and its receptor-activating NF- κ B (RANK) which is mostly expressed on mature dendritic cells, during allogeneic responses in vivo using a rodent heart allograft model. TRANCE mRNA was strongly up-regulated in acutely rejected allografts on days 4 and 5 posttransplantation whereas RANK was detected as early as day 1 but did not show further up-regulation during the first week.

Immunofluorescence analyses of heart allografts showed that 80 and 100% of TRANCE and RANK-expressing cells were T cells and APCs, respectively. We show for the first time that short-term TRANCE blockade using a mouse RANKIg fusion molecule can significantly prolong heart allograft survival in both rat and mouse models. Similarly, rat heart allografts transduced with a RANKIg encoding recombinant adenovirus exhibited a significant prolongation of survival (14.3 vs 7.6 days, $p < 0.0001$). However, TRANCE blockade using RANKIg did not appear to inhibit allogeneic T and B cell priming humoral responses against RANKIg. Interestingly, TRANCE blockade induced strong up-regulation of CD40 ligand (CD40L) mRNA in allografts. Combined CD40L and TRANCE blockade resulted in significantly decreased chronic allograft rejection lesions as well as allogeneic humoral responses compared with CD40L blockade alone. We conclude that TRANCE-RANK interactions play an important role during acute allograft rejection and that CD40L-independent allogeneic immune responses can be, at least in part, dependent on the TRANCE pathway of costimulation.

Hasan, U., C. Chaffois, et al. (2005). "Human TLR10 Is a Functional Receptor, Expressed by B Cells and Plasmacytoid Dendritic Cells, Which Activates Gene Transcription through MyD88." J. Immunol. **174**(5): 2942-2950.

<http://www.jimmunol.org/cgi/content/abstract/174/5/2942>

Human TLR10 is an orphan member of the TLR family. Genomic studies indicate that TLR10 is in a locus that also contains TLR1 and TLR6, two receptors known to function as coreceptors for TLR2. We have shown that TLR10 was not only able to homodimerize but also heterodimerized with TLRs 1 and 2. In addition, unlike TLR1 and TLR6, TLR10 was expressed in a highly restricted fashion as a highly N-glycosylated protein, which we detected in B cell lines, B cells from peripheral blood, and plasmacytoid dendritic cells from tonsil. We were also able to detect TLR10 in a CD1a⁺ DC subset derived from CD34⁺ progenitor cells which resemble Langerhans cells in the epidermis. Although we were unable to identify a specific ligand for TLR10, by using a recombinant CD4TLR10 molecule we also demonstrated that TLR10 directly associates with MyD88, the common Toll IL-1 receptor domain adapter. Additionally, we have characterized regions in the Toll IL-1 receptor domain of TLR10 that are essential in the activation of promoters from certain inflammatory cytokines. Even though TLR10 expression has not been detected in mice, we have identified a partial genomic sequence of the TLR10 gene that was present but nonfunctional and disrupted by a retroviral insertion in all mouse strains tested. However, a complete TLR10 sequence could be detected in the rat genome, indicating that a functional copy may be preserved in this species.

He, B., A. Chadburn, et al. (2004). "Lymphoma B Cells Evade Apoptosis through the TNF Family Members BAFF/BLyS and APRIL." J. Immunol. **172**(5): 3268-3279.

<http://www.jimmunol.org/cgi/content/abstract/172/5/3268>

The mechanisms underlying the autonomous accumulation of malignant B cells remain elusive. We show in this study that non-Hodgkin's lymphoma (NHL) B cells express B cell-activating factor of the TNF family (BAFF) and a proliferation-inducing ligand (APRIL), two powerful B cell-activating molecules usually expressed by myeloid cells. In addition, NHL B cells express BAFF receptor, which binds BAFF, as well as transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) and B cell maturation Ag (BCMA), which bind both BAFF and APRIL. Neutralization of endogenous BAFF and APRIL by soluble TACI and BCMA decoy receptors attenuates the survival of NHL B cells, decreases activation of the prosurvival transcription factor NF- κ B, down-regulates the antiapoptotic proteins Bcl-2 and Bcl-xL, and up-regulates the proapoptotic protein Bax. Conversely, exposure of NHL B cells to recombinant

or myeloid cell-derived BAFF and APRIL attenuates apoptosis, increases NF- κ B activation, up-regulates Bcl-2 and Bcl-xL, and down-regulates Bax. In some NHLs, exogenous BAFF and APRIL up-regulate c-Myc, an inducer of cell proliferation; down-regulate p53, an inhibitor of cell proliferation; and increase Bcl-6, an inhibitor of B cell differentiation. By showing that nonmalignant B cells up-regulate BAFF and APRIL upon stimulation by T cell CD40 ligand, our findings indicate that NHL B cells deregulate an otherwise physiological autocrine survival pathway to evade apoptosis. Thus, neutralization of BAFF and APRIL by soluble TACI and BCMA decoy receptors could be useful to dampen the accumulation of malignant B cells in NHL patients.

Hewison, M., L. Freeman, et al. (2003). "Differential Regulation of Vitamin D Receptor and Its Ligand in Human Monocyte-Derived Dendritic Cells." J. Immunol. **170**(11): 5382-5390.

<http://www.jimmunol.org/cgi/content/abstract/170/11/5382>

The functions of dendritic cells (DCs) are tightly regulated such that protective immune responses are elicited and unwanted immune responses are prevented. 1 α 25(OH)2D3 has been identified as a major factor that inhibits the differentiation and maturation of DCs, an effect dependent upon its binding to the nuclear vitamin D receptor (VDR). Physiological control of 1 α 25(OH)2D3 levels is critically dependent upon 25-hydroxyvitamin D3-1 α -hydroxylase (1 α OHase), a mitochondrial cytochrome P450 enzyme that catalyzes the conversion of inactive precursor 25-hydroxyvitamin D3 (25(OH)D3) to the active metabolite 1 α 25(OH)2D3. Using a human monocyte-derived DC (moDC) model, we have examined the relationship between DC VDR expression and the impact of exposure to its ligand, 1 α 25(OH)2D3. We show for the first time that moDCs are able to synthesize 1 α 25(OH)2D3 in vitro as a consequence of increased 1 α OHase expression. Following terminal differentiation induced by a diverse set of maturation stimuli, there is marked transcriptional up-regulation of 1 α OHase leading to increased 1 α OHase enzyme activity. Consistent with this finding is the observation that the development and function of moDCs is inhibited at physiological concentrations of the inactive metabolite 25(OH)D3. In contrast to 1 α OHase, VDR expression is down-regulated as monocytes differentiate into immature DCs. Addition of 1 α 25(OH)2D3 to moDC cultures at different time points indicates that its inhibitory effects are greater in monocyte precursors than in immature DCs. In conclusion, differential regulation of endogenous 1 α 25(OH)2D3 ligand and its nuclear receptor appear to be important regulators of DC biology and represent potential targets for the manipulation of DC function.

Holling, T. M., N. van der Stoep, et al. (2002). "Activated Human T Cells Accomplish MHC Class II Expression Through T Cell-Specific Occupation of Class II Transactivator Promoter III." J. Immunol. **168**(2): 763-770.

<http://www.jimmunol.org/cgi/content/abstract/168/2/763>

Activated human T cells express HLA-DR, HLA-DQ, and HLA-DP on their surface, but the regulation and functioning of MHC class II molecules in T lymphocytes are poorly understood. Because the MHC class II transactivator (CIITA) is essential for MHC class II expression, we have investigated transcriptional activation of CIITA in activated T cells. In this study, we show that in human activated CD4⁺ T cells, CIITA promoter III (CIITA-PIII) drives the expression of CIITA. The in vivo genomic footprint analysis revealed activated T cell-specific occupation of CIITA-PIII. Subsequent EMSA analysis of several promoter regions showed differences in banding pattern among activated T cells, naive T cells, primary B cells, and Raji B cells. Activating response element (ARE)-1 is shown to interact with the acute myeloid leukemia 2

transcription factor in nuclear extracts derived from both T and B cells. Interestingly, the acute myeloid leukemia 3 transcription factor was bound in nuclear extracts of T cells only. The ARE-2 sequence is able to bind CREB/activating transcription factor family members in both T and B cells. In addition, a yet unidentified Ets family member was found to interact with site C in activated T cells, whereas in B cells site C was bound by PU.1 and Pip/IFN regulatory factor 4/IFN consensus sequence binding protein for activated T cells. In Jurkat T cells, both ARE-1 and ARE-2 are crucial for CIITA-PIII activity, similar to Raji B cells. The differential banding pattern in *in vivo* genomic footprinting and transcription factor binding at the ARE-1 and site C between T cells and B cells probably reflects differences in CIITA-PIII activation pathways employed by these cell types.

Holtmeier, W., J. Kaller, et al. (2002). "Development and Compartmentalization of the Porcine TCR {delta} Repertoire at Mucosal and Extraintestinal Sites: The Pig as a Model for Analyzing the Effects of Age and Microbial Factors." *J. Immunol.* **169**(4): 1993-2002.

<http://www.jimmunol.org/cgi/content/abstract/169/4/1993>

{gamma}{delta} T cells are an important component of the mucosal immune system. Previously, we have shown that the TCR {delta} repertoire in human intestine is polyclonal at birth and becomes increasingly restricted with age. In this study, we expand those studies to the pig which allows more extensive experiments including several organs. Tissues from different mucosal sites like the stomach, duodenum, ileum, Peyer's patches, jejunum, and colon, and also extraintestinal sites like the lung, spleen, thymus and mesenteric lymph nodes, were obtained from conventionally reared pigs aged 2 wk to 5.5 years. In addition, tissues were also obtained from 10-wk-old specified pathogen- and germ-free pigs. TCRDV1-DV5 transcripts were amplified by RT-PCR after which complementarity-determining region 3 spectratyping was performed. Individual bands were excised from the gels and directly sequenced. The intestinal TCR {delta} repertoire showed increasing restriction with age and was highly oligoclonal in the adult 2- to 5.5-year-old pigs. In old pigs, we observed a striking compartmentalization. Different TCR {delta} repertoires were present between the lungs and the intestinal mucosa but also within different parts of the gastrointestinal tract. However, occasionally we observed identical TCR {delta} transcripts in the intestine and the lungs and shared clones could be detected also along the entire gastrointestinal tract. Thus, subsets of {gamma}{delta} T cells are likely to transport immunological information between different compartments of the immune system. Furthermore, these data support the hypothesis that in each mucosal site, different Ags are responsible for selecting and maintaining the {gamma}{delta} TCR over time.

Horowitz, M. C., Y. Xi, et al. (2004). "Pax5-Deficient Mice Exhibit Early Onset Osteopenia with Increased Osteoclast Progenitors." *J. Immunol.* **173**(11): 6583-6591.

<http://www.jimmunol.org/cgi/content/abstract/173/11/6583>

Pax5 encodes BSAP, a member of the paired box domain transcription factors, whose expression is restricted to B lymphocyte lineage cells. Pax5^{-/-} mice have a developmental arrest of the B cell lineage at the pro-B cell stage. We show here that Pax5^{-/-} mice are severely osteopenic, missing 60% of their bone mass. The osteopenia can be accounted for by a >100% increase in the number of osteoclasts in bone measured histomorphometrically. This is not due to a lack of B cells, because other strains of B cell-deficient mice do not exhibit this phenotype. There was no difference in the number of osteoclasts produced *in vitro* by wild-type and Pax5^{-/-} bone marrow cells. In contrast, spleen cells from Pax5^{-/-} mice produce as much as five times the number of osteoclasts as control spleen cells. Culture of Pax5^{-/-} spleen cells yields a population of adherent cells that grow spontaneously in culture without added growth factors for >4 wk. These cells have

a monocyte phenotype, produce large numbers of osteoclasts when induced in vitro, and therefore are highly enriched in osteoclast precursors. These data demonstrate a previously unsuspected connection between B cell and osteoclast development and a key role for Pax5 in the control of osteoclast development.

Huang, C.-Y., R. Golub, et al. (2002). "Superantigen-Induced TCR {alpha} Locus Secondary Rearrangement: Role in Tolerance Induction." J. Immunol. **168**(7): 3259-3265.

<http://www.jimmunol.org/cgi/content/abstract/168/7/3259>

Immunization with superantigen in vivo induces transient activation of superantigen-specific T cells, followed by a superantigen-nonresponsive state. In this study, using a TCR {alpha} knock-in mouse in which the knock-in {alpha}-chain can be replaced with endogenous {alpha}-chain through secondary rearrangement, we show that immunization of superantigen changes the TCR {alpha}-chain expression on peripheral superantigen-specific T cells, induces expression of recombination-activating genes, and generates DNA double-strand breaks at the TCR {alpha}-chain locus. These results suggest that viral superantigens are capable of inducing peripheral TCR revision. Our findings thus provide a new perspective on pathogen-immune system interaction.

Hubert, F.-X., C. Voisine, et al. (2004). "Rat Plasmacytoid Dendritic Cells Are an Abundant Subset of MHC Class II+ CD4+CD11b- OX62- and Type I IFN-Producing Cells That Exhibit Selective Expression of Toll-Like Receptors 7 and 9 and Strong Responsiveness to CpG." J. Immunol. **172**(12): 7485-7494.

<http://www.jimmunol.org/cgi/content/abstract/172/12/7485>

We have identified in the rat a new subset of MHC class II+ CD4+CD3-CD11b- leukocytes that produce high amounts of type I IFN upon viral stimulation and that appeared homologous to plasmacytoid DC (pDC) previously described in humans and mice. These cells exhibited the following phenotype: CD5+, CD90+, CD45R+, CD45RC+, CD11c-, CD161a+, CD200+, CD172a+, CD32+, CD86+. Rat pDC did not express the DC-specific marker OX62 and were more abundant in the spleen than the classical CD4+ and CD4- subsets of OX62+CD11b+ DC we previously described that produced very little, if any, type I IFN. Spleen pDC exhibited an undifferentiated morphology and rapidly died in vitro, but showed extensive dendrite formation, survival, maturation, and moderate type I IFN production upon stimulation by oligonucleotides containing type B CpG motifs (CpG ODN). Type A CpG ODN and CD40 ligand induced pDC to produce large amounts of type I IFN, but did not promote maturation. CpG ODN and CD40 ligand, but not influenza virus, induced IL-12p40 and IL-6 secretion. Spleen pDC did not produce IL-12p70, TNF- α , IL-1 β , or IL-10 using these stimulation conditions. Correlating with their strong responsiveness to virus and CpG ODN, rat pDC specifically expressed Toll-like receptor 7 and 9 mRNA. Fresh spleen pDC were poor stimulators of allogenic CD4+ and CD8+ T cells, but became potent inducers of allogenic T cell proliferation as well as Th1 differentiation after stimulation by type B CpG. Therefore, rat pDC appear very similar to human pDC, indicating that the specific phenotype and functions of pDC have been highly conserved between species.

Hug, A., M. Korporal, et al. (2003). "Thymic Export Function and T Cell Homeostasis in Patients with Relapsing Remitting Multiple Sclerosis." J. Immunol. **171**(1): 432-437.

<http://www.jimmunol.org/cgi/content/abstract/171/1/432>

Multiple sclerosis (MS) is an inflammatory and possibly autoimmune mediated demyelinating disease of the CNS. Autoimmunity within the CNS may be triggered by dysfunction of peripheral immune tolerance mechanisms via changes in the homeostatic composition of peripheral T cells. We have assessed the release of naive T lymphocytes from the thymus in patients with relapsing remitting MS (RRMS) to identify alterations in the equilibrium of the peripheral T cell compartment. Thymic T cell production was estimated by measuring TCR excision circles (TRECs) as a traceable molecular marker in recent thymic emigrants. A total of 46 treatment-naive patients with active RRMS and 49 gender- and age-matched healthy persons were included in the study. The levels of TREC-expressing CD4+ and CD8+ T lymphocytes were significantly decreased in MS patients, and TREC quantities overall matched those of 30 years older healthy individuals. The average concentrations of TRECs/106 CD4+ and CD8+ T lymphocytes derived from MS patients and healthy donors were $26 \times 103/106$ and $28 \times 103/106$ vs $217 \times 103/106$ and $169 \times 103/106$, respectively. To account for any influence of T cell proliferation on TREC levels, we assayed T lymphocytes from additional patients with MS and normal individuals for telomere length ($n = 20$) and telomerase activity (8 MS patients, 16 controls), respectively. There were no significant differences between CD4+ and CD8+ T cells from MS patients and controls. Altogether, our findings suggest that an impaired thymic export function and, as a consequence, altered ability to maintain T cell homeostasis and immune tolerance may play an important pathogenic role in RRMS.

Iizasa, H., H. Yoneyama, et al. (2005). "Exacerbation of Granuloma Formation in IL-1 Receptor Antagonist-Deficient Mice with Impaired Dendritic Cell Maturation Associated with Th2 Cytokine Production." *J. Immunol.* **174**(6): 3273-3280.

<http://www.jimmunol.org/cgi/content/abstract/174/6/3273>

Dendritic cell (DC) maturation at the site of inflammation and migration into draining lymph nodes is fundamental to initiate Ag-specific immune responses. Although several proinflammatory cytokines, including IL-1, are known to promote DC maturation in vitro, their contributions to DC activation and migration within peripheral inflamed tissue compartments are not yet fully understood. We show here that endogenous IL-1 receptor antagonist (IL-1ra) controls the activation state of liver-recruited DCs and their migration in a *Propionibacterium acnes*-induced murine granulomatous liver disease model. After *P. acnes* treatment, formation of portal tract-associated lymphoid tissue was conversely impaired in IL-1ra-deficient mice. IL-1ra-deficient mice developed hepatic granulomas within 3 days after *P. acnes* administration and showed a more pronounced granuloma formation than wild-type mice. Although sinusoidal granulomas contained numerous CD11c+ DCs at day 7, expressions of CCR7, IL-12p40 by these DCs were dramatically decreased in IL-1ra-deficient mice, suggesting aberrant DC maturation and sinusoid portal migration in the absence of endogenous IL-1ra. This was accompanied with enhanced intrahepatic Th2 cytokine production and severe hepatocellular damage. Thus, hepatocyte-derived IL-1ra may control optimal activation and migration of inflammatory DCs within the liver and thereby determine the local immune responses in granulomatous liver disease.

Inoue, T., D. Hammaker, et al. (2005). "Regulation of p38 MAPK by MAPK Kinases 3 and 6 in Fibroblast-Like Synoviocytes." *J. Immunol.* **174**(7): 4301-4306.

<http://www.jimmunol.org/cgi/content/abstract/174/7/4301>

The p38 MAPK signal transduction pathway is a key regulator of IL-1 and TNF- α production

in rheumatoid arthritis. Previous studies demonstrated that upstream MAPK kinases (MKK3 and MKK6) that regulate p38 are activated in rheumatoid arthritis synovium. However, their functional relevance in fibroblast-like synoviocytes (FLS) has not been determined. To investigate the relative contribution of MKK3 and MKK6 to p38 activation, the effect of dominant-negative (DN) MKK3 and MKK6 constructs on cultured FLS was evaluated. Cultured FLS were stimulated with medium or IL-1{beta}, and immunoblotting was performed. In some experiments, cells were lysed and immunoprecipitated with anti-p38 Ab, followed by in vitro kinase assay with [gamma]-32P]ATP and GST-activating transcription factor-2 as substrate. IL-1{beta} rapidly induced p38 phosphorylation in cells transfected with empty vector (pcDNA3.1), but was inhibited by 25% in cells expressing DN MKK3 or DN MKK6. Cotransfection with both DN plasmids decreased phospho-p38 by almost 75%. In vitro kinase assays on IL-1-stimulated FLS also showed that the combination of DN MKK3 and DN MKK6 markedly decreased kinase activity compared with empty vector or the individual DN plasmids. Furthermore, IL-1{beta}-induced IL-8, IL-6, and matrix metalloproteinase-3 protein production was significantly inhibited in DN MKK3/DN MKK6-transfected cells. The constructs had no effect on the respective mediator mRNA levels. These data demonstrate that MKK3 and MKK6 make individual contributions to p38 activation in FLS after cytokine stimulation, but that both must be blocked for maximum inhibition.

Ioannidis, V., B. Kunz, et al. (2003). "Initiation and Limitation of Ly-49A NK Cell Receptor Acquisition by T Cell Factor-1." *J. Immunol.* **171**(2): 769-775.

<http://www.jimmunol.org/cgi/content/abstract/171/2/769>

The establishment of clonally variable expression of MHC class I-specific receptors by NK cells is not well understood. The Ly-49A receptor is used by {approx}20% of NK cells, whereby most cells express either the maternal or paternal allele and few express simultaneously both alleles. We have previously shown that NK cells expressing Ly-49A were reduced or almost absent in mice harboring a single or no functional allele of the transcription factor T cell factor-1 (TCF-1), respectively. In this study, we show that enforced expression of TCF-1 in transgenic mice yields an expanded Ly-49A subset. Even though the frequencies of Ly-49A+ NK cells varied as a function of the TCF-1 dosage, the relative abundance of mono- and biallelic Ly-49A cells was maintained. Mono- and biallelic Ly-49A NK cells were also observed in mice expressing exclusively a transgenic TCF-1, i.e., expressing a fixed amount of TCF-1 in all NK cells. These findings suggest that Ly-49A acquisition is a stochastic event due to limiting TCF-1 availability, rather than the consequence of clonally variable expression of the endogenous TCF-1 locus. Efficient Ly-49A acquisition depended on the expression of a TCF-1 isoform, which included a domain known to associate with the TCF-1 coactivator {beta}-catenin. Indeed, the proximal Ly-49A promoter was {beta}-catenin responsive in reporter gene assays. We thus propose that Ly-49A receptor expression is induced from a single allele in occasional NK cells due to a limitation in the amount of a transcription factor complex requiring TCF-1.

Ishida, I., H. Kubo, et al. (2002). "Hypoxia Diminishes Toll-Like Receptor 4 Expression Through Reactive Oxygen Species Generated by Mitochondria in Endothelial Cells." *J. Immunol.* **169**(4): 2069-2075.

<http://www.jimmunol.org/cgi/content/abstract/169/4/2069>

Hypoxia and inflammation often occur simultaneously due to prevention of adequate gas exchange. Understanding the influence of hypoxia on the inflammatory response is important because hypoxia directly regulates expression of many genes, including those regulating inflammation, and plays a role in modulating the resolution of an inflammatory response. LPS is a major mediator of cellular injury and inflammation that induces its effects through Toll-like

receptor 4 (TLR4). The aim of this study was to evaluate the effect of hypoxia on TLR4 expression. Hypoxia decreased TLR4 expression on cultured endothelial cells. Furthermore, LPS-induced ICAM-1 up-regulation was decreased by hypoxia. Because reactive oxygen species (ROS) generated from mitochondria are one of the signaling molecules induced by hypoxia, the role of ROS in hypoxia-induced TLR4 down-regulation was evaluated. Our data showed that hypoxia increased ROS generation and that hypoxia-induced TLR4 down-regulation was inhibited by myxothiazol, a mitochondrial site III electron transport inhibitor. Hypoxia also inhibited AP-1 translocation. Since the TLR4 promoter has a binding site for AP-1, hypoxia-induced TLR4 down-regulation may be due to an ROS-mediated decrease in AP-1-binding activity. We conclude that hypoxia decreases TLR4 expression in endothelial cells and that this change is mediated by mitochondrial ROS leading to attenuation of AP-1 transcriptional activity.

Jaatinen, T., M. Eholuoto, et al. (2002). "Characterization of a De Novo Conversion in Human Complement C4 Gene Producing a C4B5-Like Protein." *J. Immunol.* **168**(11): 5652-5658.

<http://www.jimmunol.org/cgi/content/abstract/168/11/5652>

Complement C4 is a highly polymorphic protein essential for the activation of the classical complement pathway. Most of the allelic variation of C4 resides in the C4d region. Four polymorphic amino acid residues specify the isotype and an additional four specify the Rodgers and Chido determinants of the protein. Rare C4 allotypes have been postulated to originate from recombination between highly homologous C4 genes through gene conversions. Here we describe the development of a de novo C4 hybrid protein with allotypic and antigenic diversity resulting from nonhomologous intra or interchromosomal recombination of the maternal chromosomes. A conversion was observed between maternal C4A3a and C4B1b genes producing a functional hybrid gene in one of the children. The codons determining the isotype, Asp1054, Leu1101, Ser1102, Ile1105 and His1106, were characteristic of C4B gene, whereas the polymorphic sites in exon and intron 28 were indicative of C4A3a sequence. The protein produced by this hybrid gene was electrophoretically similar to C4B5 allotype. It also possesses reversed antigenicity being Rodgers 1, 2, 3 and Chido-1, -2, -3, 4, -5, and -6. Our case describes the development of a rare bimodular C4B-C4B haplotype containing a functional de novo C4 hybrid gene arisen through gene conversion from C4A to C4B. Overall the data supports the hypothesis of gene conversions as an ongoing process increasing allelic diversity in the C4 locus.

Jensen, L. E. and A. S. Whitehead (2003). "Pellino3, a Novel Member of the Pellino Protein Family, Promotes Activation of c-Jun and Elk-1 and May Act as a Scaffolding Protein." *J. Immunol.* **171**(3): 1500-1506.

<http://www.jimmunol.org/cgi/content/abstract/171/3/1500>

Toll-like receptors and the IL-1R are part of the innate immune response aimed at mobilizing defense mechanisms in response to infections or injury. These receptors can initiate common intracellular signaling cascades. One intermediate component in these signaling cascades is Pellino, which was first identified in *Drosophila* and shown to interact with IL-1R-associated kinase. Two homologues, Pellino1 and Pellino2, have been identified in mammals. A novel member of the Pellino protein family has been identified and named Pellino3. Pellino3 shares 84 and 85% amino acid identity with Pellino1 and Pellino2, respectively. Two alternatively spliced Pellino3 mRNAs, Pellino3a and Pellino3b, are widely expressed. Pellino3 physically interacts with IL-1R-associated kinase-1, TNF receptor-associated factor-6, TGF- β -activated kinase-1, and NF- κ B-inducing kinase in an IL-1-dependent manner, suggesting that it plays a role as a scaffolding protein. In reporter assays Pellino3 leads to activation of c-Jun and Elk-1, but not NF- κ B. Pellino3 also leads to activation of c-Jun N-terminal kinase. These data suggest that

Pellino3 plays an important role in the innate immune response.

Jiankuo, M., W. Xingbing, et al. (2003). "Peptide Nucleic Acid Antisense Prolongs Skin Allograft Survival by Means of Blockade of CXCR3 Expression Directing T Cells into Graft." *J. Immunol.* **170**(3): 1556-1565.

<http://www.jimmunol.org/cgi/content/abstract/170/3/1556>

CXCR3, predominantly expressed on memory/activated T cells, is a receptor for both IFN- γ -inducible protein 10/CXC chemokine ligand (CXCL)10 and monokine induced by IFN- γ /CXCL9. It was reported that CXC chemokines IFN- γ -inducible protein 10/CXCL10 and monokine induced by IFN- γ /CXCL9 play a critical role in the allograft rejection. We report that CXCR3 is a dominant factor directing T cells into mouse skin allograft, and that peptide nucleic acid (PNA) CXCR3 antisense significantly prolongs skin allograft survival by means of blockade of CXCR3 expression directing T cells into allografts in mice. We found that CXCR3 is highly up-regulated in spleen T cells and allografts from BALB/c recipients by day 7 of receiving transplantation, whereas CCR5 expression is moderately increased. We designed PNA CCR5 and PNA CXCR3 antisenses, and i.v. treated mice that received skin allograft transplantations. The PNA CXCR3 at a dosage of 10 mg/kg/day significantly prolonged mouse skin allograft survival (17.1 $\pm$ 2.4 days) compared with physiological saline treatment (7.5 $\pm$ 0.7 days), whereas PNA CCR5 (10 mg/kg/day) marginally prolonged skin allograft survival (10.7 $\pm$ 1.1 days). The mechanism of prolongation of skin allograft survival is that PNA CXCR3 directly blocks the CXCR3 expression in T cells, which is responsible for directing T cells into skin allograft to induce acute rejection, without interfering with other functions of the T cells. These results were obtained at mRNA and protein levels by flow cytometry and real-time quantitative RT-PCR technique, and confirmed by chemotaxis, Northern and Western blot assays, and histological evaluation of skin grafts. The present study indicates the therapeutic potential of PNA CXCR3 to prevent acute transplantation rejection.

Jinquan, T., H. H. Jacobi, et al. (2003). "CCR3 Expression Induced by IL-2 and IL-4 Functioning as a Death Receptor for B Cells." *J. Immunol.* **171**(4): 1722-1731.

<http://www.jimmunol.org/cgi/content/abstract/171/4/1722>

We report that CCR3 is not expressed on freshly isolated peripheral and germinal B cells, but is up-regulated after stimulation with IL-2 and IL-4 ($\sim$ 98% CCR3+). Ligation of CCR3 by eotaxin/chemokine ligand (CCL) 11 induces apoptosis in IL-2- and IL-4-stimulated primary CD19+ ($\sim$ 40% apoptotic cells) B cell cultures as well as B cell lines, but has no effect on chemotaxis or cell adhesion. Freshly isolated B cells express low levels of CD95 and CD95 ligand (CD95L) (19 and 21%, respectively). Expression is up-regulated on culture in the presence of a combination of IL-2, IL-4, and eotaxin/CCL11 (88% CD95 and 84% CD95L). We therefore propose that ligation of such newly induced CCR3 on peripheral and germinal B cells by eotaxin/CCL11 leads to the enhanced levels of CD95 and CD95L expression. Ligation of CD95 by its CD95L expressed on neighboring B cells triggers relevant death signaling pathways, which include an increase in levels of Bcl-2 expression, its functional activity, and the release of cytochrome c from the mitochondria into the cytosol. These events initiate a cascade of enzymatic processes of the caspase family, culminating in programmed cell death. Interaction between CCR3 and eotaxin/CCL11 may, besides promoting allergic reactions, drive activated B cells to apoptosis, thereby reducing levels of Ig production, including IgE, and consequently limit the development of the humoral immune response. The apoptotic action of eotaxin/CCL11 suggests a therapeutic modality in the treatment of B cell lymphoma.

Johansson-Lindbom, B. and C. A. K. Borrebaeck (2002). "Germinal Center B Cells Constitute a Predominant Physiological Source of IL-4: Implication for Th2 Development In Vivo." J. Immunol. **168**(7): 3165-3172.

<http://www.jimmunol.org/cgi/content/abstract/168/7/3165>

Protective immunity depends upon the capability of the immune system to properly adapt the response to the nature of an infectious agent. CD4⁺ Th cells are implicated in this orchestration by secreting a polarized pattern of cytokines. Although Th2 development in animal models and in human cells in vitro to a large extent depends on IL-4, the nature of the cells that provide the initial IL-4 in vivo is still elusive. In this report, we describe the anatomical localization as well as the identity of IL-4-producing cells in human tonsil, a representative secondary lymphoid organ. We demonstrate that IL-4 production is a normal and intrinsic feature of germinal center (GC) B cells. We also show that expression of IL-4 is highly confined to the GCs, in which the B cells constitute the prevalent cellular source. Furthermore, immunofluorescence analysis of colon mucosa reveals a strikingly similar pattern of IL-4-expressing cells compared with tonsils, demonstrating that IL-4 production from GC B cells is not a unique feature of the upper respiratory tract. Our results show that GCs provide the most appropriate microenvironment for IL-4-dependent Th2 polarization in vivo and imply a critical role for GC B cells in this differentiation process.

Johnson, D. R. (2003). "Locus-Specific Constitutive and Cytokine-Induced HLA Class I Gene Expression." J. Immunol. **170**(4): 1894-1902.

<http://www.jimmunol.org/cgi/content/abstract/170/4/1894>

Cytokine induction of the MHC class I genes increases the nascent molecules available for binding potentially antigenic peptides. The human H chain loci, HLA-A, -B, and -C, encode highly homologous and polymorphic mRNAs. Here, these transcripts were resolved and measured by competitive PCR of cDNA using locus-specific primers. Endothelial cells expressed many HLA-A and -B, but fewer HLA-C, transcripts. In contrast, HeLa cells expressed many HLA-A and -C, but fewer HLA-B, transcripts. The inflammatory cytokines TNF- α , IFN- β , and IFN- γ induced HLA-B strongly, but HLA-A and -C weakly in both cell types. Combined treatment with IFNs and TNF further increased HLA-A and -B, but not HLA-C transcripts. The constitutive and inducible activities of transfected promoters correlated well with mRNA levels. The weak IFN response of the HLA-A2 promoter was not due to variations in the IFN consensus sequence, the site α , or a 3-bp insertion between them. The HLA-Cw6 promoter was less TNF responsive due to a variant κ B enhancer, which also reduced the IFN responses. The NF- κ B subunit RelA strongly activated the HLA-A2 and -B7 promoters but only weakly activated the HLA-Cw6 promoter due to the variant κ B. Cotransfecting NF- κ B1 with RelA further increased activity of the HLA-A2 and -B7, but not HLA-Cw6, promoters. All three promoters were activated by MHC class II trans-activator, but not CREB-binding protein, whereas IFN regulatory factor-1 and -2 weakly activated the HLA-B7 and -Cw6, but not HLA-A2, promoters. These studies illustrate common and locus-specific mechanisms that may be targeted to modulate immune reactions.

Johnson, E. N., E. R. Appelbaum, et al. (2004). "Neuromedin Elicits Cytokine Release in Murine Th2-Type T Cell Clone D10.G4.1." J. Immunol. **173**(12): 7230-7238.

<http://www.jimmunol.org/cgi/content/abstract/173/12/7230>

Neuromedin U (NmU), originally isolated from porcine spinal cord and later from other species, is a novel peptide that potently contracts smooth muscle. NmU interacts with two G protein-coupled receptors designated as NmU-1R and NmU-2R. This study demonstrates a potential proinflammatory role for NmU. In a mouse Th2 cell line (D10.G4.1), a single class of high affinity saturable binding sites for ¹²⁵I-labeled NmU (K_D 364 pM and B_{max} 1114 fmol/mg protein) was identified, and mRNA encoding NmU-1R, but not NmU-2R, was present. Competition binding analysis revealed equipotent, high affinity binding of NmU isopeptides to membranes prepared from D10.G4.1 cells. Exposure of these cells to NmU isopeptides resulted in an increase in intracellular Ca²⁺ concentration (EC₅₀ 4.8 nM for human NmU). In addition, NmU also significantly increased the synthesis and release of cytokines including IL-4, IL-5, IL-6, IL-10, and IL-13. Studies using pharmacological inhibitors indicated that maximal NmU-evoked cytokine release required functional phospholipase C, calcineurin, MEK, and PI3K pathways. These data suggest a role for NmU in inflammation by stimulating cytokine production by T cells.

Kanayama, N., T. Kimoto, et al. (2002). "B Cell Selection and Affinity Maturation During an Antibody Response in the Mouse with Limited B Cell Diversity." *J. Immunol.* **169**(12): 6865-6874.

<http://www.jimmunol.org/cgi/content/abstract/169/12/6865>

The quasi-monoclonal mouse has limited B cell diversity, whose major ([~]80%) B cell Ag receptors are comprised of the knockin VH 17.2.25 (VHT)-encoded H chain and the {lambda}1 or {lambda}2 L chain, thereby being specific for 4-hydroxy-3-nitrophenylacetyl. The p-nitrophenylacetyl (pNP) was found to be a low affinity analog of nitrophenylacetyl. We examined affinity maturation of anti-pNP IgG by analyzing mAbs obtained from quasi-monoclonal mice that were immunized with this low affinity Ag. The results are: 1) Although VHT/{lambda}1 and VHT/{lambda}2 IgM were equally produced, VHT/{lambda}2 IgG almost exclusively underwent affinity maturation toward pNP. 2) A common mutation in complementarity-determining region 3 of VHT (T313A) mainly contributed to generating the specificity for pNP. 3) Because mutated VHT-encoded {gamma}-chains could form {lambda}1-bearing IgG in Chinese hamster ovary cells, apparent absence of VHT/{lambda}1 anti-pNP IgG may not be due to the incompatibility between the {gamma}-chains and the {lambda}1-chain, but may be explained by the fact that VHT/{lambda}1 B cells showed 50- to 100-fold lower affinity for pNP than VHT/{lambda}2 B cells. 4) Interestingly, a pNP-specific IgM mAb that shared common mutations including T313A with high affinity anti-pNP IgG was isolated, suggesting that a part of hypermutation coupled with positive selection can occur before isotype switching. Thus, even weak B cell receptor engagement can elicit an IgM response, whereas only B cells that received signals stronger than a threshold may be committed to an affinity maturation process.

Katavic, V., I. K. Lukic, et al. (2003). "Increased Bone Mass Is a Part of the Generalized Lymphoproliferative Disorder Phenotype in the Mouse." *J. Immunol.* **170**(3): 1540-1547.

<http://www.jimmunol.org/cgi/content/abstract/170/3/1540>

We investigated the bone phenotype of mice with generalized lymphoproliferative disorder (gld) due to a defect in the Fas ligand-mediated apoptotic pathway. C57BL/6-gld mice had greater whole body bone mineral density and greater trabecular bone volume than their wild-type controls. gld mice lost 5-fold less trabecular bone and had less osteoclasts on bone surfaces after ovariectomy-induced bone resorption. They also formed more bone in a model of osteogenic regeneration after bone marrow ablation, had less osteoclasts on bone surfaces and less

apoptotic osteoblasts. gld and wild-type mice had similar numbers of osteoclasts in bone marrow cultures, but marrow stromal fibroblasts from gld mice formed more alkaline phosphatase-positive colonies. Bone diaphyseal shafts and bone marrow stromal fibroblasts produced more osteoprotegerin mRNA and protein than wild-type mice. These findings provide evidence that the disturbance of the bone system is a part of generalized lymphoproliferative syndrome and indicates the possible role of osteoprotegerin as a regulatory link between the bone and immune system.

Kawauchi, K., K. Ihjima, et al. (2005). "IL-2 Increases Human Telomerase Reverse Transcriptase Activity Transcriptionally and Posttranslationally through Phosphatidylinositol 3'-Kinase/Akt, Heat Shock Protein 90, and Mammalian Target of Rapamycin in Transformed NK Cells." J. Immunol. **174**(9): 5261-5269.

<http://www.jimmunol.org/cgi/content/abstract/174/9/5261>

Human telomerase activity is induced by Ag receptor ligation in T and B cells. However, it is unknown whether telomerase activity is increased in association with activation and proliferation of NK cells. We found that telomerase activity in a human NK cell line (NK-92), which requires IL-2 for proliferation, was increased within 24 h after stimulation with IL-2. Levels of human telomerase reverse transcriptase (hTERT) mRNA and protein correlated with telomerase activity. ERK1/2 and Akt kinase (Akt) were activated by IL-2 stimulation. LY294002, an inhibitor of PI3K, abolished expression of hTERT mRNA and protein expression and abolished hTERT activity, whereas PD98059, which inhibits MEK1/2 and thus ERK1/2, had no effect. In addition, radicicol, an inhibitor of heat shock protein 90 (Hsp90), and rapamycin, an inhibitor of the mammalian target of rapamycin (mTOR), blocked IL-2-induced hTERT activity and nuclear translocation of hTERT but not hTERT mRNA expression. hTERT was coimmunoprecipitated with Akt, Hsp90, mTOR, and p70 S6 kinase (S6K), suggesting that these molecules form a physical complex. Immunoprecipitates of Akt, Hsp90, mTOR, and S6K from IL-2-stimulated NK-92 cells contained telomerase activity. Furthermore, the findings that Hsp90 and mTOR immunoprecipitates from primary samples contained telomerase activity are consistent with the results from NK-92 cells. These results indicate that IL-2 stimulation induces hTERT activation and that the mechanism of IL-2-induced hTERT activation involves transcriptional or posttranslational regulation through the pathway including PI3K/Akt, Hsp90, mTOR, and S6K in NK cells.

Khan, N., N. Shariff, et al. (2002). "Cytomegalovirus Seropositivity Drives the CD8 T Cell Repertoire Toward Greater Clonality in Healthy Elderly Individuals." J. Immunol. **169**(4): 1984-1992.

<http://www.jimmunol.org/cgi/content/abstract/169/4/1984>

The deterioration in immune function with aging is thought to make a major contribution to the increased morbidity and mortality from infectious disease in old age. One aspect of immune senescence is the reduction in CD8 T cell repertoire as due to the accumulation of oligoclonal, memory T cells and a reduction in the naive T cell pool. CD8 T cell clonal expansions accumulate with age, but their antigenic specificity remains unknown. In this study, we show that in elderly individuals seropositivity for human CMV leads to the development of oligoclonal populations of CMV-specific CTL that can constitute up to one-quarter of the total CD8 T cell population. Furthermore, CMV-specific CTL have a highly polarized membrane phenotype that is typical of effector memory cells (CD28⁻, CD57⁺, CCR7⁻). TCR analyses show that CMV-specific CTL have highly restricted clonality with greater restriction in the larger expansions. Clonal analysis of the total CD8 T cell repertoire was compared between CMV-seropositive and CMV-seronegative donors. Thirty-three percent more clonal expansions were observed in CMV-seropositive donors in comparison with seronegative individuals. These data implicate CMV as a major factor in

driving oligoclonal expansions in old age. Such a dramatic accumulation of virus-specific effector CTL might impair the ability to respond to heterologous infection and may underlie the negative influence of CMV seropositivity on survival in the very elderly.

Kikuchi-Maki, A., S.-i. Yusa, et al. (2003). "KIR2DL4 Is an IL-2-Regulated NK Cell Receptor That Exhibits Limited Expression in Humans but Triggers Strong IFN- γ Production." *J. Immunol.* **171**(7): 3415-3425.

<http://www.jimmunol.org/cgi/content/abstract/171/7/3415>

Killer cell Ig-like receptor (KIR)2DL4 (2DL4, CD158d) was previously described as the only KIR expressed by every human NK cell. It is also structurally atypical among KIRs because it possesses a basic transmembrane residue, which is characteristic of many activating receptors, but also contains a cytoplasmic immunoreceptor tyrosine-based inhibitory motif (ITIM). We expressed epitope-tagged 2DL4 in an NK-like cell line to study receptor function. Three distinct 2DL4 cDNA clones were analyzed: one encoding the "conventional" 2DL4 with the cytoplasmic ITIM (2DL4.1) and two encoding different cytoplasmic truncated forms lacking the ITIM (2DL4.2 and 2DL4*). Surprisingly, one truncated receptor (2DL4.2), which is the product of a prevalent human 2DL4 allele, was not expressed on the cell surface, indicating that some individuals may lack functional 2DL4 protein expression. Conversely, both 2DL4.1 and 2DL4* were expressed on the cell surface and up-regulated by IL-2. Analysis of primary NK cells with anti-2DL4 mAb confirmed the lack of surface expression in a donor with the 2DL4.2 genotype. Donors with the 2DL4.1 genotype occasionally expressed receptor only on CD56^{high} NK cells, although their expression was up-regulated by IL-2. Interestingly, Ab engagement of epitope-tagged 2DL4 triggered rapid and robust IFN- γ production, but weak redirected cytotoxicity in an NK-like cell line, which was the opposite pattern to that observed upon engagement of another NK cell activating receptor, NKp44. Importantly, both 2DL4.1 and 2DL4* exhibited similar activation potential, indicating that the ITIM does not influence 2DL4.1 activating function. The unique activation properties of 2DL4 suggest linkage to a distinct signaling pathway.

Kim, H., J. M. Suh, et al. (2003). "Thyrotropin-Mediated Repression of Class II Trans-Activator Expression in Thyroid Cells: Involvement of STAT3 and Suppressor of Cytokine Signaling." *J. Immunol.* **171**(2): 616-627.

<http://www.jimmunol.org/cgi/content/abstract/171/2/616>

It has been suggested that class I and class II MHC are contributing factors for numerous diseases including autoimmune thyroid diseases, type 1 diabetes, rheumatoid arthritis, Alzheimer's disease, and multiple sclerosis. The class II trans-activator (CIITA), which is a non-DNA-binding regulator of class II MHC transcription, regulates the constitutive and inducible expression of the class I and class II genes. FRTL-5 thyroid cells incubated in the presence of IFN- γ have a significantly higher level of cell surface rat MHC class II RT1.B. However, the IFN- γ -induced RT1.B expression was suppressed significantly in cells incubated in the presence of thyrotropin. Thyrotropin (TSH) represses IFN- γ -induced CIITA expression by inhibiting type IV CIITA promoter activity through the suppression of STAT1 activation and IFN regulatory factor 1 induction. This study found that TSH induces transcriptional activation of the STAT3 gene through the phosphorylation of STAT3 and CREB activation. TSH induces SOCS-1 and SOCS-3, and TSH-mediated SOCS-3 induction was dependent on STAT3. The cell line stably expressing the wild-type STAT3 showed a higher CIITA induction in response to IFN- γ and also exhibited TSH repression of the IFN- γ -mediated induction of CIITA. However, TSH repression of the IFN- γ -induced CIITA expression was not observed in FRTL-5 thyroid cells, which stably expresses the dominant negative forms of STAT3, STAT3-

Y705F, and STAT3-S727A. This report suggests that TSH is also engaged in immunomodulation through signal cross-talk with the cytokines in thyroid cells.

Kirkiles-Smith, N. C., K. Mahboubi, et al. (2004). "IL-11 Protects Human Microvascular Endothelium from Alloinjury In Vivo by Induction of Survivin Expression." J. Immunol. **172**(3): 1391-1396.

<http://www.jimmunol.org/cgi/content/abstract/172/3/1391>

IL-11 can reduce tissue injury in animal models of inflammation but the mechanism(s) is unknown. When C.B-17 SCID/beige mice bearing human skin grafts are injected i.p. with human PBMC allogeneic to the donor skin, infiltrating T cells destroy human microvessels by day 21. Intradermal injection of human IL-11 (500 ng/day) delays the time course of graft microvessel loss without reducing the extent of T cell infiltration. Protective actions of IL-11 are most pronounced on day 15. IL-11 has no effect on T cell activation marker, effector molecule, cytokine expression, or endothelial ICAM-1 expression. IL-11 up-regulates the expression of survivin, a cytoprotective protein, in graft keratinocytes and endothelial cells. Topical application of survivin antisense oligonucleotide down-regulates survivin expression in both cell types and largely abrogates the protective effect of IL-11. We conclude that in this human transplant model, IL-11 exerts a cytoprotective rather than anti-inflammatory or immunomodulatory effect mediated through induction of survivin.

Kohler, R. E., A. C. Caon, et al. (2003). "A Role for Macrophage Inflammatory Protein-3{alpha}/CC Chemokine Ligand 20 in Immune Priming During T Cell-Mediated Inflammation of the Central Nervous System." J. Immunol. **170**(12): 6298-6306.

<http://www.jimmunol.org/cgi/content/abstract/170/12/6298>

Chemokines are a family of cytokines that exhibit selective chemoattractant properties for target leukocytes and play a significant role in leukocyte migration. In this study, we have investigated the role of the C-C chemokine, macrophage inflammatory protein (MIP)-3{alpha}/CC chemokine ligand 20, in the pathogenesis of experimental autoimmune encephalomyelitis (EAE), a model of T cell-dependent inflammation. Expression in the CNS of MIP-3{alpha}, as determined by RT-PCR, increased in a time-dependent manner such that peak expression correlated with peak clinical disease. Similarly, levels of immunoreactive MIP-3{alpha} in the draining lymph nodes increased up to 10-fold 9 days postimmunization and remained elevated for up to 21 days postimmunization. The increased production of MIP-3{alpha} coincided with onset of clinical disease. Treatment of mice with specific neutralizing anti-MIP-3{alpha} Abs significantly reduced the severity of both clinical EAE and neuroinflammation by inhibiting the sensitization of lymphocytes to the specific Ag and release of lymphocytes from the draining lymph nodes. In contrast, adoptive transfer experiments indicated that MIP-3{alpha} was not essential for the effector phase of EAE. Together, these data demonstrate that MIP-3{alpha} plays a critical role in the sensitization phase of EAE.

Kondo, E., M. S. Topp, et al. (2002). "Efficient Generation of Antigen-Specific Cytotoxic T Cells Using Retrovirally Transduced CD40-Activated B Cells." J. Immunol. **169**(4): 2164-2171.

<http://www.jimmunol.org/cgi/content/abstract/169/4/2164>

The development of rapid, efficient, and safe methods for generating Ag-specific T cells is

necessary for the clinical application of adoptive immunotherapy. We show that B cells stimulated with CD40 ligand and IL-4 (CD40-B cells) can be efficiently transduced with retroviral vectors encoding a model Ag, CMV tegument protein pp65 gene, and maintain high levels of costimulatory molecules after gene transfer. CTL lines specific for pp65 were readily generated in all four healthy CMV-seropositive donors by stimulating autologous CD8⁺ T cells with these transduced CD40-B cells, both of which were derived from 10 ml peripheral blood. ELISPOT assays revealed that the CTL lines used multiple HLA alleles as restricting elements. Thus, CD40-B cells transduced retrovirally with Ag-encoding cDNA can be potent APC and facilitate to generate Ag-specific CTL in vitro.

Kwok, L.-Y., S. Lutjen, et al. (2003). "The Induction and Kinetics of Antigen-Specific CD8 T Cells Are Defined by the Stage Specificity and Compartmentalization of the Antigen in Murine Toxoplasmosis." *J. Immunol.* **170**(4): 1949-1957.

<http://www.jimmunol.org/cgi/content/abstract/170/4/1949>

Toxoplasma gondii forms different life stages, fast-replicating tachyzoites and slow-growing bradyzoites, in mammalian hosts. CD8 T cells are of crucial importance in toxoplasmosis, but it is unknown which parasite stage is recognized by CD8 T cells. To analyze stage-specific CD8 T cell responses, we generated various recombinant *Toxoplasma gondii* expressing the heterologous Ag {beta}-galactosidase ({beta}-gal) and studied whether 1) secreted or cytoplasmic Ags and 2) tachyzoites or bradyzoites, which persist intracerebrally, induce CD8 T cells. We monitored the frequencies and kinetics of {beta}-gal-specific CD8 T cells in infected mice by MHC class I tetramer staining. Upon oral infection of B6C (H-2bxd) mice, only {beta}-gal-secreting tachyzoites induced {beta}-gal-specific CD8 T cells. However, upon secondary infection of mice that had received a primary infection with tachyzoites secreting {beta}-gal, {beta}-gal-secreting tachyzoites and bradyzoites transiently increased the frequency of intracerebral {beta}-gal-specific CD8 T cells. Frequencies of splenic and cerebral {beta}-gal-specific CD8 T cells peaked at day 23 after infection, thereafter persisting at high levels in the brain but declining in the spleen. Splenic and cerebral {beta}-gal-specific CD8 T cells produced IFN- γ and were cytolytic upon specific restimulation. Thus, compartmentalization and stage specificity of an Ag determine the induction of CD8 T cells in toxoplasmosis.

Laan, M., S. Bozinovski, et al. (2004). "Cigarette Smoke Inhibits Lipopolysaccharide-Induced Production of Inflammatory Cytokines by Suppressing the Activation of Activator Protein-1 in Bronchial Epithelial Cells." *J. Immunol.* **173**(6): 4164-4170.

<http://www.jimmunol.org/cgi/content/abstract/173/6/4164>

Chronic smoking is characterized by immunosuppressive changes in the airways, leading to chronic colonization with bacteria, which in turn may contribute to the chronic obstructive pulmonary disease. The mechanisms causing this immunosuppression, however, are poorly characterized. This study evaluated whether cigarette smoke can inhibit endotoxin (LPS)-induced inflammatory cytokine production in bronchial epithelial cells and, if so, what the mechanisms are behind this effect. Pretreatment with cigarette smoke extract (CSE) concentration dependently inhibited the LPS-induced GM-CSF and IL-8 protein release, which was accompanied by decreased expression of mRNA in human bronchial epithelial cells (Beas-2B). The increase of neutrophil chemotaxis induced by conditioned medium from LPS-treated Beas-2B cells was also suppressed by CSE. In addition, the activity of LPS-induced transcription factor AP-1, but not NF- κ B, was down-regulated by CSE. Notably, at the concentrations used, CSE had no effect on number or viability of Beas-2B cells. These data indicate that cigarette smoke possesses immunosuppressive properties by down-regulating the bacterial pathogen-induced neutrophil-

mobilizing cytokine production via suppression of AP-1 activation in the airways. Hence, this study suggests a novel mechanism by which cigarette smoke may contribute to chronic colonization and chronic obstructive pulmonary disease in smokers.

LaBonte, M. L., E. I. Choi, et al. (2004). "Molecular Determinants Regulating the Pairing of NKG2 Molecules with CD94 for Cell Surface Heterodimer Expression." J. Immunol. **172**(11): 6902-6912.

<http://www.jimmunol.org/cgi/content/abstract/172/11/6902>

The lytic capacity of a NK cell is regulated, in part, by the balance in cell surface expression between inhibitory CD94/NKG2A and activating CD94/NKG2C heterodimers. We demonstrate that, in the absence of DAP12, rhesus monkey NKG2A is preferentially expressed at the cell surface with CD94 due to a single amino acid difference in the transmembrane of NKG2A and NKG2C. Furthermore, in the context of an NKG2A transmembrane, the stalk domain of NKG2C was found to enhance heterodimer formation with CD94 compared with the stalk domain of NKG2A. In the presence of DAP12, the ability of NKG2C to compete for cell surface CD94 heterodimerization is enhanced and approaches that of NKG2A. Finally, allelic differences that affect the ability of rhesus NKG2A to reach the cell surface with CD94 could also be mapped to the transmembrane. These differences in the ability of inhibitory and activating NKG2 molecules to reach the cell surface provide a mechanism for the regulation of NK cell activity.

Lafont, B. A. P., A. Buckler-White, et al. (2003). "Characterization of Pig-Tailed Macaque Classical MHC Class I Genes: Implications for MHC Evolution and Antigen Presentation in Macaques." J. Immunol. **171**(2): 875-885.

<http://www.jimmunol.org/cgi/content/abstract/171/2/875>

MHC-dependent CD8⁺ T cell responses have been associated with control of viral replication and slower disease progression during lentiviral infections. Pig-tailed macaques (*Macaca nemestrina*) and rhesus monkeys (*Macaca mulatta*), two nonhuman primate species commonly used to model HIV infection, can exhibit distinct clinical courses after infection with different primate lentiviruses. As an initial step in assessing the role of MHC class I restricted immune responses to these infections, we have cloned and characterized classical MHC class I genes of pig-tailed macaques and have identified 19 MHC class I alleles (Mane) orthologous to rhesus macaque MHC-A, -B, and -I genes. Both Mane-A and Mane-B loci were found to be duplicated, and no MHC-C locus was detected. Pig-tailed and rhesus macaque MHC-A alleles form two groups, as defined by 14 polymorphisms affecting mainly their B peptide-binding pockets. Furthermore, an analysis of multiple pig-tailed monkeys revealed the existence of three MHC-A haplotypes. The distribution of these haplotypes in various Old World monkeys provides new insights about MHC-A evolution in nonhuman primates. An examination of B and F peptide-binding pockets in rhesus and pig-tailed macaques suggests that their MHC-B molecules present few common peptides to their respective CTLs.

Langdon, C., C. Kerr, et al. (2003). "Oncostatin M Regulates Eotaxin Expression in Fibroblasts and Eosinophilic Inflammation in C57BL/6 Mice." J. Immunol. **170**(1): 548-555.

<http://www.jimmunol.org/cgi/content/abstract/170/1/548>

Oncostatin M (OSM) is a member of the IL-6/LIF (or gp130) cytokine family, and its potential role

in inflammation is supported by a number of activities identified in vitro. In this study, we investigate the action of murine OSM on expression of the CC chemokine eotaxin by fibroblasts in vitro and on mouse lung tissue in vivo. Recombinant murine OSM stimulated eotaxin protein production and mRNA levels in the NIH 3T3 fibroblast cell line. IL-6 could regulate a small induction of eotaxin in NIH 3T3 cells, but other IL-6/LIF cytokines (LIF, cardiotrophin-1 (CT-1)) had no effect. Cell signaling studies showed that murine OSM, LIF, IL-6, and CT-1 stimulated the tyrosine phosphorylation of STAT-3, suggesting STAT-3 activation is not sufficient for eotaxin induction in NIH 3T3 cells. OSM induced ERK-1,2 and p38 mitogen-activated protein kinase phosphorylation in NIH 3T3 cells, and inhibitors of ERK (PD98059) or p38 (SB203580) could partially reduce OSM-induced eotaxin production, suggesting partial dependence on mitogen-activated protein kinase signaling. OSM (but not LIF, IL-6, or CT-1) also induced eotaxin release by mouse lung fibroblast cultures derived from C57BL/6 mice. Overexpression of murine OSM in lungs of C57BL/6 mice using an adenovirus vector encoding murine OSM resulted in a vigorous inflammatory response by day 7 after intranasal administration, including marked extracellular matrix accumulation and eosinophil infiltration. Elevated levels of eotaxin mRNA in whole lung were detected at days 4 and 5. These data strongly support a role of OSM in lung inflammatory responses that involve eosinophil infiltration.

Langerak, A. W., B. Nadel, et al. (2004). "Unraveling the Consecutive Recombination Events in the Human IGK Locus." *J. Immunol.* **173**(6): 3878-3888.

<http://www.jimmunol.org/cgi/content/abstract/173/6/3878>

In addition to the classical V{kappa}-J{kappa}, V{kappa}-{kappa} deleting element (Kde), and intron-Kde gene rearrangements, atypical recombinations involving J{kappa} recombination signal sequence (RSS) or intronRSS elements can occur in the Ig{kappa} (IGK) locus, as observed in human B cell malignancies. In-depth analysis revealed that atypical J{kappa}RSS-intronRSS, V{kappa}-intronRSS, and J{kappa}RSS-Kde recombinations not only occur in B cell malignancies, but rather reflect physiological gene rearrangements present in normal human B cells as well. Excision circle analysis and recombination substrate assays can discriminate between single-step vs multistep rearrangements. Using this combined approach, we unraveled that the atypical V{kappa}-intronRSS and J{kappa}RSS-Kde pseudohybrid joints most probably result from ongoing recombination following an initial aberrant J{kappa}RSS-intronRSS signal joint formation. Based on our observations in normal and malignant human B cells, a model is presented to describe the sequential (classical and atypical) recombination events in the human IGK locus and their estimated relative frequencies (0.2-1.0 vs <0.03). The initial J{kappa}RSS-intronRSS signal joint formation (except for J{kappa}1RSS-intronRSS) might be a side event of an active V(D)J recombination mechanism, but the subsequent formation of V{kappa}-intronRSS and J{kappa}RSS-Kde pseudohybrid joints can represent an alternative pathway for IGK allele inactivation and allelic exclusion, in addition to classical C{kappa} deletions. Although usage of this alternative pathway is limited, it seems essential for inactivation of those IGK alleles that have undergone initial aberrant recombinations, which might otherwise hamper selection of functional Ig L chain proteins.

Lausen, B. F., L. Hougs, et al. (2004). "Human Memory B Cells Transferred by Allogeneic Bone Marrow Transplantation Contribute Significantly to the Antibody Repertoire of the Recipient." *J. Immunol.* **172**(5): 3305-3318.

<http://www.jimmunol.org/cgi/content/abstract/172/5/3305>

The bone marrow is an important source of Abs involved in long-term protection from recurrence of infections. Allogeneic bone marrow transplantation (BMT) fails to restore this working memory.

Attempts to overcome this immunodeficiency by immunization of the donor have not been very successful. More needs to be known about transfer of B cell memory by BMT. We tracked memory B cells from the donor to the recipient during BMT of a girl with leukocyte adhesion deficiency. Vaccination of her HLA-identical sibling donor 7 days before harvest induced *Haemophilus influenzae* type b (Hib) capsular polysaccharide (HibCP)-specific B cells readily detectable in marrow and blood. BMT did not lead to spontaneous production of HibCP Abs, but the recipient responded well to booster immunizations 9 and 11 mo after BMT. HibCP-specific B cells were obtained 7 days after the vaccinations, and their VH genes were sequenced and analyzed for rearrangements and unique patterns of somatic hypermutations identifying clonally related cells. Ninety (74%) of 121 sequences were derived from only 16 precursors. Twelve clones were identified in the donor, and representatives from all of them were detected in the recipient where they constituted 61 and 68% of the responding B cells after the first and second vaccinations, respectively. No evidence for re-entry of memory clones into the process of somatic hypermutation was seen in the recipient. Thus, memory B cells were transferred from the donor, persisted for at least 9 mo in the recipient, and constituted the major part of the HibCP-specific repertoire.

Lauw, F. N., J. Branger, et al. (2002). "IL-18 Improves the Early Antimicrobial Host Response to Pneumococcal Pneumonia." *J. Immunol.* **168**(1): 372-378.

<http://www.jimmunol.org/cgi/content/abstract/168/1/372>

To determine the role of endogenous IL-18 during pneumonia, IL-18 gene-deficient (IL-18^{-/-}) mice and wild-type (WT) mice were intranasally inoculated with *Streptococcus pneumoniae*, the most common causative agent of community-acquired pneumonia. Infection with *S. pneumoniae* increased the expression of IL-18 mRNA and was associated with elevated concentrations of both precursor and mature IL-18 protein within the lungs. IL-18^{-/-} mice had significantly more bacteria in their lungs and were more susceptible for progressing to systemic infection at 24 and 48 h postinoculation. Similarly, treatment of WT mice with anti-IL-18 was associated with enhanced outgrowth of pneumococci. In contrast, the clearance of pneumococci from lungs of IL-12^{-/-} mice was unaltered when compared with WT mice. Furthermore, anti-IL-12 did not influence bacterial clearance in either IL-18^{-/-} or WT mice. These data suggest that endogenous IL-18, but not IL-12, plays an important role in the early antibacterial host response during pneumococcal pneumonia.

Lebbink, R. J., T. de Ruiter, et al. (2004). "The Mouse Homologue of the Leukocyte-Associated Ig-Like Receptor-1 Is an Inhibitory Receptor That Recruits Src Homology Region 2-Containing Protein Tyrosine Phosphatase (SHP)-2, but Not SHP-1." *J. Immunol.* **172**(9): 5535-5543.

<http://www.jimmunol.org/cgi/content/abstract/172/9/5535>

We report the molecular cloning and characterization of the first leukocyte-associated Ig-like receptor 1 (LAIR-1) homologue in mice that we have named mouse LAIR-1 (mLAIR-1). The mLAIR-1 gene maps to the proximal end of mouse chromosome 7 in a region syntenic with human chromosome 19q13.4 where the leukocyte receptor cluster is located. The protein shares 40% sequence identity with human LAIR-1, has a single Ig-like domain, and contains two immunoreceptor tyrosine-based inhibitory motif-like structures in its cytoplasmic tail. Mouse LAIR-1 is broadly expressed on various immune cells, and cross-linking of the molecule on stably transfected RBL-2H3 and YT.2C2 cells results in strong inhibition of their degranulation and cytotoxic activities, respectively. Upon pervanadate stimulation, the mLAIR-1 cytoplasmic tail becomes phosphorylated, thereby recruiting Src homology region 2-containing tyrosine phosphatase-2. Interestingly, unlike human LAIR-1, Src homology region 2-containing tyrosine

phosphatase-1 is not recruited to the mLAIR-1 cytoplasmic tail. Screening human and mouse cell lines for mLAIR-1 and human LAIR-1 binding partners identified several lines expressing putative ligand(s) for both receptors.

Lee, S.-K., J. Kalinowski, et al. (2002). "1,25 (OH)₂ Vitamin D₃-Stimulated Osteoclast Formation in Spleen-Osteoblast Cocultures Is Mediated in Part by Enhanced IL-1{alpha} and Receptor Activator of NF-{kappa}B Ligand Production in Osteoblasts." *J. Immunol.* **169**(5): 2374-2380.

<http://www.jimmunol.org/cgi/content/abstract/169/5/2374>

We examined the ability of 1,25 (OH)₂ vitamin D₃ (Vit D) to stimulate osteoclast-like cell (OCL) formation in cocultures of spleen cells and primary calvarial osteoblasts from wild-type (WT) and IL-1R type 1-deficient (knockout; KO) mice. Vit D dose dependently increased OCL in cocultures containing WT osteoblasts. In contrast, there was a 90% reduction in OCL numbers in cocultures containing KO osteoblasts. In cocultures with either WT or KO osteoblasts, treatment with Vit D increased receptor activator of NF-{kappa}B ligand mRNA by 17-, 19-, or 3.5-fold, respectively. Vit D decreased osteoprotegerin mRNA to undetectable in all groups. Intracellular IL-1{alpha} protein increased after Vit D treatment in cocultures containing WT, but not KO osteoblasts. We also examined direct effects of Vit D, IL-1{alpha}, and their combination on gene expression in primary osteoblasts. In WT cells, Vit D and IL-1 stimulated receptor activator of NF-{kappa}B ligand mRNA expression by 3- and 4-fold, respectively, and their combination produced a 7-fold increase. Inhibition of osteoprotegerin mRNA in WT cells was partial with either agent alone and greatest with their combination. In KO cells, only Vit D stimulated a response. IL-1 alone increased IL-1{alpha} protein expression in WT osteoblasts. However, in combination with Vit D, there was a synergistic response (100-fold increase). In KO cultures, there were no effects of IL-1, Vit D, or their combination on IL-1{alpha} protein. These results demonstrate interactions between IL-1 and Vit D in primary osteoblasts that appear important in both regulation of IL-1{alpha} production and the ability of Vit D to support osteoclastogenesis.

Li, J. S.-y., F. Chu, et al. (2002). "Antibodies Highly Effective in SCID Mice During Infection by the Intracellular Bacterium *Ehrlichia chaffeensis* Are of Picomolar Affinity and Exhibit Preferential Epitope and Isotype Utilization." *J. Immunol.* **169**(3): 1419-1425.

<http://www.jimmunol.org/cgi/content/abstract/169/3/1419>

Although often considered to be ineffective against intracellular bacteria, Abs, in the absence of lymphocytes, have been shown previously to protect SCID mice from lethal infection by the obligate intracellular bacterium *Ehrlichia chaffeensis*, even when administered well after infection has been established. To identify characteristics of Abs that are critical for host defense during this intracellular infection, a panel of *Ehrlichia*-specific mAbs was generated and analyzed. Among 100 Abs recovered, 39 recognized an amino-terminal hypervariable region of an outer membrane protein (OMP), demonstrating that the OMPs are both antigenically variable and immunodominant. A subset of 16 representative OMP-specific Abs was further examined to identify characteristics that were essential for in vivo efficacy. The highly effective Abs recognized a linear epitope within the first hypervariable region of OMP-1g. Only IgG were found to be effective, and among the effective IgG, the following hierarchy was observed: IgG2a > IgG3 = IgG2b. The most striking characteristics of the highly effective Abs were their picomolar binding affinities and long binding t_{1/2}. Thus, although epitope recognition and isotype use may contribute to efficacy, high affinity may be a critical characteristic of Abs that can act effectively during this intracellular bacterial infection.

Linard, B., S. Bezieau, et al. (2002). "A ras-Mutated Peptide Targeted by CTL Infiltrating a Human Melanoma Lesion." J. Immunol. **168**(9): 4802-4808.

<http://www.jimmunol.org/cgi/content/abstract/168/9/4802>

Aggs derived from commonly mutated oncogenic proteins seem ideally suited as targets for tumor immunotherapy. Nonetheless, only a few mutated epitopes efficiently presented by human tumors have thus far been identified. We describe here an approach to identify such epitopes. This approach involves: 1) identifying tumors expressing a ras mutation and isolating the tumor-infiltrating lymphocytes (TIL); 2) transfecting COS cells to induce expression of unknown mutated peptides in the context of a patient's HLA class I molecules; and 3) screening epitope recognition by using TIL from the tumors expressing a ras mutation. By using this approach, there appeared to be a N-ras mutation (a glutamine-to-arginine exchange at residue 61 (Q61R)), detected in a melanoma lesion, which was recognized specifically by the autologous TIL in the HLA-A*0101 context. The ras peptide 55-64Q61R was the epitope of these TIL and was regularly presented by Q61R-mutated HLA-A*0101+ melanoma cell lines. This peptide and its wild-type homolog (55-64wt) bound to HLA-A*0101 with similar affinities. However, only the mutated peptide could induce specific CTL expansion from PBL. All the CTL clones specific to the mutated peptide, failed to recognize the wild-type sequence on both COS and melanoma cells. These data thus show that oncogenic protein mutations can create shared tumor-specific CTL epitopes, efficiently presented by tumor cells, and that screening for oncogene-transfected COS cell recognition by TIL (from tumors containing mutations) is a powerful approach for the identification of these epitopes.

Locati, M., U. Deuschle, et al. (2002). "Analysis of the Gene Expression Profile Activated by the CC Chemokine Ligand 5/RANTES and by Lipopolysaccharide in Human Monocytes." J. Immunol. **168**(7): 3557-3562.

<http://www.jimmunol.org/cgi/content/abstract/168/7/3557>

The gene expression profile induced by the CC chemokine ligand (CCL) 5/RANTES in human monocytes was examined using the oligonucleotide array technology. Of 5600 transcripts examined, 42 were consistently induced by CCL5, and none were suppressed. Chemokine-inducible transcripts could be clustered in functional groups, including selected cytokines and receptors (e.g., IL-1{beta}, CCL2/monocyte chemotactic protein-1, and the CCL5 receptor CCR1) and molecules involved in extracellular matrix recognition and digestion (e.g., CD44 splice transcripts, urokinase-type plasminogen activator receptor, matrix metalloprotease (MMP)-9, and MMP-19). Transcript expression, confirmed by quantitative real-time PCR analysis for selected genes, was associated with protein induction for some (e.g., CCL2), but not all (e.g., IL-1{beta}), transcripts examined. The chemokine-induced gene profile was distinct from that activated by LPS, a prototypic phagocyte activator. Although certain transcripts were stimulated by both agonists (e.g., IL-1{beta} and CCL2), others were induced only by either LPS (e.g., TNF-{alpha} and IL-6) or CCL5 (e.g., MMP-19) or were divergently regulated (e.g., CCR1). Thus, CCL5, a prototypic CC inflammatory chemokine, activates a restricted transcriptional program in monocytes distinct from that induced by the prototypic pathogen-derived proinflammatory stimulant LPS. Chemokine-induced chemokines production could represent a novel amplification loop of leukocyte recruitment, while a subset of chemokine-inducible transcripts could be involved in monocyte extravasation and tissue invasion.

Lu, G., B. M. Janjic, et al. (2002). "Innate Direct Anticancer Effector Function of Human Immature Dendritic Cells. II. Role of TNF, Lymphotoxin- α 1 β 2, Fas Ligand, and TNF-Related Apoptosis-Inducing Ligand." J. Immunol. **168**(4): 1831-1839.

<http://www.jimmunol.org/cgi/content/abstract/168/4/1831>

Our recent studies have demonstrated that human immature dendritic cells (DCs) are able to directly and effectively mediate apoptotic killing against a wide array of cultured and freshly-isolated cancer cells without harming normal cells. In the present study, we demonstrate that this tumoricidal activity is mediated by multiple cytotoxic TNF family ligands. We determine that human immature DCs express on their cell surface four different cytotoxic TNF family ligands: TNF, lymphotoxin- α 1 β 2, Fas ligand, and TNF-related apoptosis inducing ligand; while cancer cells express the corresponding death receptors. Disruptions of interactions between the four ligands expressed on DCs and corresponding death-signaling receptors expressed on cancer cells using specific Abs or R:Fc fusion proteins block the cytotoxic activity of DCs directed against cancer cells. The novel findings suggest that DC killing of cancer cells is mediated by the concerted engagement of four TNF family ligands of DCs with corresponding death receptors of cancer cells. Overall, our data demonstrate that DCs are fully equipped for an efficient direct apoptotic killing of cancer cells and suggest that this mechanism may play a critical role in both afferent and efferent anti-tumor immunity.

Maaser, C., J. Heidemann, et al. (2004). "Human Intestinal Microvascular Endothelial Cells Express Toll-Like Receptor 5: A Binding Partner for Bacterial Flagellin." J. Immunol. **172**(8): 5056-5062.

<http://www.jimmunol.org/cgi/content/abstract/172/8/5056>

Bacterial flagellin has recently been identified as a ligand for Toll-like receptor 5 (TLR5). Human sites known to specifically express TLR5 include macrophages and gastric and intestinal epithelium. Because infection of intestinal epithelial cells with *Salmonella* leads to an active transport of flagellin to the subepithelial compartment in proximity to microvessels, we hypothesized that human intestinal endothelial cells functionally express TLR5, thus enabling an active inflammatory response upon binding of translocated flagellin. Endothelial expression of TLR5 in human macro- and microvascular endothelial cells was examined by RT-PCR, immunoblot analysis, and immunofluorescence. Endothelial expression of TLR5 in vivo was verified by immunohistochemistry. Endothelial modulation of ICAM-1 expression was quantitated using flow cytometry, and leukocyte transmigration in vitro was assessed by an endothelial transmigration assay. Epithelial-endothelial cellular interactions upon infection with viable *Salmonella* were investigated using a coculture system in vitro. We found that *Salmonella*-infected intestinal epithelial cells induce endothelial ICAM-1 expression in cocultured human endothelial cells. Both macro- (HUVEC) and microvascular endothelial cells derived from human skin (human dermal microvascular endothelial cell 1) and human colon (human intestinal microvascular endothelial cells) were found to express high constitutive amounts of TLR5 mRNA and protein. These findings were paralleled by strong immunoreactivity for TLR5 of normal human colonic microvessels in vivo. Furthermore, incubation of human dermal microvascular endothelial cells with flagellin from clinical isolates of *Escherichia* and *Salmonella* strains led to a marked up-regulation of ICAM-1, as well as to an enhanced leukocyte transendothelial cell migration. These results suggest that endothelially expressed TLR5 might play a previously unrecognized role in the innate immune response toward bacterial Ags.

Mackay, G. A., Z. Liu, et al. (2004). "Protection Against Late-Onset AIDS in Macaques Prophylactically Immunized with a Live Simian HIV Vaccine Was Dependent on Persistence of the Vaccine Virus." J. Immunol. **173**(6): 4100-4107.

<http://www.jimmunol.org/cgi/content/abstract/173/6/4100>

This is a 5-year follow-up study on 12 macaques that were immunized orally with two live SHIV vaccines, six with V1 and six with V2. All 12 macaques became persistently infected after transient replication of the vaccine viruses; all were challenged vaginally 6 mo later with homologous pathogenic SHIVKU-1. Two of the V1 group developed full-blown AIDS without evidence of vaccine virus DNA in tissues. The data on the 10 vaccinated survivors showed that all 10 became infected with SHIVKU-1 and that DNA of both vaccine and SHIVKU-1 viruses were present 6 mo postchallenge, with minimal replication of SHIVKU-1. During the following 5 years, these animals remained persistently infected, but with only one of the two viruses. Six animals eliminated their vaccine virus after variable periods of time and four of these succumbed to reactivation of the challenge virus and AIDS. Five years after challenge, four latently infected animals, two with V2 and two with SHIVKU-1, were reinoculated with SHIVKU-1. This resulted in transient superinfection and the animals promptly returned to their prechallenge status. Immunosuppression of the four animals 1 year later with Abs to CD8⁺ lymphocytes resulted in transiently productive replication of their respective latent viruses, and upon recovery of CD8⁺ lymphocytes, they reverted to their latent virus status. The major finding was that of eight animals that eliminated the vaccine virus, six developed AIDS. The two others harboring SHIVKU-1 remain at risk for developing late-onset disease. The primary correlate against AIDS was persistence of the vaccine virus.

Maertzdorf, J., A. D. M. E. Osterhaus, et al. (2002). "IL-17 Expression in Human Herpetic Stromal Keratitis: Modulatory Effects on Chemokine Production by Corneal Fibroblasts." J. Immunol. **169**(10): 5897-5903.

<http://www.jimmunol.org/cgi/content/abstract/169/10/5897>

Herpetic stromal keratitis (HSK) is an immunopathologic disease triggered by infection of the cornea with HSV. Key events in HSK involve the interaction between cornea-infiltrating inflammatory cells and resident cells. This interaction, in which macrophages, producing IL-1 and TNF- α , and IFN- γ -producing Th1 cells play a crucial role, results in the local secretion of immune-modulatory factors and a major influx of neutrophils causing corneal lesions and blindness. The Th1-derived cytokine IL-17 has been shown to play an important role in several inflammatory diseases characterized by a massive infiltration of neutrophils into inflamed tissue. Here we show that IL-17 is expressed in corneas from patients with HSK and that the IL-17R is constitutively expressed by human corneal fibroblasts (HCF). IL-17 exhibited a strong synergistic effect with TNF- α on the induction of IL-6 and IL-8 secretion by cultured HCF. Secreted IL-8 in these cultures had a strong chemotactic effect on neutrophils. IL-17 also enhanced TNF- α - and IFN- γ -induced secretion of macrophage-inflammatory proteins 1 α and 3 α , while inhibiting the induced secretion of RANTES. Furthermore, considerable levels of IFN- γ -inducible protein 10 and matrix metalloproteinase 1 were measured in stimulated HCF cultures, while the constitutive secretion of monocyte chemotactic protein 1 remained unaffected. The data presented suggest that IL-17 may play an important role in the induction and/or perpetuation of the immunopathologic processes in human HSK by modulating the secretion of proinflammatory and neutrophil chemotactic factors by corneal resident fibroblasts.

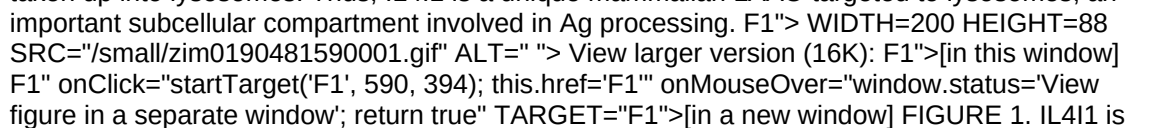
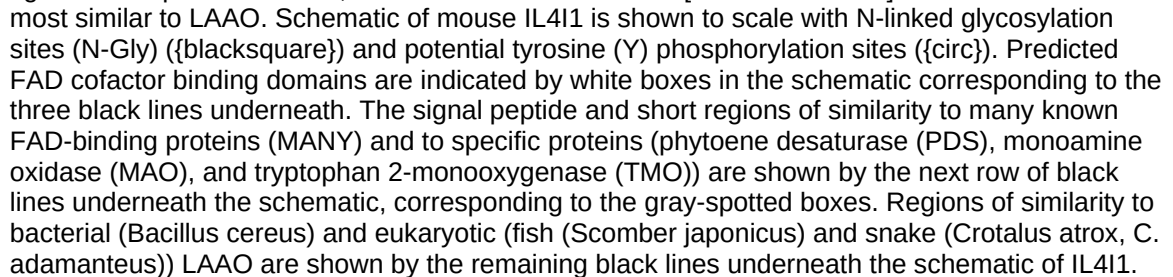
Manne, J., M. J. Mastrangelo, et al. (2002). "TCR Rearrangement in Lymphocytes Infiltrating Melanoma Metastases After Administration of Autologous Dinitrophenyl-Modified Vaccine." J. Immunol. **169**(6): 3407-3412.

<http://www.jimmunol.org/cgi/content/abstract/169/6/3407>

Administration of a vaccine consisting of autologous melanoma cells modified with a hapten, dinitrophenyl (DNP), induces T cell infiltration of metastatic sites. We have reported an analysis of these infiltrating T cells, indicating that certain TCR-V{beta} gene segments are greatly overexpressed. In this study, we investigate the rearrangement of the TCR-V{beta} as well as the junctional diversity in T cells infiltrating melanoma metastases following treatment with DNP vaccine. In 19 of 26 control specimens, V-D-J length analysis showed the expected polyclonal patterns. In contrast, postvaccine tumors from 9 of 10 patients showed dominant peaks of V-D-J junction size in one or more V{beta} families. Dominant peaks were seen most frequently in six V{beta} families (V{beta}7, 12, 13, 14, 16, and 23) and were never seen in seven others. Further analysis of the oligoclonal V{beta} products showed dominant peaks in the J region as well. Of particular interest was the finding that V{beta} and J{beta} peaks were similar in inflamed metastases obtained at different times or from different sites from the same patient. Although 6 of 10 patients expressed HLA-A1, there was no common pattern of TCR rearrangements among them. Finally, the amplified PCR products from seven of these specimens were cloned and sequenced and the amino acid sequence of the complementarity-determining region 3 was deduced. In six of seven specimens, the same complementarity-determining region 3 sequence was repeated in at least two clones and in five of seven in at least three clones. Our study indicates that DNP vaccine induces the expansion of particular T cell clones that may be agents of its antitumor effects.

Mason, J. M., M. D. Naidu, et al. (2004). "IL-4-Induced Gene-1 Is a Leukocyte L-Amino Acid Oxidase with an Unusual Acidic pH Preference and Lysosomal Localization." *J. Immunol.* **173**(7): 4561-4567.

<http://www.jimmunol.org/cgi/content/abstract/173/7/4561>

IL-4-induced gene-1 (IL4i1 or Fig1) initially isolated as a gene of unknown function from mouse B lymphocytes, is limited in expression to primarily immune tissues and genetically maps to a region of susceptibility to autoimmune disease. The predicted IL4i1 protein (IL4I1) sequence is most similar to apoptosis-inducing protein and Apoxin I, both L-amino acid oxidases (LAAO; Enzyme Commission 1.4.3.2). We demonstrate that IL4I1 has unique LAAO properties. IL4I1 has preference for aromatic amino acid substrates, having highest specific activity with phenylalanine. In support of this selectivity, IL4I1 is inhibited by aromatic competitors (benzoic acid and para-aminobenzoic acid), but not by nonaromatic LAAO inhibitors. IL4i1 protein and enzyme activity is found in the insoluble fraction of transient transfections, implying an association with cell membrane and possibly intracellular organelles. Indeed, IL4I1 has the unique property of being most active at acidic pH (pH 4), suggesting it may reside preferentially in lysosomes. IL4I1 is N-linked glycosylated, a requirement for lysosomal localization. Confocal microscopy of cells expressing IL4I1 translationally fused to red fluorescent protein demonstrated that IL4I1 colocalized with GFP targeted to lysosomes and with acriflavine, a green fluorescent dye that is taken up into lysosomes. Thus, IL4I1 is a unique mammalian LAAO targeted to lysosomes, an important subcellular compartment involved in Ag processing.   FIGURE 1. IL4I1 is most similar to LAAO. Schematic of mouse IL4I1 is shown to scale with N-linked glycosylation sites (N-Gly) (◼) and potential tyrosine (Y) phosphorylation sites (◉). Predicted FAD cofactor binding domains are indicated by white boxes in the schematic corresponding to the three black lines underneath. The signal peptide and short regions of similarity to many known FAD-binding proteins (MANY) and to specific proteins (phytoene desaturase (PDS), monoamine oxidase (MAO), and tryptophan 2-monooxygenase (TMO)) are shown by the next row of black lines underneath the schematic, corresponding to the gray-spotted boxes. Regions of similarity to bacterial (*Bacillus cereus*) and eukaryotic (fish (*Scomber japonicus*) and snake (*Crotalus atrox*, *C. adamanteus*)) LAAO are shown by the remaining black lines underneath the schematic of IL4I1.

Masuda, A., Y. Yoshikai, et al. (2002). "Th2 Cytokine Production from Mast Cells Is Directly Induced by Lipopolysaccharide and Distinctly Regulated by c-Jun N-Terminal Kinase and p38 Pathways." J. Immunol. **169**(7): 3801-3810.

<http://www.jimmunol.org/cgi/content/abstract/169/7/3801>

Mast cells secrete multiple cytokines and play an important role in allergic inflammation. Although it is widely accepted that bacteria infection occasionally worsens allergic airway inflammation, the mechanism has not been defined. In this study, we show that LPS induced Th2-associated cytokine production such as IL-5, IL-10, and IL-13 from mast cells and also synergistically enhanced production of these cytokines induced by IgE cross-linking. LPS-mediated Th2-type cytokine production was abolished in mouse bone marrow-derived mast cells derived from C3H/HeJ mice, suggesting that Toll-like receptor 4 is essential for the cytokine production. Furthermore, we found that mitogen-activated protein kinases including extracellular signal-regulated kinase 1/2, c-Jun N-terminal kinase, and p38 kinase were activated by LPS stimulation in bone marrow-derived mast cells. Inhibition of extracellular signal-regulated kinase activation has little effect on LPS-mediated cytokine production. In contrast, inhibition of c-Jun N-terminal kinase activation significantly suppressed both IL-10 and IL-13 expression at both mRNA and protein levels. Interestingly, although inhibition of p38 did not down-regulate the mRNA induction, it moderately decreased all three cytokine productions by LPS. These results indicate that LPS-mediated production of IL-5, IL-10, and IL-13 was distinctly regulated by mitogen-activated protein kinases. Our findings may indicate a clue to understanding the mechanisms of how bacteria infection worsens the clinical features of asthma.

Masuda, A., Y. Yoshikai, et al. (2004). "The Interaction between GATA Proteins and Activator Protein-1 Promotes the Transcription of IL-13 in Mast Cells." J. Immunol. **173**(9): 5564-5573.

<http://www.jimmunol.org/cgi/content/abstract/173/9/5564>

IL-13 is considered to be a key modulator in the pathogenesis of Th2-induced allergic inflammation, although little is known about the regulation of IL-13 transcription in mast cells. In T cells, involvement of GATA-3 in cell type-specific expression of the IL-13 gene has been reported. However, the mechanisms that induce rapid transactivation of the IL-13 gene in response to various types of stimulation have hitherto remained unknown. In this report, we describe our investigation of the promoter region necessary for IL-13 transcription; we have found that both AP-1 and GATA proteins are indispensable for IL-13 transcription in mouse mast cells. In our investigation, we focused on the functional interaction between GATA and AP-1 in the IL-13 promoter context. Transfection experiments have revealed that GATA-1 and GATA-2 proteins are able to associate with AP-1 proteins. We have also shown that overexpression of GATA-1 induced excess AP-1 binding to the IL-13 promoter as well as a significant increase in IL-13 production in mast cells. The results of the present study have shown that direct interaction between AP-1 and GATA proteins plays an important role in IL-13 transcription in mast cells.

Matsumoto, Y., Y. Tsukada, et al. (2004). "C Protein-Induced Myocarditis and Subsequent Dilated Cardiomyopathy: Rescue from Death and Prevention of Dilated Cardiomyopathy by Chemokine Receptor DNA Therapy." J. Immunol. **173**(5): 3535-3541.

<http://www.jimmunol.org/cgi/content/abstract/173/5/3535>

Severe experimental autoimmune myocarditis and subsequent dilated cardiomyopathy (DCM) were successfully produced in Lewis rats by immunization with recombinant cardiac C protein. Seventy-five percent of immunized rats died between days 15 and 49 postimmunization, and all of the survived rats showed typical DCM characterized by the presence of ventricular dilatation and extensive fibrosis. Immunopathological and chemokine analysis during the acute phase revealed that there were marked macrophage infiltration with myocyte necrosis and up-regulation of MCP-1 and IFN- γ -inducible protein-10 (IP-10). Based on these findings, we prepared plasmid DNAs encoding the binding site of CCR2 and CXCR3, which are receptors for MCP-1 and IP-10, respectively. The culture supernatant of cells transfected with these DNAs inhibited the migration of T cells and macrophages induced by MCP-1 and IP-10. Remarkably, administration of the DNAs to C protein-immunized rats prevented the disease progression and rescued animals from death. The present study has demonstrated for the first time that gene therapy targeting the chemokine receptor could be a powerful tool for the control of experimental autoimmune myocarditis and DCM.

McKay, P. F., J. E. Schmitz, et al. (2002). "Vaccine Protection Against Functional CTL Abnormalities in Simian Human Immunodeficiency Virus-Infected Rhesus Monkeys." *J. Immunol.* **168**(1): 332-337.

<http://www.jimmunol.org/cgi/content/abstract/168/1/332>

Accumulating evidence suggests that HIV-specific CD8⁺ CTL are dysfunctional in HIV-infected individuals with progressive clinical disease. In the present studies, cytokine production by virus-specific CTL was assessed in the rhesus monkey model for AIDS to determine its contribution to the functional impairment of CTL. CTL from monkeys infected with nonpathogenic isolates of simian and simian-human immunodeficiency virus expressed high levels of IFN- γ , TNF- α , and IL-2 after in vitro exposure to a nonspecific mitogen or the optimal peptide representing a dominant virus-specific CTL epitope. However, similarly performed studies assessing these capabilities in CTL from monkeys infected with pathogenic immunodeficiency virus isolates demonstrated a significant dysfunction in the ability of the CTL to produce IL-2 and TNF- α . Importantly, CTL from vaccinated monkeys that effectively controlled the replication of a highly pathogenic simian-human immunodeficiency virus isolate following challenge demonstrated a preserved capacity to produce these cytokines. These experiments suggest that defects in cytokine production may contribute to CTL dysfunction in chronic HIV or SIV infection. Moreover, an AIDS vaccine that confers protection against clinical disease evolution in this experimental model also preserves the functional capacity of these CTL to produce both IL-2 and TNF- α .

Mencacci, A., C. Montagnoli, et al. (2002). "CD80+Gr-1⁺ Myeloid Cells Inhibit Development of Antifungal Th1 Immunity in Mice with Candidiasis." *J. Immunol.* **169**(6): 3180-3190.

<http://www.jimmunol.org/cgi/content/abstract/169/6/3180>

To find out whether polymorphonuclear neutrophils (PMN), abundantly recruited in disseminated *Candida albicans* infection, could directly affect the activation of Th cells we addressed the issues as to whether murine PMN, like their human counterparts, express costimulatory molecules and the functional consequence of this expression in terms of antifungal immune resistance. To this purpose, we assessed 1) the expression of CD80 (B7-1) and CD86 (B7-2) molecules on peripheral, splenic, and inflammatory murine Gr-1⁺ PMN; 2) its modulation upon interaction with *C. albicans* in vitro, in vivo, and in human PMN; 3) the effect of *Candida* exposure on the ability of murine PMN to affect CD4⁺ Th1 cell proliferation and cytokine production; and 4) the mechanism responsible for this effect. Murine PMN constitutively expressed CD80 molecules on both the surface and intracellularly; however, in both murine and human PMN, CD80 expression was

differentially modulated upon interaction with *Candida* yeasts or hyphae in vitro as well as in infected mice. The expression of the CD86 molecule was neither constitutive nor inducible upon exposure to the fungus. In vitro, Gr-1+ PMN were found to inhibit the activation of IFN- γ -producing CD4+ T cells and to induce apoptosis through a CD80/CD28-dependent mechanism. A population of CD80+Gr-1+ myeloid cells was found to be expanded in conventional as well as in bone marrow-transplanted mice with disseminated candidiasis, but its depletion increased the IFN- γ -mediated antifungal resistance. These data indicate that alternatively activated PMN expressing CD80 may adversely affect Th1-dependent resistance in fungal infections.

Meusel, T. R. and F. Imani (2003). "Viral Induction of Inflammatory Cytokines in Human Epithelial Cells Follows a p38 Mitogen-Activated Protein Kinase-Dependent but NF- κ B-Independent Pathway." *J. Immunol.* **171**(7): 3768-3774.

<http://www.jimmunol.org/cgi/content/abstract/171/7/3768>

The initial step in an immune response toward a viral infection is the induction of inflammatory cytokines. This innate immune response is mediated by expression of a variety of cytokines exemplified by TNF- α and IL-1 β . A key signal for the recognition of intracellular viral infections is the presence of dsRNA. Viral infections and dsRNA treatment can activate several signaling pathways including the protein kinase R pathway, mitogen-activated protein kinase (MAPK) pathways, and NF- κ B, which are important in the expression of inflammatory cytokines. We previously reported that activation of protein kinase R was required for dsRNA induction of TNF- α , but not for IL-1 β . In this study, we report that activation of the p38 MAPK pathway by respiratory viral infections is necessary for induction of inflammatory cytokines in human bronchial epithelial cells. Inhibition of p38 MAPK by two different pharmacological inhibitors showed that expression of both TNF- α and IL-1 β required activation of this signaling pathway. Interestingly, inhibition of NF- κ B did not significantly reduce viral induction of either cytokine. Our data show that, during the initial infections of epithelial cells with respiratory viruses, activation of the p38 MAPK pathway is associated with induction of inflammation, and NF- κ B activation may be less important than previously suggested.

Meusel, T. R., K. E. Kehoe, et al. (2002). "Protein Kinase R Regulates Double-Stranded RNA Induction of TNF- α But Not IL-1 β mRNA in Human Epithelial Cells." *J. Immunol.* **168**(12): 6429-6435.

<http://www.jimmunol.org/cgi/content/abstract/168/12/6429>

Epithelial cells represent the initial site of respiratory viral entry and the first line of defense against such infections. This early antiviral response is characterized by an increase in the production of proinflammatory cytokines such as TNF- α and IL-1 β . dsRNA, which is a common factor present during the life cycle of both DNA and RNA viruses, is known to induce TNF- α and IL-1 β in a variety of cells. In this work we provide data showing that dsRNA treatment induces TNF- α and IL-1 β in human lung epithelial cells via two different mechanisms. Our data show that dsRNA activation of dsRNA-activated protein kinase (PKR) is associated with induction of TNF- α but not IL-1 β expression. An inhibitor of PKR activation blocked the dsRNA-induced elevations in TNF- α but not IL-1 β mRNA in epithelial cells. Data obtained from infection of epithelial cells with a vaccinia virus lacking the PKR inhibitory polypeptide, E3L, revealed that PKR activation was essential for TNF- α but not for IL-1 β expression. In this report, we provide experimental support for the differential regulation of proinflammatory cytokine expression by dsRNA and viral infections in human airway epithelial cells.

Michalec, L., B. K. Choudhury, et al. (2002). "CCL7 and CXCL10 Orchestrate Oxidative Stress-Induced Neutrophilic Lung Inflammation." *J. Immunol.* **168**(2): 846-852.

<http://www.jimmunol.org/cgi/content/abstract/168/2/846>

Oxidative stress from ozone (O₃) exposure augments airway neutrophil recruitment and chemokine production. We and others have shown that severe and sudden asthma is associated with airway neutrophilia, and that O₃ oxidative stress is likely to augment neutrophilic airway inflammation in severe asthma. However, very little is known about chemokines that orchestrate oxidative stress-induced neutrophilic airway inflammation in vivo. To identify these chemokines, three groups of BALB/c mice were exposed to sham air, 0.2 ppm O₃, or 0.8 ppm O₃ for 6 h. Compared with sham air, 0.8 ppm O₃, but not 0.2 ppm O₃, induced pronounced neutrophilic airway inflammation that peaked at 18 h postexposure. The 0.8 ppm O₃ up-regulated lung mRNA of CXCL1,2,3 (mouse growth-related oncogene- α) and macrophage-inflammatory protein-2), CXCL10 (IFN- γ -inducible protein-10), CCL3 (macrophage-inflammatory protein-1 α), CCL7 (monocyte chemoattractant protein-3), and CCL11 (eotaxin) at 0 h postexposure, and expression of CXCL10, CCL3, and CCL7 mRNA was sustained 18 h postexposure. O₃ increased lung protein levels of CXCL10, CCL7, and CCR3 (CCL7R). The airway epithelium was identified as a source of CCL7. The role of up-regulated chemokines was determined by administering control IgG or IgG Abs against six murine chemokines before O₃ exposure. As expected, anti-mouse growth-related oncogene- α inhibited neutrophil recruitment. Surprisingly, Abs to CCL7 and CXCL10 also decreased neutrophil recruitment by 63 and 72%, respectively. These findings indicate that CCL7 and CXCL10, two chemokines not previously reported to orchestrate neutrophilic inflammation, play a critical role in mediating oxidative stress-induced neutrophilic airway inflammation. These observations may have relevance in induction of neutrophilia in severe asthma.

Miyakoshi, A., W. K. Yoon, et al. (2003). "Characterization of the Antigen Specificity and TCR Repertoire, and TCR-Based DNA Vaccine Therapy in Myelin Basic Protein-Induced Autoimmune Encephalomyelitis in DA Rats." *J. Immunol.* **170**(12): 6371-6378.

<http://www.jimmunol.org/cgi/content/abstract/170/12/6371>

Like Lewis rats, DA rats are an experimental autoimmune encephalomyelitis (EAE)-susceptible strain and develop severe EAE upon immunization with myelin basic protein (MBP). However, there are several differences between the two strains. In the present study we induced acute EAE in DA rats by immunization with MBP and MBP peptides and examined the Ag specificity and TCR repertoire of encephalitogenic T cells. It was found that although immunization with MBP and a peptide corresponding to its 62-75 sequence (MBP62-75) induced clinical EAE, the responses of lymph node T cells isolated from MBP-immunized rats to MBP62-75 was marginal, indicating that this peptide contains major encephalitogenic, but not immunodominant, epitopes. The TCR analysis by CDR3 spectratyping of spinal cord T cells revealed that V β 10 and V β 15 spectratype expansion was always found in MBP62-75-immunized symptomatic rats. On the basis of these findings, we examined the encephalitogenicity of V β 10- and V β 15-positive T cells. First, the adoptive transfer experiments revealed that V β 10-positive T line cells derived from MBP62-75-immunized rats induced clinical EAE in recipients. Second, administration of DNA vaccines encoding V β 10 and V β 15, alone or in combination, ameliorated MBP62-75-induced EAE. Collectively, it was strongly suggested that V β 10- and V β 15-positive T cells are encephalitogenic. Analyses of the Ag specificity and T cell repertoire of pathogenic T cells performed in this study provide useful information for designing specific immunotherapies against autoimmune diseases.

Nakajima, P. B. and M. J. Bosma (2002). "Variable Diversity Joining Recombination: Nonhairpin Coding Ends in Thymocytes of SCID and Wild-Type Mice." J. Immunol. **169**(6): 3094-3104.

<http://www.jimmunol.org/cgi/content/abstract/169/6/3094>

Initiation of V(D)J recombination results in broken DNA molecules with blunt recombination signal ends and covalently sealed (hairpin) coding ends. In SCID mice, coding joint formation is severely impaired and hairpin coding ends accumulate as a result of a deficiency in the catalytic subunit of DNA-dependent protein kinase, an enzyme involved in the repair of DNA double-strand breaks. In this study, we report that not all SCID coding ends are hairpinned. We have detected open J δ 1 and D δ 2 coding ends at the TCR δ locus in SCID thymocytes. Approximately 25% of 5'D δ 2 coding ends were found to be open. Large deletions and abnormally long P nucleotide additions typical of SCID D δ 2-J δ 1 coding joints were not observed. Most J δ 1 and D δ 2 coding ends exhibited 3' overhangs, but at least 20% had unique 5' overhangs not previously detected in vivo. We suggest that the SCID DNA-dependent protein kinase deficiency not only reduces the efficiency of hairpin opening, but also may affect the specificity of hairpin nicking, as well as the efficiency of joining open coding ends.

Nakamura, Y., S. Esnault, et al. (2004). "Ets-1 Regulates TNF- α -Induced Matrix Metalloproteinase-9 and Tenascin Expression in Primary Bronchial Fibroblasts." J. Immunol. **172**(3): 1945-1952.

<http://www.jimmunol.org/cgi/content/abstract/172/3/1945>

Increased subepithelial deposition of extracellular matrix proteins is a key feature in bronchial asthma. Matrix metalloproteinase-9 (MMP-9) is a proteolytic enzyme that degrades the extracellular matrix. Tenascin is an extracellular matrix glycoprotein that is abundant in thickened asthmatic subbasement membrane. The expression of MMP-9 and tenascin reflects disease activity in asthma and airway remodeling. The molecular mechanisms regulating the expression of these proteins remain unknown. Both MMP-9 and tenascin promoters contain an Ets binding site, suggesting control by Ets-1. Thus, we hypothesized that Ets-1 expression is increased in asthma and that it contributed to enhanced MMP-9 and tenascin expression. To test this hypothesis, we determined the expression of Ets-1 in bronchial biopsies obtained from asthmatic subjects and determined the expression of Ets-1, MMP-9, and tenascin by bronchial fibroblasts activated ex vivo. We observed that nuclear extracts from TNF- α -activated fibroblasts showed increased Ets-binding activity. In addition, TNF- α -activated fibroblasts had increased expression of Ets-1 mRNA and protein, which preceded an increase in MMP-9 and tenascin mRNA. Furthermore, treatment of fibroblasts with Ets-1 antisense oligonucleotides down-regulated TNF- α -induced Ets-1, MMP-9, and, to a lesser extent, tenascin protein expression or activity. Taken together, these data demonstrate that TNF- α increases MMP-9 and tenascin expression in bronchial fibroblasts via the transcription factor Ets-1, and suggest a role for Ets-1 in airway remodeling in asthma.

Nomura, I., E. Goleva, et al. (2003). "Cytokine Milieu of Atopic Dermatitis, as Compared to Psoriasis, Skin Prevents Induction of Innate Immune Response Genes." J. Immunol. **171**(6): 3262-3269.

<http://www.jimmunol.org/cgi/content/abstract/171/6/3262>

Atopic dermatitis (AD) and psoriasis are the two most common chronic skin diseases. However patients with AD, but not psoriasis, suffer from frequent skin infections. To understand the

molecular basis for this phenomenon, skin biopsies from AD and psoriasis patients were analyzed using GeneChip microarrays. The expression of innate immune response genes, human β defensin (HBD)-2, IL-8, and inducible NO synthetase (iNOS) was found to be decreased in AD, as compared with psoriasis, skin (HBD-2, $p = 0.00021$; IL-8, $p = 0.044$; iNOS, $p = 0.016$). Decreased expression of the novel antimicrobial peptide, HBD-3, was demonstrated at the mRNA level by real-time PCR ($p = 0.0002$) and at the protein level by immunohistochemistry ($p = 0.0005$). By real-time PCR, our data confirmed that AD, as compared with psoriasis, is associated with elevated skin production of Th2 cytokines and low levels of proinflammatory cytokines such as TNF- α , IFN- γ , and IL-1 β . Because HBD-2, IL-8, and iNOS are known to be inhibited by Th2 cytokines, we examined the effects of IL-4 and IL-13 on HBD-3 expression in keratinocyte culture in vitro. We found that IL-13 and IL-4 inhibited TNF- α - and IFN- γ -induced HBD-3 production. These studies indicate that decreased expression of a constellation of antimicrobial genes occurs as the result of local up-regulation of Th2 cytokines and the lack of elevated amounts of TNF- α and IFN- γ under inflammatory conditions in AD skin. These observations could explain the increased susceptibility of AD skin to microorganisms, and suggest a new fundamental rule that may explain the mechanism for frequent infection in other Th2 cytokine-mediated diseases.

Nonaka, S., T. Naito, et al. (2005). "Intestinal $\gamma\delta$ T Cells Develop in Mice Lacking Thymus, All Lymph Nodes, Peyer's Patches, and Isolated Lymphoid Follicles." *J. Immunol.* **174**(4): 1906-1912.

<http://www.jimmunol.org/cgi/content/abstract/174/4/1906>

Through analysis of athymic (nu/nu) mice carrying a transgenic gene encoding GFP instead of RAG-2 product, it has recently been reported that, in the absence of thymopoiesis, mesenteric lymph nodes and Peyer's patches (PP) but not gut cryptopatches are pivotal birthplace of mature T cells such as the thymus-independent intestinal intraepithelial T cells (IEL). To explore and evaluate this important issue, we generated nu/nu mice lacking all lymph nodes (LN) and PP by administration of lymphotoxin- β receptor-Ig and TNF receptor 55-Ig fusion proteins into the timed pregnant nu/+ mice that had been mated with male nu/nu mice (nu/nu LNP- mice). We also generated nu/nu aly/aly (aly, alymphoplasia) double-mutant mice that inherently lacked all LN, PP, and isolated lymphoid follicles. Although $\gamma\delta$ -IEL were slightly smaller in number than those in nu/nu mice, substantial colonization of $\gamma\delta$ -IEL was found to take place in the intestinal epithelia of nu/nu LNP- and nu/nu aly/aly mice. Notably, the population size of a major CD8 $\alpha\alpha$ + $\gamma\delta$ -IEL subset was maintained, the use of TCR- γ -chain variable gene segments by these $\gamma\delta$ -IEL was unaltered, and the development of cryptopatches remained intact in these nu/nu LNP- and nu/nu aly/aly mice. These findings indicate that all LN, including mesenteric LN, PP, and isolated lymphoid follicles, are not an absolute requirement for the development of $\gamma\delta$ -IEL in athymic nu/nu mice.

Oberholzer, A., C. Oberholzer, et al. (2002). "Increased Survival in Sepsis by In Vivo Adenovirus-Induced Expression of IL-10 in Dendritic Cells." *J. Immunol.* **168**(7): 3412-3418.

<http://www.jimmunol.org/cgi/content/abstract/168/7/3412>

The dendritic cell (DC) is the most potent APC of the immune system, capable of stimulating naive T cells to proliferate and differentiate into effector T cells. Recombinant adenovirus (Adv) readily transduces DCs in vitro allowing directed delivery of transgenes that modify DC function and immune responses. In this study we demonstrate that footpad injection of a recombinant Adv readily targets transduction of myeloid and lymphoid DCs in the draining popliteal lymph node, but not in other lymphoid organs. Popliteal DCs transduced with an empty recombinant Adv

undergo maturation, as determined by high MHC class II and CD86 expression. However, transduction with vectors expressing human IL-10 limit DC maturation and associated T cell activation in the draining lymph node. The extent of IL-10 expression is dose dependent; transduction with low particle numbers (105) yields only local expression, while transduction with higher particle numbers (107 and 1010) leads additionally to IL-10 appearance in the circulation. Furthermore, local DC expression of human IL-10 following in vivo transduction with low particle numbers (105) significantly improves survival following cecal ligation and puncture, suggesting that compartmental modulation of DC function profoundly alters the sepsis-induced immune response.

Ortega-Cava, C. F., S. Ishihara, et al. (2003). "Strategic Compartmentalization of Toll-Like Receptor 4 in the Mouse Gut." J. Immunol. **170**(8): 3977-3985.

<http://www.jimmunol.org/cgi/content/abstract/170/8/3977>

Pattern recognition receptors (PRRs), which include the Toll-like receptors (TLRs), are involved in the innate immune response to infection. TLR4 is a model for the TLR family and is the main LPS receptor. We wanted to determine the expression of TLR4 and compare it with that of TLR2 and CD14 along the gastrointestinal mucosa of normal and colitic BALB/c mice. Colitis was induced with 2.5% dextran sodium sulfate (DSS). Mucosa from seven segments of the digestive tract (stomach, small intestine in three parts, and colon in three parts) was isolated by two different methods. Mucosal TLR4, CD14, TLR2, MyD88, and IL-1 β mRNA were semiquantified by Northern blotting. TLR4 protein was determined by Western blotting. TLR4/MD-2 complex and CD14 were evaluated by immunohistochemistry. PRR genes were constitutively expressed and were especially stronger in colon. TLR4 and CD14 mRNA were increased in the distal colon, but TLR2 mRNA was expressed more strongly in the proximal colon, and MyD88 had a uniform expression throughout the gut. Accordingly, TLR4 and CD14 protein levels were higher in the distal colon. TLR4/MD-2 and CD14 were localized at crypt bottom epithelial cells. TLR4/MD2, but not CD14, was found in mucosal mononuclear cells. Finally, DSS-induced inflammation was localized in the distal colon. All genes studied were up-regulated during DSS-induced inflammation, but the normal colon-stressed gut distribution was preserved. Our findings demonstrate that TLR4, CD14, and TLR2 are expressed in a compartmentalized manner in the mouse gut and provide novel information about the in vivo localization of PRRs.

Petersson, K., H. Pettersson, et al. (2003). "Staphylococcal Enterotoxin H Induces V α -Specific Expansion of T Cells." J. Immunol. **170**(8): 4148-4154.

<http://www.jimmunol.org/cgi/content/abstract/170/8/4148>

Staphylococcal enterotoxin H (SEH) is a bacterial superantigen secreted by *Staphylococcus aureus*. Superantigens are presented on the MHC class II and activate large amounts of T cells by cross-linking APC and T cells. In this study, RT-PCR was used to show that SEH stimulates human T cells via the V α domain of TCR, in particular V α 10 (TRAV27), while no TCR V β -specific expansion was seen. This is in sharp contrast to all other studied bacterial superantigens, which are highly specific for TCR V β . It was further confirmed by flow cytometry that SEH stimulation does not alter the levels of certain TCR V β . In a functional assay addressing cross-reactivity, V β binding superantigens were found to form one group, whereas SEH has different properties that fit well with V α reactivity. As SEH binds on top of MHC class II, an interaction between MHC and TCR upon SEH binding is not likely. This concludes that the specific expansion of TCR V α is not due to contacts between MHC and TCR, instead we suggest that SEH directly interacts with the TCR V α domain.

Pewe, L. L., J. M. Netland, et al. (2004). "Very Diverse CD8 T Cell Clonotypic Responses after Virus Infections." J. Immunol. **172**(5): 3151-3156.

<http://www.jimmunol.org/cgi/content/abstract/172/5/3151>

We measured CD8 T cell clonotypic diversity to three epitopes recognized in C57BL/6 mice infected with mouse hepatitis virus, strain JHM, or lymphocytic choriomeningitis virus. We isolated epitope-specific T cells with an IFN- γ capture assay or MHC class I/peptide tetramers and identified different clonotypes by V β chain sequence analysis. In agreement with our previous results, the number of different clonotypes responding to all three epitopes fit a log-series distribution. From these distributions, we estimated that >1000 different clonotypes responded to each immunodominant CD8 T cell epitope; the response to a subdominant CD8 T cell epitope was modestly less diverse. These results suggest that T cell response diversity is greater by 1-2 orders of magnitude than predicted previously.

Podojil, J. R. and V. M. Sanders (2003). "Selective Regulation of Mature IgG1 Transcription by CD86 and β 2-Adrenergic Receptor Stimulation." J. Immunol. **170**(10): 5143-5151.

<http://www.jimmunol.org/cgi/content/abstract/170/10/5143>

Stimulation of CD86 and the β 2-adrenergic receptor (β 2AR) on a B cell, either alone or together, is known to increase the level of IgG1 protein produced by a CD40 ligand/IL-4-activated B cell. It is also known that the mechanism by which CD40 and IL-4R stimulation on a B cell increases the level of IgG1 protein is by increasing germline γ 1 transcription, IgG1 class switching, and mature IgG1 transcription, while the molecular mechanism responsible for mediating the CD86- and β 2AR-induced effect remains unknown. In the present study using real-time PCR we show that the level of mature IgG1 transcription increases in CD40 ligand/IL-4-activated B cells following stimulation of either CD86 and/or β 2AR, and that this increase reflects the increase in IgG1 protein. Furthermore, we show that the CD86- and/or β 2AR-induced increase in mature IgG1 transcript is due to an increase in the rate of mature IgG1 transcription, as determined by nuclear run-on analysis. This effect is additive when both receptors are stimulated and is lost when B cells from CD86- and β 2AR-deficient mice are used. In contrast, the level of germline γ 1 transcription, the stability of mature IgG1 transcript, the number of IgG1-positive B cells, and the number of IgG1-secreting B cells did not change. These results provide the first evidence that CD86 and/or β 2AR stimulation on a CD40 ligand/IL-4-activated B cell increases the level of IgG1 protein produced per cell by increasing the rate of mature IgG1 transcription.

Precopio, M. L., J. L. Sullivan, et al. (2003). "Differential Kinetics and Specificity of EBV-Specific CD4+ and CD8+ T Cells During Primary Infection." J. Immunol. **170**(5): 2590-2598.

<http://www.jimmunol.org/cgi/content/abstract/170/5/2590>

The generation and maintenance of virus-specific CD4+ T cells in humans are not well understood. We used short in vitro stimulation assays followed by intracellular cytokine staining to characterize the timing, magnitude, and Ag specificity of CD4+ T cells over the course of primary EBV infection. Lytic and latent protein-specific CD4+ T cells were readily detected at presentation with acute infectious mononucleosis and declined rapidly thereafter. Responses to BZLF-1, BMLF-1, and Epstein-Barr nuclear Ag-3A were more commonly detected than responses to

Epstein-Barr nuclear Ag-1. Concurrent analyses of BZLF-1-specific CD4+ and CD8+ T cells revealed differences in the expansion, specificity, and stability of CD4+ and CD8+ T cell-mediated responses over time. Peripheral blood EBV load directly correlated with the frequency of EBV-specific CD4+ T cell responses at presentation and over time, suggesting that EBV-specific CD4+ T cell responses are Ag-driven.

Qiao, Y., S. Prabhakar, et al. (2004). "Posttranscriptional Inhibition of Gene Expression by Mycobacterium tuberculosis Offsets Transcriptional Synergism with IFN- γ and Posttranscriptional Up-Regulation by IFN- γ ." *J. Immunol.* **172**(5): 2935-2943.

<http://www.jimmunol.org/cgi/content/abstract/172/5/2935>

Host defense against Mycobacterium tuberculosis requires the cytokine IFN- γ and IFN regulatory factor 1 (IRF-1), a transcription factor that is induced to high levels by IFN- γ . Therefore, we chose to study regulation of IRF-1 expression as a model for effects of M. tuberculosis on response to IFN- γ . We found that IRF-1 mRNA abundance increased far more than transcription rate in human monocytic THP-1 cells stimulated by IFN- γ , but less than transcription rate in cells infected by M. tuberculosis. IFN- γ stimulation of infected cells caused a synergistic increase in IRF-1 transcription, yet IRF-1 mRNA abundance was similar in uninfected and infected cells stimulated by IFN- γ , as was the IRF-1 protein level. Comparable infection by Mycobacterium bovis bacillus Calmette-Guerin failed to induce IRF-1 expression and had no effect on the response to IFN- γ . We also examined the kinetics of transcription, the mRNA t_{1/2}, and the distribution of IRF-1 transcripts among total nuclear RNA, poly(A) nuclear RNA, and poly(A) cytoplasmic RNA pools in cells that were infected by M. tuberculosis and/or stimulated by IFN- γ . Our data suggest that infection by M. tuberculosis inhibits RNA export from the nucleus. Moreover, the results indicate that regulated entry of nascent transcripts into the pool of total nuclear RNA affects IRF-1 expression and that this process is stimulated by IFN- γ and inhibited by M. tuberculosis. The ability of infection by M. tuberculosis to limit the increase in IRF-1 mRNA expression that typically follows transcriptional synergism may contribute to the pathogenicity of M. tuberculosis.

Raynard, S. J., L. R. Read, et al. (2002). "Evidence for the Murine IgH {micro} Locus Acting as a Hot Spot for Intrachromosomal Homologous Recombination." *J. Immunol.* **168**(5): 2332-2339.

<http://www.jimmunol.org/cgi/content/abstract/168/5/2332>

Homologous recombination accomplishes the exchange of genetic information between two similar or identical DNA duplexes. It can occur either by gene conversion, a process of unidirectional genetic exchange, or by reciprocal crossing over. Homologous recombination is well known for its role in generating genetic diversity in meiosis and, in mitosis, as a DNA repair mechanism. In the immune system, the evidence suggests a role for homologous recombination in Ig gene evolution and in the diversification of Ab function. Previously, we reported the occurrence of homologous recombination between repeated, donor and recipient alleles of the Ig H chain {micro} gene C (C{micro}) region residing at the Ig {micro} locus in mouse hybridoma cells. In this study, we constructed mouse hybridoma cell lines bearing C{micro} region heteroalleles to learn more about the intrachromosomal homologous recombination process. A high frequency of homologous recombination (gene conversion) was observed for markers spanning the entire recipient C{micro} region, suggesting that recombination might initiate at random sites within the C{micro} region. The C{micro} region heteroalleles were equally proficient as either conversion donors or recipients. Remarkably, when the same C{micro} heteroalleles were tested for recombination in ectopic genomic positions, the mean frequency of gene conversion was reduced by at least 65-fold. These results are consistent with the murine IgH

{micro} locus behaving as a hot spot for intrachromosomal homologous recombination.

Ruckert, R., K. Brandt, et al. (2005). "Blocking IL-15 Prevents the Induction of Allergen-Specific T Cells and Allergic Inflammation In Vivo." *J. Immunol.* **174**(9): 5507-5515.

<http://www.jimmunol.org/cgi/content/abstract/174/9/5507>

IL-15 has been shown to accelerate and boost allergic sensitization in mice. Using a murine model of allergic sensitization to OVA, we present evidence that blocking endogenous IL-15 during the sensitization phase using a soluble IL-15R{alpha} (sIL-15R{alpha}) suppresses the induction of Ag-specific, Th2-differentiated T cells. This significantly reduces the production of OVA-specific IgE and IgG and prevents the induction of a pulmonary inflammation. Release of proinflammatory TNF-{alpha}, IL-1{beta}, IL-6, and IL-12 as well as that of Th2 cytokines IL-4, IL-5, and IL-13 into the bronchi are significantly reduced, resulting in suppressed recruitment of eosinophils and lymphocytes after allergen challenge. It is of clinical relevance that the airway hyper-responsiveness, a major symptom of human asthma bronchiale, is significantly reduced by sIL-15R{alpha} treatment. Ex vivo analysis of the draining lymph nodes revealed reduced numbers of CD8, but not CD4, memory cells and the inability of T cells of sIL-15R{alpha}-treated mice to proliferate and to produce Th2 cytokines after in vitro OVA restimulation. This phenomenon is not mediated by enhanced numbers of CD4+/CD25+ T cells. These results show that IL-15 is important for the induction of allergen-specific, Th2-differentiated T cells and induction of allergic inflammation in vivo.

Rutella, S., G. Bonanno, et al. (2003). "Identification of a Novel Subpopulation of Human Cord Blood CD34-CD133-CD7-CD45+Lineage- Cells Capable of Lymphoid/NK Cell Differentiation After In Vitro Exposure to IL-15." *J. Immunol.* **171**(6): 2977-2988.

<http://www.jimmunol.org/cgi/content/abstract/171/6/2977>

The hemopoietic stem cell (HSC) compartment encompasses cell subsets with heterogeneous proliferative and developmental potential. Numerous CD34- cell subsets that might reside at an earlier stage of differentiation than CD34+ HSCs have been described and characterized within human umbilical cord blood (UCB). We identified a novel subpopulation of CD34-CD133-CD7-CD45dimlin- (lin)- HSCs contained within human UCB that were endowed with low but measurable extended long-term culture-initiating cell activity. Exposure of CD34-CD133-CD7-CD45dimlin- HSCs to stem cell factor preserved cell viability and was associated with the following: 1) concordant expression of the stem cell-associated Ags CD34 and CD133, 2) generation of CFU-granulocyte-macrophage, burst-forming unit erythroid, and megakaryocytic aggregates, 3) significant extended long-term culture-initiating cell activity, and 4) up-regulation of mRNA signals for myeloperoxidase. At variance with CD34+lin- cells, CD34-CD133-CD7-CD45dimlin- HSCs maintained with IL-15, but not with IL-2 or IL-7, proliferated vigorously and differentiated into a homogeneous population of CD7+CD45brightCD25+CD44+ lymphoid progenitors with high expression of the T cell-associated transcription factor GATA-3. Although they harbored nonclonally rearranged TCR{gamma} genes, IL-15-primed CD34-CD133-CD7-CD45dimlin- HSCs failed to achieve full maturation, as manifested in their CD3-TCR{alpha}{beta}-{gamma}{delta}- phenotype. Conversely, culture on stromal cells supplemented with IL-15 was associated with the acquisition of phenotypic and functional features of NK cells. Collectively, CD34-CD133-CD7-CD45dimlin- HSCs from human UCB displayed an exquisite sensitivity to IL-15 and differentiated into lymphoid/NK cells. Whether the transplantation of CD34-lin- HSCs possessing T/NK cell differentiation potential may impact on immunological reconstitution and control of minimal residual disease after HSC transplantation for autoimmune or malignant diseases remains to be determined.

Ryan, A. F., R. L. Grendell, et al. (2002). "A Soluble Isoform of the Rhesus Monkey Nonclassical MHC Class I Molecule Mamu-AG Is Expressed in the Placenta and the Testis." J. Immunol. **169**(2): 673-683.

<http://www.jimmunol.org/cgi/content/abstract/169/2/673>

The nonclassical MHC class I locus HLA-G is expressed primarily in the placenta, although other sites of expression have been noted in normal and pathological situations. In addition, soluble HLA-G isoforms have been detected in the serum of pregnant and nonpregnant women as well as men. The rhesus monkey placenta expresses a novel nonclassical MHC class I molecule Mamu-AG, which has features remarkably similar to those of HLA-G. We determined that the rhesus placenta expresses Mamu-AG mRNA (Mamu-AG5), retaining intron 4 as previously noted in HLA-G5. Immunostaining experiments with Ab 16G1 against the soluble HLA-G5 intron 4 peptide demonstrated that an immunoreactive protein(s) was present in the syncytiotrophoblasts of the chorionic villi of the rhesus placenta, within villous cytotrophoblasts, and occasionally within cells of the villous stroma. The Mamu-AG5 mRNA was readily detected in rhesus testis (although not in ejaculated sperm). Whereas an Ab against membrane-bound Mamu-AG stained few cells, primarily in the interstitium of the testis, there was consistent immunostaining for Mamu-AG5 in cells within the seminiferous tubules, which was corroborated by localization of Mamu-AG mRNA by in situ hybridization. While primary spermatocytes were negative, Sertoli cells, spermatocytes, and spermatids were consistently positive for 16G1 immunostaining. The specific recognition of the soluble Mamu-AG isoform was confirmed by Western blotting of Mamu-AG5 expressed in heterologous cells. The results demonstrate that a soluble nonclassical MHC class I molecule is expressed in the rhesus monkey placenta and testis, and confirm and extend the unique homology between HLA-G and the rhesus nonclassical molecule Mamu-AG.

Sacedon, R., B. Diez, et al. (2005). "Sonic Hedgehog Is Produced by Follicular Dendritic Cells and Protects Germinal Center B Cells from Apoptosis." J. Immunol. **174**(3): 1456-1461.

<http://www.jimmunol.org/cgi/content/abstract/174/3/1456>

The Hedgehog (Hh) signaling pathway is involved in the development of many tissues during embryogenesis, but has also been described to function in adult self-renewing tissues. In the immune system, Sonic Hedgehog (Shh) regulates intrathymic T cell development and modulates the effector functions of peripheral CD4⁺ T cells. In this study we investigate whether Shh signaling is involved in peripheral B cell differentiation in mice. Shh is produced by follicular dendritic cells, mainly in germinal centers (GCs), and GC B cells express both components of the Hh receptor, Patched and Smoothened. Blockade of the Hh signaling pathway reduces the survival, and consequently the proliferation and Ab secretion, of GC B cells. Furthermore, Shh rescues GC B cells from apoptosis induced by Fas ligation. Taken together, our data suggest that Shh is one of the survival signals provided by follicular dendritic cells to prevent apoptosis in GC B cells.

Saeed, R. W., S. Varma, et al. (2004). "Ethanol Blocks Leukocyte Recruitment and Endothelial Cell Activation In Vivo and In Vitro." J. Immunol. **173**(10): 6376-6383.

<http://www.jimmunol.org/cgi/content/abstract/173/10/6376>

Immune system impairment and increased susceptibility to infection among alcohol abusers is a

significant but not well-understood problem. We hypothesized that acute ethanol administration would inhibit leukocyte recruitment and endothelial cell activation during inflammation and infection. Using LPS and carrageenan air pouch models in mice, we found that physiological concentrations of ethanol (1-5 g/kg) significantly blocked leukocyte recruitment (50-90%). Because endothelial cell activation and immune cell-endothelial cell interactions are critical regulators of leukocyte recruitment, we analyzed the effect of acute ethanol exposure on endothelial cell activation in vivo using the localized Shwartzman reaction model. In this model, ethanol markedly suppressed leukocyte accumulation and endothelial cell adhesion molecule expression in a dose-dependent manner. Finally, we examined the direct effects of ethanol on endothelial cell activation and leukocyte-endothelial cell interactions in vitro. Ethanol, at concentrations within the range found in human blood after acute exposure and below the levels that induce cytotoxicity (0.1-0.5%), did not induce endothelial cell activation, but significantly inhibited TNF-mediated endothelial cell activation, as measured by adhesion molecule (E-selectin, ICAM-1, VCAM-1) expression and chemokine (IL-8, MCP-1, RANTES) production and leukocyte adhesion in vitro. Studies exploring the potential mechanism by which ethanol suppresses endothelial cell activation revealed that ethanol blocked NF- κ B nuclear entry in an I κ B α -dependent manner. These findings support the hypothesis that acute ethanol overexposure may increase the risk of infection and inhibit the host inflammatory response, in part, by blocking endothelial cell activation and subsequent immune cell-endothelial cell interactions required for efficient immune cell recruitment.

Santangelo, S., D. J. Cousins, et al. (2002). "DNA Methylation Changes at Human Th2 Cytokine Genes Coincide with DNase I Hypersensitive Site Formation During CD4⁺ T Cell Differentiation." J. Immunol. **169**(4): 1893-1903.

<http://www.jimmunol.org/cgi/content/abstract/169/4/1893>

The differentiation of naive CD4⁺ T lymphocytes into Th1 and Th2 lineages generates either cellular or humoral immune responses. Th2 cells express the cytokines IL-4, -5, and -13, which are implicated in asthma and atopy. Much has been published about the regulation of murine Th2 cytokine expression, but studies in human primary T cells are less common. We have developed a method for differentiating human CD45RA⁺ (naive) T cells into Th1 and Th2 populations that display distinct cytokine expression profiles. We examined both CpG methylation, using bisulfite DNA modification and sequencing, and chromatin structure around the IL-4 and IL-13 genes before and after human T cell differentiation and in normal human skin fibroblasts. In naive cells, the DNA was predominantly methylated. After Th2 differentiation, DNase I hypersensitive sites (DHS) appeared at IL-4 and IL-13 and CpG demethylation occurred only around the Th2-specific DHS. Both DHS and CpG demethylation coincided with consensus binding sites for the Th2-specific transcription factor GATA-3. Although fibroblasts, like naive and Th1 cells, did not express IL-4 or IL-13, DHS and unmethylated CpG sites that were distinct from the Th2-specific sites were observed, suggesting that chromatin structure in this cluster not only varies in T cells according to IL-4/IL-13 expression but is also tissue specific.

Sasso, E. H., M. Martinez, et al. (2004). "Frequent Joining of Bcl-2 to a JH6 Gene in Hepatitis C Virus-Associated t(14;18)." J. Immunol. **173**(5): 3549-3556.

<http://www.jimmunol.org/cgi/content/abstract/173/5/3549>

The t(14;18) chromosomal translocation, which joins the Bcl-2 proto-oncogene to an Ig JH gene, has increased prevalence in patients chronically infected with hepatitis C virus (HCV). We now establish a link between the molecular structure and clinical occurrence of HCV-associated t(14;18). A t(14;18) was detected by PCR in leukocytes from 22 of 46 HCV-infected patients

(48%) and 11 of 54 healthy controls (20%) ($p = 0.0053$). Nucleotide sequence analysis of the Bcl-2/JH joins found a JH6 gene in 18 of 22 (82%) t(14;18) from HCV+ patients, and 3 of 8 (38%) from controls ($p = 0.031$). The t(14;18) rarely contained JH gene mutations, or an intervening region sequence suggestive of D gene rearrangement or templated nucleotide insertion. Analysis of published t(14;18) nucleotide sequences established that the JH6 prevalence in t(14;18) from normal/nonneoplastic controls (48%) was significantly lower than in t(14;18) from our HCV+ patients ($p = 0.004$) or from non-Hodgkin's lymphomas (66%, $p = 0.003$). We conclude that the increased prevalence of t(14;18) in HCV+ patients occurs with a strong bias for Bcl-2/JH6 joins. In this regard, HCV-associated t(14;18) more closely resemble t(14;18) in lymphomas than t(14;18) from normal subjects.

Seiffert, K., J. Hosoi, et al. (2002). "Catecholamines Inhibit the Antigen-Presenting Capability of Epidermal Langerhans Cells." *J. Immunol.* **168**(12): 6128-6135.

<http://www.jimmunol.org/cgi/content/abstract/168/12/6128>

The sympathetic nervous system modulates immune function at a number of levels. Within the epidermis, APCs (Langerhans cells (LC)) are frequently anatomically associated with peripheral nerves. Furthermore, some neuropeptides have been shown to regulate LC Ag-presenting function. We explored the expression of adrenergic receptors (AR) in murine LC and assessed their functional role on Ag presentation and modulation of cutaneous immune responses. Both purified LC and the LC-like cell lines XS52-4D and XS106 expressed mRNA for the ARs {alpha}1A and {beta}2. XS106 cells and purified LC also expressed {beta}1-AR mRNA. Treatment of murine epidermal cell preparations with epinephrine (EPI) or norepinephrine inhibited Ag presentation in vitro. Furthermore, pretreatment of epidermal cells with EPI or norepinephrine in vitro suppressed the ability of these cells to present Ag for elicitation of delayed-type hypersensitivity in previously immunized mice. This effect was blocked by use of the {beta}2-adrenergic antagonist ICI 118,551 but not by the {alpha}-antagonist phentolamine. Local intradermal injection of EPI inhibited the induction of contact hypersensitivity to epicutaneously administered haptens. Surprisingly, injection of EPI at a distant site also suppressed induction of contact hypersensitivity. Thus, catecholamines may have both local and systemic effects. We conclude that specific ARs are expressed on LC and that signaling through these receptors can decrease epidermal immune reactions.

Shiina, T., S. Shimizu, et al. (2004). "Comparative Genomic Analysis of Two Avian (Quail and Chicken) MHC Regions." *J. Immunol.* **172**(11): 6751-6763.

<http://www.jimmunol.org/cgi/content/abstract/172/11/6751>

We mapped two different quail Mhc haplotypes and sequenced one of them (haplotype A) for comparative genomic analysis with a previously sequenced haplotype of the chicken Mhc. The quail haplotype A spans 180 kb of genomic sequence, encoding a total of 41 genes compared with only 19 genes within the 92-kb chicken Mhc. Except for two gene families (B30 and tRNA), both species have the same basic set of gene family members that were previously described in the chicken "minimal essential" Mhc. The two Mhc regions have a similar overall organization but differ markedly in that the quail has an expanded number of duplicated genes with 7 class I, 10 class IIB, 4 NK, 6 lectin, and 8 B-G genes. Comparisons between the quail and chicken Mhc class I and class II gene sequences by phylogenetic analysis showed that they were more closely related within species than between species, suggesting that the quail Mhc genes were duplicated after the separation of these two species from their common ancestor. The proteins encoded by the NK and class I genes are known to interact as ligands and receptors, but unlike in the quail and the chicken, the genes encoding these proteins in mammals are found on different

chromosomes. The finding of NK-like genes in the quail Mhc strongly suggests an evolutionary connection between the NK C-type lectin-like superfamily and the Mhc, providing support for future studies on the NK, lectin, class I, and class II interaction in birds.

Shilling, H. G., L. A. Guethlein, et al. (2002). "Allelic Polymorphism Synergizes with Variable Gene Content to Individualize Human KIR Genotype." J. Immunol. **168**(5): 2307-2315.

<http://www.jimmunol.org/cgi/content/abstract/168/5/2307>

Killer Ig-like receptor (KIR) genes are a multigene family on human chromosome 19. KIR genes occur in various combinations on different haplotypes. Additionally, KIR genes are polymorphic. To examine how allelic polymorphism diversifies KIR haplotypes with similar or identical combinations of KIR genes, we devised methods for discriminating alleles of KIR2DL1, -2DL3, -3DL1, and -3DL2. These methods were applied to 143 individuals from 34 families to define 98 independent KIR haplotypes at the allele level. Three novel 3DL2 alleles and a chimeric 3DL1/3DL2 sequence were also identified. Among the A group haplotypes were 22 different combinations of 2DL1, 2DL3, 3DL1, and 3DL2 alleles. Among the B group haplotypes that were unambiguously determined were 15 distinct haplotypes involving 9 different combinations of KIR genes. A and B haplotypes both exhibit strong linkage disequilibrium (LD) between 2DL1 and 2DL3 alleles, and between 3DL1 and 3DL2 alleles. In contrast, there was little LD between the 2DL1/2DL3 and 3DL1/3DL2 pairs that define the two halves of the KIR gene complex. The synergistic combination of allelic polymorphism and variable gene content individualize KIR genotype to an extent where unrelated individuals almost always have different KIR types. This level of diversity likely reflects strong pressure from pathogens on the human NK cell response.

Shimoda, K., K. Kamesaki, et al. (2002). "Cutting Edge: Tyk2 Is Required for the Induction and Nuclear Translocation of Daxx Which Regulates IFN- α -Induced Suppression of B Lymphocyte Formation." J. Immunol. **169**(9): 4707-4711.

<http://www.jimmunol.org/cgi/content/abstract/169/9/4707>

IFN- α inhibits B lymphocyte development, and the nuclear protein Daxx has been reported to be essential for this biological activity. We show in this study that IFN- α inhibits the clonal proliferation of B lymphocyte progenitors in response to IL-7 in wild-type, but not in tyk2-deficient, mice. In addition, the IFN- α -induced up-regulation and nuclear translocation of Daxx are completely abrogated in the absence of tyk2. Therefore, tyk2 is directly involved in IFN- α signaling for the induction and translocation of Daxx, which may result in B lymphocyte growth arrest and/or apoptosis.

Shin, J.-S., J. Yu, et al. (2002). "Peptide Mimotopes of Pneumococcal Capsular Polysaccharide of 6B Serotype: A Peptide Mimotope Can Bind to Two Unrelated Antibodies." J. Immunol. **168**(12): 6273-6278.

<http://www.jimmunol.org/cgi/content/abstract/168/12/6273>

Two groups of bacteriophage clones displaying the antigenic properties of serotype 6B pneumococcal capsular polysaccharide (PS) were obtained from different phage libraries expressing random heptameric peptides. One group, biopanned with a mouse mAb (Hyp6BM1), is comprised of 17 phage clones expressing 10 unique sequences of linear peptides. The other

group, selected with another mAb (Hyp6BM8), contained six clones, all of which expressed the identical circular peptide. Phage clones expressing the linear peptides (e.g., PhaM1L3) bound only to Hyp6BM1, but not other 6B PS-specific mAb, and their binding could be inhibited with pneumococcal capsular type 6B PS only. In contrast, a phage clone expressing the circular peptide (PhaM8C1) cross-reacted with several other 6B PS-specific mAbs, and their binding could be inhibited with pneumococcal capsular PS of 6A and 6B serotypes. Two short peptides, PepM1L3 and PepM8C1, reflecting the peptide inserts of the corresponding phage clones, could inhibit the binding of the two clones to their respective mAb. Interestingly, the peptide insert in PhaM8C1 was identical to that in PhaB3C4, a previously reported mimotope of $\alpha(2\rightarrow 8)$ polysialic acid, *Neisseria meningitidis* group B PS. Indeed, PhaM8C1 bound to HmenB3 (a meningococcal Ab), and their association could be inhibited with $\alpha(2\rightarrow 8)$ polysialic acid, but not with 6B PS. Conversely, $\alpha(2\rightarrow 8)$ polysialic acid could not inhibit the binding of PhaM8C1 to Hyp6BM8. The two-dimensional nuclear magnetic resonance studies indicate that PepM8C1 peptide can assume several conformations in solution. The ability of this peptide to assume multiple conformations might account for its ability to mimic more than one Ag type.

Shoham, T., R. Rajapaksa, et al. (2003). "The Tetraspanin CD81 Regulates the Expression of CD19 During B Cell Development in a Postendoplasmic Reticulum Compartment." *J. Immunol.* **171**(8): 4062-4072.

<http://www.jimmunol.org/cgi/content/abstract/171/8/4062>

CD81 is a widely expressed tetraspanin that associates in B cells with CD19 in the CD19-CD21-CD81 signaling complex. CD81 is necessary for normal CD19 expression; *cd81*^{-/-} B cells express lower levels of CD19, especially *cd81*^{-/-} small pre-BII cells, which are almost devoid of surface CD19. The dependence of CD19 expression on CD81 is specific to this particular tetraspanin since *cd9*^{-/-} B cells express normal levels of CD19. Furthermore, expression of human CD81 in mouse *cd81*^{-/-} B cells restored surface CD19 to normal levels. Quantitative analysis of CD19 mRNA demonstrated normal levels, even in *cd81*^{-/-} pre-BII cells. Analysis of CD19 at the protein level identified two CD19 glycoforms in both wild-type and *cd81*^{-/-} B cells. The higher Mr glycoform is significantly reduced in *cd81*^{-/-} B cells and is endoglycosidase H (endo-H) resistant. In contrast, the low Mr glycoform is comparably expressed in *cd81*^{-/-} and in wild-type B cells and is endo-H sensitive. Because endo-H sensitivity is tightly correlated with endoplasmic reticulum localization, we suggest that the dependency of CD19 expression on CD81 occurs in a postendoplasmic reticulum compartment where CD81 is necessary for normal trafficking or for surface membrane stability of CD19.

Shum, B. P., L. R. Flodin, et al. (2002). "Conservation and Variation in Human and Common Chimpanzee CD94 and NKG2 Genes." *J. Immunol.* **168**(1): 240-252.

<http://www.jimmunol.org/cgi/content/abstract/168/1/240>

To assess polymorphism and variation in human and chimpanzee NK complex genes, we determined the coding-region sequences for CD94 and NKG2A, C, D, E, and F from several human (*Homo sapiens*) donors and common chimpanzees (*Pan troglodytes*). CD94 is highly conserved, while the NKG2 genes exhibit some polymorphism. For all the genes, alternative mRNA splicing variants were frequent among the clones obtained by RT-PCR. Alternative splicing acts similarly in human and chimpanzee to produce the CD94B variant from the CD94 gene and the NKG2B variant from the NKG2A gene. Whereas single chimpanzee orthologs for CD94, NKG2A, NKG2E, and NKG2F were identified, two chimpanzee paralogs of the human NKG2C gene were defined. The chimpanzee Pt-NKG2CI gene encodes a protein similar to human NKG2C, whereas in the chimpanzee Pt-NKG2CII gene the translation frame changes

near the beginning of the carbohydrate recognition domain, causing premature termination. Analysis of a panel of chimpanzee NK cell clones showed that Pt-NKG2CI and Pt-NKG2CII are independently and clonally expressed. Pt-NKG2CI and Pt-NKG2CII are equally diverged from human NKG2C, indicating that they arose by gene duplication subsequent to the divergence of chimpanzee and human ancestors. Genomic DNA from 80 individuals representing six primate species were typed for the presence of CD94 and NKG2. Each species gave distinctive typing patterns, with NKG2A and CD94 being most conserved. Seven different NK complex genotypes within the panel of 48 common chimpanzees were due to differences in Pt-NKG2C and Pt-NKG2D genes.

Stehlik, C., S. H. Lee, et al. (2003). "Apoptosis-Associated Speck-Like Protein Containing a Caspase Recruitment Domain Is a Regulator of Procaspace-1 Activation." J. Immunol. **171**(11): 6154-6163.

<http://www.jimmunol.org/cgi/content/abstract/171/11/6154>

Apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC)/target of methylation-induced silencing/PYCARD represents one of only two proteins encoded in the human genome that contains a caspase recruitment domain (CARD) together with a pyrin, AIM, ASC, and death domain-like (PAAD)/PYRIN/DAPIN domain. CARDS regulate caspase family proteases. We show here that ASC binds by its CARD to procaspase-1 and to adapter proteins involved in caspase-1 activation, thereby regulating cytokine pro-IL-1{beta} activation by this protease in THP-1 monocytes. ASC enhances IL-1{beta} secretion into the cell culture supernatants, at low concentrations, while suppressing at high concentrations. When expressed in HEK293 cells, ASC interferes with Cardiak/Rip2/Rick-mediated oligomerization of procaspase-1 and suppresses activation this protease, as measured by protease activity assays. Moreover, ASC also recruits procaspase-1 into ASC-formed cytosolic specks, separating it from Cardiak. We also show that expression of the PAAD/PYRIN family proteins pyrin or cryopyrin/PYPAF1/NALP3 individually inhibits IL-1{beta} secretion but that coexpression of ASC with these proteins results in enhanced IL-1{beta} secretion. However, expression of ASC uniformly interferes with caspase-1 activation and IL-1{beta} secretion induced by proinflammatory stimuli such as LPS and TNF, suggesting pathway competition. Moreover, LPS and TNF induce increases in ASC mRNA and protein expression in cells of myeloid/monocytic origin, revealing another level of cross-talk of cytokine-signaling pathways with the ASC-controlled pathway. Thus, our results suggest a complex interplay of the bipartite adapter protein ASC with PAAD/PYRIN family proteins, LPS (Toll family receptors), and TNF in the regulation of procaspase-1 activation, cytokine production, and control of inflammatory responses.

Stievano, L., V. Tosello, et al. (2003). "CD8+{alpha}{beta}+ T Cells That Lack Surface CD5 Antigen Expression Are a Major Lymphotactin (XCL1) Source in Peripheral Blood Lymphocytes." J. Immunol. **171**(9): 4528-4538.

<http://www.jimmunol.org/cgi/content/abstract/171/9/4528>

To better characterize the cellular source of lymphotactin (XCL1), we compared XCL1 expression in different lymphocyte subsets by real-time PCR. XCL1 was constitutively expressed in both PBMC and CD4+ cells, but its expression was almost 2 log higher in CD8+ cells. In vitro activation was associated with a substantial increase in XCL1 expression in both PBMC and CD8+ cells, but not in CD4+ lymphocytes. The preferential expression of XCL1 in CD8+ cells was confirmed by measuring XCL1 production in culture supernatants, and a good correlation was found between figures obtained by real-time PCR and XCL1 contents. XCL1 expression was mostly confined to a CD3+CD8+ subset not expressing CD5, where XCL1 expression equaled

that shown by $\gamma\delta$ + T cells. Compared with the CD5+ counterpart, CD3+CD8+CD5- cells, which did not express CD5 following in vitro activation, showed preferential expression of the $\alpha\alpha$ form of CD8 and a lower expression of molecules associated with a noncommitted/naive phenotype, such as CD62L. CD3+CD8+CD5- cells also expressed higher levels of the XCL1 receptor; in addition, although not differing from CD3+CD8+CD5+ cells in terms of the expression of most α - and β -chemokines, they showed higher expression of CCL3/macrophage inflammatory protein-1 α . These data show that TCR $\alpha\beta$ -expressing lymphocytes that lack CD5 expression are a major XCL1 source, and that the contribution to its synthesis by different TCR $\alpha\beta$ -expressing T cell subsets, namely CD4+ lymphocytes, is negligible. In addition, they point to the CD3+CD8+CD5- population as a particular T cell subset within the CD8+ compartment, whose functional properties deserve further attention.

Sugita, S., T. F. Ng, et al. (2004). "CTLA-4+CD8+ T Cells That Encounter B7-2+ Iris Pigment Epithelial Cells Express Their Own B7-2 to Achieve Global Suppression of T Cell Activation." J. Immunol. **172**(7): 4184-4194.

<http://www.jimmunol.org/cgi/content/abstract/172/7/4184>

Pigment epithelial (PE) cells cultured from the eye possess the novel property of suppressing TCR-dependent activation of T cells in vitro. Iris PE (IPE) cells accomplish this suppression by a direct cell contact mechanism in which B7-2 expressed by the PE cells interacts with CTLA-4 on responding T cells. Because CTLA-4 expression is constitutively expressed on a very small proportion of naive splenic T cells and since exposure of splenic T cells to IPE leads to global T cell suppression, we have inquired into the mechanism by which suppression is achieved. Using splenic T cells and IPE from donor mice with disrupted genes for CD80 (B7-1), CD86 (B7-2), CTLA-4, and/or CD28, we report that B7-2+ IPE in the presence of anti-CD3 supported selectively the activation of CTLA-4+ CD8+ T cells that express their own B7-2 and secrete enhanced amounts of active TGF β . By contrast, activation of CTLA-4-negative T cells, especially CD4+ cells, in these cultures was profoundly suppressed. Because global suppression of T cell activation in these cultures was obtained only when both IPE and T cells possessed B7-2 genes and expressed the costimulators as surface molecules, we propose that T cells activated in the presence of parenchymal cells from the eye (an immune privileged site) express B7-2 in a manner that equips them to suppress bystander T cells. Thus, B7-2 expression on T cells participates in their eventual ability to function as regulators in vitro.

Swanson, P. J., S. L. Kuskal, et al. (2004). "Fatal Acute Lymphoblastic Leukemia in Mice Transgenic for B Cell-Restricted bcl-xL and c-myc." J. Immunol. **172**(11): 6684-6691.

<http://www.jimmunol.org/cgi/content/abstract/172/11/6684>

Expression of the c-myc gene is frequently dysregulated in malignant tumors and translocations of c-myc into the Ig H chain locus are associated with Burkitt's-type lymphoma. There is indirect evidence that bcl-x, an anti-apoptotic member of the bcl-2 gene family, may also contribute to a variety of B lymphoid tumors. In this study, we show that mice transgenic for both B cell-restricted c-myc and bcl-xL developed aggressive, acute leukemias expressing early B lineage and stem cell surface markers. Of interest, the tumor cells proliferated and differentiated down the B cell developmental pathway following in vitro treatment with IL-7. Analysis of sorted leukemic cells from spleen indicated constitutive expression of sterile μ and κ transcripts in combination with evidence for D-JH DNA rearrangements. Several B cell-specific genes were either not expressed or were expressed at low levels in primary tumor cells and were induced following culture with IL-7. IL-7 also increased V-J κ and V-DJH rearrangements. These

data demonstrate oncogenic synergy between c-myc and bcl-xL in a new mouse model for acute lymphoblastic leukemia. Tumors in these animals target an early stage in B cell development characterized by the expression of both B lineage and stem cell genes.

Tagawa, T., H. Nishimura, et al. (2004). "V δ 1+ $\gamma\delta$ T Cells Producing CC Chemokines May Bridge a Gap between Neutrophils and Macrophages in Innate Immunity during Escherichia coli Infection in Mice." *J. Immunol.* **173**(8): 5156-5164.

<http://www.jimmunol.org/cgi/content/abstract/173/8/5156>

An influx of neutrophils followed a short time later by an influx of macrophages to the infected site plays a key role in innate immunity against Escherichia coli infection. We found in this study that V δ 1^{-/-} mice exhibited impaired accumulation of peritoneal macrophages but not neutrophils and delayed bacterial clearance after i.p. inoculation with E. coli. Peritoneal $\gamma\delta$ T cells from E. coli-infected wild-type mice produced CCL3/MIP-1 α and CCL5/RANTES in response to $\gamma\delta$ TCR triggering in vitro, whereas such production was not evident in $\gamma\delta$ T cells from E. coli-infected V δ 1^{-/-} mice. Neutralization of CCL3/MIP-1 α by a specific mAb in vivo significantly inhibited the accumulation of macrophages in the peritoneal cavity after E. coli infection, resulting in exacerbated bacterial growth in the peritoneal cavity. These results suggest that V δ 1+ $\gamma\delta$ T cells bridge a gap between neutrophils and macrophages in innate immunity during E. coli infection mediated by production of CC chemokines, enhancing macrophage trafficking to the site of infection.

Tao, D., L. Shangwu, et al. (2005). "CD226 Expression Deficiency Causes High Sensitivity to Apoptosis in NK T Cells from Patients with Systemic Lupus Erythematosus." *J. Immunol.* **174**(3): 1281-1290.

<http://www.jimmunol.org/cgi/content/abstract/174/3/1281>

Humans and mice with systemic lupus erythematosus (SLE) and related autoimmune diseases have reduced numbers of NK T cells. An association between NK T cell deficiency and autoimmune disease has been identified. However, the mechanisms for reduction of NK T cell number in patients with SLE are unknown. In the present study we report that NK T cells from active SLE patients are highly sensitive to anti-CD95-induced apoptosis compared with those from normal subjects and inactive SLE patients. CD226 expression is deficient on NK T cells from active SLE patients. The expression of one antiapoptotic member protein, survivin, is found to be selectively deficient in freshly isolated NK T cells from active SLE patients. CD226 preactivation significantly up-regulates survivin expression and activation, which can rescue active SLE NK T cells from anti-CD95-induced apoptosis. In transfected COS7 cells, we confirm that anti-CD95-mediated death signals are inhibited by activation of the CD226 pathway through stabilization of caspase-8 and caspase-3 and through activation of survivin. We therefore conclude that deficient expression of CD226 and survivin in NK T cells from active SLE is a molecular base of high sensitivity of the cells to anti-CD95-induced apoptosis. These observations offer a potential explanation for high apoptotic sensitivity of NK T cells from active SLE, and provide a new insight into the mechanism of reduction of NK T cell number in SLE and understanding the association between NK T cell deficiency and autoimmune diseases.

Teusch, N., E. Lombardo, et al. (2004). "The Low Molecular Weight GTPase RhoA and Atypical Protein Kinase C ζ Are Required for TLR2-Mediated Gene Transcription." *J. Immunol.* **173**(1): 507-514.

<http://www.jimmunol.org/cgi/content/abstract/173/1/507>

The Rho GTPases are molecular switches that regulate many essential cellular processes, including actin dynamics, gene transcription, cell cycle progression, cell adhesion, and motility. In this study, we report that stimulation of TLR2 in human epithelial and monocytic cells leads to rapid and transient activation of RhoA. RhoA cooperated with the canonical I- κ B kinase-mediated pathway that induces the release of NF- κ B, in regulating the trans activation of the NF- κ B subunit p65/RelA by affecting Ser311 phosphorylation, and subsequent cytokine production. Another consequence of TLR2 stimulation by bacterial derived products was the activation of atypical protein kinase C (PKC) ζ and association of this protein kinase with RhoA. Inhibition of PKC ζ decreased NF- κ B activation and p65/RelA trans activation without affecting I- κ B α degradation. The observation of a transient, stimulus-dependent association of RhoA with PKC ζ suggests that RhoA mediates at least partially its effect on gene transcription through atypical PKC. In contrast to previous studies, identifying Rac1-PI3K as an upstream element in TLR2-initiated response to NF- κ B, PI3K signaling was not required for RhoA or PKC ζ activity. These results indicate that multiple GTPase-regulated pathways emerge from stimulated Toll receptors, controlling different aspects of NF- κ B-mediated gene transcription.

Thorn, C. F. and A. S. Whitehead (2002). "Differential Glucocorticoid Enhancement of the Cytokine-Driven Transcriptional Activation of the Human Acute Phase Serum Amyloid A Genes, SAA1 and SAA2." *J. Immunol.* **169**(1): 399-406.

<http://www.jimmunol.org/cgi/content/abstract/169/1/399>

The human acute phase serum amyloid A (A-SAA) genes, SAA1 and SAA2, have a high degree of sequence identity that extends $\sim$ 450 bp upstream of their transcription start sites. Each promoter contains analogously positioned functional binding sites for the transcription factors NF- κ B and NF-IL6. In human HepG2 hepatoma cells transfected with SAA promoter luciferase reporter constructs, administration of IL-1 and IL-6, singly or in combination, induced SAA1 and SAA2 transcriptional readouts that were qualitatively indistinguishable. However, under induced conditions, the SAA2 promoter had a significant quantitative transcriptional advantage over the SAA1 promoter. The application of the synthetic glucocorticoid dexamethasone in the context of cytokine stimulation enhanced the transcriptional activity of the SAA1, but not the SAA2, promoter such that readout from the former became equivalent to that from the latter. A putative glucocorticoid response element (GRE) is present (between residues -208 and -194) only in the SAA1 gene; a similar sequence in the corresponding region of the SAA2 gene is disrupted by a nine-residue insertion. The SAA1 GRE was shown to be functionally active and the SAA2 disrupted GRE was shown to be functionally inactive in experiments using reporter constructs carrying SAA1 and SAA2 promoters that had been modified by site-specific mutagenesis. Quantitative analysis of transcript-specific RT-PCR products, derived from SAA1 and SAA2 mRNAs after treatment of HepG2 cells with cytokines in the presence or absence of dexamethasone, confirmed that the endogenous SAA1 gene has a cytokine-driven transcriptional disadvantage that is superseded by a marginal transcriptional advantage when glucocorticoids are present.

Thornton, M. V., D. Kudo, et al. (2004). "Degradation of NF- κ B in T Cells by Gangliosides Expressed on Renal Cell Carcinomas." *J. Immunol.* **172**(6): 3480-3490.

<http://www.jimmunol.org/cgi/content/abstract/172/6/3480>

T cells from cancer patients are often functionally impaired, which imposes a barrier to effective immunotherapy. Most pronounced are the alterations characterizing tumor-infiltrating T cells, which in renal cell carcinomas includes defective NF- κ B activation and a heightened sensitivity to apoptosis. Coculture experiments revealed that renal tumor cell lines induced a time-dependent decrease in RelA(p65) and p50 protein levels within both Jurkat T cells and peripheral blood T lymphocytes that coincided with the onset of apoptosis. The degradation of RelA/p50 is critical for SK-RC-45-induced apoptosis because overexpression of RelA in Jurkat cells protects against cell death. The loss of RelA/p50 coincided with a decrease in expression of the NF- κ B regulated antiapoptotic protein Bcl-xL at both the protein and mRNA level. The disappearance of RelA/p50 protein was mediated by a caspase-dependent pathway because pretreatment of T lymphocytes with a pan caspase inhibitor before coculture with SK-RC-45 blocked RelA and p50 degradation. SK-RC-45 gangliosides appear to mediate this degradative pathway, as blocking ganglioside synthesis in SK-RC-45 cells with the glucosylceramide synthase inhibitor, PPPP, protected T cells from tumor cell-induced RelA degradation and apoptosis. The ability of the Bcl-2 transgene to protect Jurkat cells from RelA degradation, caspase activation, and apoptosis implicates the mitochondria in these SK-RC-45 ganglioside-mediated effects.

Torikai, H., Y. Akatsuka, et al. (2004). "A Novel HLA-A*3303-Restricted Minor Histocompatibility Antigen Encoded by an Unconventional Open Reading Frame of Human TMSB4Y Gene." J. Immunol. **173**(11): 7046-7054.

<http://www.jimmunol.org/cgi/content/abstract/173/11/7046>

Female-to-male hemopoietic stem cell transplantation (HSCT) elicits T cell responses against male-specific minor histocompatibility (H-Y) Ags encoded by the Y chromosome. All previously identified H-Y Ags are encoded by conventional open reading frames, but we report in this study the identification of a novel H-Y Ag encoded in the 5'-untranslated region of the TMSB4Y gene. An HLA-A*3303-restricted CD8⁺ CTL clone was isolated from a male patient after an HSCT from his HLA-identical sister. Using a panel of cell lines carrying Y chromosome terminal deletions, a narrow region controlling the susceptibility of these target cells to CTL recognition was localized. Minigene transfection and epitope reconstitution assays identified an 11-mer peptide, EVLLRPGLHFR, designated TMSB4Y/A33, whose first amino acid was located 405 bp upstream of the TMSB4Y initiation codon. Analysis of the precursor frequency of CTL specific for recipient minor histocompatibility Ags in post-HSCT peripheral blood T cells revealed that a significant fraction of the total donor CTL response in this patient was directed against the TMSB4Y epitope. Tetramer analysis continued to detect TMSB4Y/A33-specific CD8⁺ T cells at least up to 700 days post-HSCT. This finding underscores the in vivo immunological relevance of minor histocompatibility Ags derived from unconventional open reading frame products.

Tsuchiya, T., S. Fukuda, et al. (2003). "Role of $\gamma\delta$ T Cells in the Inflammatory Response of Experimental Colitis Mice." J. Immunol. **171**(10): 5507-5513.

<http://www.jimmunol.org/cgi/content/abstract/171/10/5507>

We examined the severity of experimental colitis induced by dextran sulfate sodium (DSS) using immunologically manipulated mice. C57BL/6 mice showed more severe colitis than BALB/c mice, but mice of both strains recovered fully from the disease after the removal of DSS from their drinking water. The infiltrated cells at the lesions were mainly granulocytes in normal littermates. However, C.B-17 scid, IL-7R α deficient, and TCR-C $\beta\delta$ double-deficient mice showed severe colitis and did not recover from the disease even after the removal of DSS. It was found that the infiltrated cells at the lesions in the lethal strains were monocytes. Although both

TCR-C δ ^{-/-} and TCR-C β ^{-/-} mice showed severe colitis phenotypes, infiltration in the former is monocyte-dominant while that in the latter is granulocyte-dominant. Thus the type of cells that infiltrate at the lesions of DSS-induced experimental colitis may be controlled by functional T cell subsets. Immunohistological and RT-PCR analyses of the inflamed colon revealed that the murine homologue of human GRO α released by some cells under the control of $\gamma\delta$ T cells is a possible candidate determining the severity of DSS-induced experimental colitis.

Uemura, K., M. Saka, et al. (2002). "L-MBP Is Expressed in Epithelial Cells of Mouse Small Intestine." J. Immunol. **169**(12): 6945-6950.

<http://www.jimmunol.org/cgi/content/abstract/169/12/6945>

The mannan-binding proteins (L-MBP and S-MBP, also denoted MBL-C and MBL-A), mainly produced in liver and existing in liver and serum, play important roles in the innate immunity against a variety of pathogens. Total RNA from mouse tissues were screened for MBP mRNA by RT-PCR. In addition to liver, S-MBP mRNA was detected in lung, kidney, and testis, and L-MBP mRNA was detected in kidney, thymus, and small intestine. Quantitative RT-PCR revealed that the small intestine is a predominant site of extrahepatic expression of L-MBP. Western blotting with polyclonal Abs against rat L-MBP demonstrated this protein in Triton X-100 extracts of the small intestine obtained from mice that had undergone systemic perfusion. Immunohistochemical staining with an mAb against mouse L-MBP and in situ hybridization revealed that L-MBP is selectively expressed in some villous epithelial cells of the small intestine. These findings suggest that L-MBP plays a role in mucosal innate immunity.

Ueta, M., T. Nochi, et al. (2004). "Intracellularly Expressed TLR2s and TLR4s Contribution to an Immunosilent Environment at the Ocular Mucosal Epithelium." J. Immunol. **173**(5): 3337-3347.

<http://www.jimmunol.org/cgi/content/abstract/173/5/3337>

Epithelial cells are key players in the first line of defense offered by the mucosal immune system against invading pathogens. In the present study we sought to determine whether human corneal epithelial cells expressing Toll-like receptors (TLRs) function as pattern-recognition receptors in the innate immune system and, if so, whether these TLRs act as a first line of defense in ocular mucosal immunity. Incubation of human primary corneal epithelial cells and the human corneal epithelial cell line (HCE-T) with peptidoglycan or LPS did not lead to activation, at the level of DNA transcription, of NF- κ B or the secretion of inflammation-associated molecules such as IL-6, IL-8, and human β -defensin-2. However, when incubated with IL-1 α to activate NF- κ B, the production by these cells of such inflammatory mediators was enhanced. Human corneal epithelial cells were observed to express both TLR2- and TLR4-specific mRNA as well as their corresponding proteins intracellularly, but not at the cell surface. However, even when LPS was artificially introduced into the cytoplasm, it did not lead to the activation of epithelial cells. Taken together, our results demonstrate that the intracellular expression of TLR2 and TLR4 in human corneal epithelial cells fails to elicit innate immune responses and therefore, perhaps purposely, contributes to an immunosilent environment at the ocular mucosal epithelium.

van der Stoep, N., E. Quinten, et al. (2002). "Transcriptional Regulation of the MHC Class II Trans-Activator (CIITA) Promoter III: Identification of a Novel Regulatory Region in the 5'-Untranslated Region and an Important Role for cAMP-Responsive Element Binding Protein 1 and Activating

Transcription Factor-1 in CIITA-Promoter III Transcriptional Activation in B Lymphocytes." J. Immunol. **169**(9): 5061-5071.

<http://www.jimmunol.org/cgi/content/abstract/169/9/5061>

The class II trans-activator (CIITA), which acts as a master regulator for expression of MHC class II genes, is expressed constitutively in mature B cells. This constitutive expression of CIITA is driven by CIITA promoter III (CIITA-PIII). However, little is known about the factors that control the B cell-mediated trans-activation of CIITA-PIII. In this study using B cells we have identified several cAMP-responsive elements (CREs) in the proximal promoter and in the 5'-untranslated region (5'-UTR) that are involved in the activation of CIITA-PIII. We show that activating transcription factor (ATF)/CRE binding protein (CREB) factors bind to the CREs in vitro and in vivo. Notably, our results also reveal that the 5'-UTR of CIITA-PIII functions as an important regulatory region in B lymphocytes. Furthermore, transient cotransfections of a CIITA-PIII luciferase reporter construct with either CREB-1 or ATF-1 boost CIITA-PIII trans-activation in a dose-dependent manner, which was further enhanced by addition of general coactivator CREB-binding protein. Transient transfections using mutant CIITA-PIII luciferase reporter constructs that either lack the (5'-UTR) or abolish binding of CREB-1 and ATF-1 to the CRE located in activation response element-2, displayed severely reduced promoter activity in B cells. A similar successive deletion of the CREs resulted in a subsequent reduction of CREB-1-induced activity of CIITA-PIII in B cells. Together our results argue for an important role of ATF/CREB factors and the 5'-UTR of CIITA-PIII in the trans-activation of CIITA-PIII in B cells.

Veenstra, K. G., Z. L. Jonak, et al. (2002). "IL-12 Induces Monocyte IL-18 Binding Protein Expression Via IFN- γ ." J. Immunol. **168**(5): 2282-2287.

<http://www.jimmunol.org/cgi/content/abstract/168/5/2282>

IL-18 is a Th1 cytokine that synergizes with IL-12 and IL-2 in the stimulation of lymphocyte IFN- γ production. IL-18 binding protein (IL-18BP) is a recently discovered inhibitor of IL-18 that is distinct from the IL-1 and IL-18 receptor families. In this report we show that IL-18BP α , the IL-18BP isoform with the highest affinity for IL-18, was strongly induced by IL-12 in human PBMC. Other Th1 cytokines, including IFN- γ , IL-2, IL-15, and IL-18, were also capable of augmenting IL-18BP α expression. In contrast, IL-1 α , IL-1 β , TNF- α , IFN- γ -inducible protein-10, and Th2 cytokines such as IL-4 and IL-10 did not induce IL-18BP α . Although monocytes were found to be the primary source of IL-18BP α , the induction of IL-18BP α by IL-12 was mediated through IFN- γ derived predominantly from NK cells. IL-18BP α production was observed in cancer patients receiving recombinant human IL-12 and correlated with the magnitude of IFN- γ production. The IFN- γ /IL-18BP α negative feedback loop identified in this study may be capable of broadly controlling immune activation by cytokines that synergize with IL-18 to induce IFN- γ and probably plays a key role in the modulation of both innate and adaptive immunity.

Voisine, C., F.-X. Hubert, et al. (2002). "Two Phenotypically Distinct Subsets of Spleen Dendritic Cells in Rats Exhibit Different Cytokine Production and T Cell Stimulatory Activity." J. Immunol. **169**(5): 2284-2291.

<http://www.jimmunol.org/cgi/content/abstract/169/5/2284>

We recently reported that splenic dendritic cells (DC) in rats can be separated into CD4⁺ and CD4⁻ subsets and that the CD4⁻ subset exhibited a natural cytotoxic activity in vitro against tumor

cells. Moreover, a recent report suggests that CD4⁻ DC could have tolerogenic properties in vivo. In this study, we have analyzed the phenotype and in vitro T cell stimulatory activity of freshly isolated splenic DC subsets. Unlike the CD4⁻ subset, CD4⁺ splenic DC expressed CD5, CD90, and signal regulatory protein {alpha} molecules. Both fresh CD4⁻ and CD4⁺ DC displayed an immature phenotype, although CD4⁺ cells constitutively expressed moderate levels of CD80. The half-life of the CD4⁻, but not CD4⁺ DC in vitro was extremely short but cells could be rescued from death by CD40 ligand, IL-3, or GM-CSF. The CD4⁻ DC produced large amounts of the proinflammatory cytokines IL-12 and TNF- α and induced Th1 responses in allogeneic CD4⁺ T cells, whereas the CD4⁺ DC produced low amounts of IL-12 and no TNF- α , but induced Th1 and Th2 responses. As compared with the CD4⁺ DC that strongly stimulated the proliferation of purified CD8⁺ T cells, the CD4⁻ DC exhibited a poor CD8⁺ T cell stimulatory capacity that was substantially increased by CD40 stimulation. Therefore, as previously shown in mice and humans, we have identified the existence of a high IL-12-producing DC subset in the rat that induces Th1 responses. The fact that both the CD4⁺ and CD4⁻ DC subsets produced low amounts of IFN- α upon viral infection suggests that they are not related to plasmacytoid DC.

Waetzig, G. H., D. Seegert, et al. (2002). "p38 Mitogen-Activated Protein Kinase Is Activated and Linked to TNF- α Signaling in Inflammatory Bowel Disease." *J. Immunol.* **168**(10): 5342-5351.

<http://www.jimmunol.org/cgi/content/abstract/168/10/5342>

Inflammatory bowel diseases (IBD)--Crohn's disease and ulcerative colitis--are relapsing chronic inflammatory disorders which involve genetic, immunological, and environmental factors. The regulation of TNF- α , a key mediator in the inflammatory process in IBD, is interconnected with mitogen-activated protein kinase pathways. The aim of this study was to characterize the activity and expression of the four p38 subtypes (p38{alpha}-{delta}), c-Jun N-terminal kinases (JNKs), and the extracellular signal-regulated kinases (ERK)1/2 in the inflamed intestinal mucosa. Western blot analysis revealed that p38{alpha}, JNKs, and ERK1/2 were significantly activated in IBD, with p38{alpha} showing the most pronounced increase in kinase activity. Protein expression of p38 and JNK was only moderately altered in IBD patients compared with normal controls, whereas ERK1/2 protein was significantly down-regulated. Immunohistochemical analysis of inflamed mucosal biopsies localized the main expression of p38{alpha} to lamina propria macrophages and neutrophils. ELISA screening of the supernatants of Crohn's disease mucosal biopsy cultures showed that incubation with the p38 inhibitor SB 203580 significantly reduced secretion of TNF- α . In vivo inhibition of TNF- α by a single infusion of anti-TNF- α Ab (infliximab) resulted in a highly significant transient increase of p38{alpha} activity during the first 48 h after infusion. A significant infliximab-dependent p38{alpha} activation was also observed in THP-1 myelomonocytic cells. In human monocytes, infliximab enhanced TNF- α gene expression, which could be inhibited by SB 203580. In conclusion, p38{alpha} signaling is involved in the pathophysiology of IBD.

Wang, H.-W., H. P. McNeil, et al. (2002). "{delta} Tryptase Is Expressed in Multiple Human Tissues, and a Recombinant Form Has Proteolytic Activity." *J. Immunol.* **169**(9): 5145-5152.

<http://www.jimmunol.org/cgi/content/abstract/169/9/5145>

Tryptases are neutral serine proteases selectively expressed in mast cells and have been implicated in the development of a number of inflammatory diseases including asthma. It has recently been established that the number of genes encoding human mast cell tryptases is much larger than originally believed, but it is not clear how many of these genes are expressed. A recent report suggested that the transcript for at least one of these genes, originally named

mMCP-7-like tryptase, is not expressed. To further address this question, we screened tissue-specific RNA samples by RT-PCR, using primers designed to match the putative exonic sequence of this gene. We successfully generated and cloned the correctly sized RT-PCR product from mRNA isolated from the human mast cell-I cell line. Two distinct clones were identified whose nucleotide sequence matched the published sequence of the mMCP-7-like I and mMCP-7-like II genes. Transcripts were detected in a wide variety of human tissues including lung, heart, stomach, spleen, skin, and colon. A polyclonal antipeptide Ab that specifically recognizes the translated product of this transcript was used to demonstrate its expression in mast cells that reside in the colon, lung, and inflamed synovium. A recombinant form of this protein expressed in bacterial cells was able to cleave a synthetic trypsin-sensitive substrate, D-Ile-Phe-Lys pNA. These results suggest that the range of functional tryptases is larger than previously recognized. For simplicity, we suggest that the gene, transcripts, and corresponding protein product be named δ tryptase.

Weinstock-Guttman, B., D. Badgett, et al. (2003). "Genomic Effects of IFN- β in Multiple Sclerosis Patients." *J. Immunol.* **171**(5): 2694-2702.

<http://www.jimmunol.org/cgi/content/abstract/171/5/2694>

The purpose of this report was to characterize the dynamics of the gene expression cascades induced by an IFN- β -1a treatment regimen in multiple sclerosis patients and to examine the molecular mechanisms potentially capable of causing heterogeneity in response to therapy. In this open-label pharmacodynamic study design, peripheral blood was obtained from eight relapsing-remitting multiple sclerosis patients just before and at 1, 2, 4, 8, 24, 48, 120, and 168 h after i.m. injection of 30 μ g of IFN- β -1a. The total RNA was isolated from monocyte-depleted PBL and analyzed using cDNA microarrays containing probes for >4000 known genes. IFN- β -1a treatment resulted in selective, time-dependent effects on multiple genes. The mRNAs for genes implicated in the anti-viral response, e.g., double-stranded RNA-dependent protein kinase, myxovirus resistance proteins 1 and 2, and guanylate binding proteins 1 and 2 were rapidly induced within 1-4 h of IFN- β treatment. The mRNAs for several genes involved in IFN- β signaling, such as IFN- α/β receptor-2 and Stat1, were also increased. The mRNAs for lymphocyte activation markers, such as IFN-induced transmembrane protein 1 (9-27), IFN-induced transmembrane protein 2 (1-8D), β 2-microglobulin, and CD69, were also increased in a time-dependent manner. The findings demonstrate that IFN- β treatment induces specific and time-dependent changes in multiple mRNAs in lymphocytes of multiple sclerosis patients that could provide a framework for rapid monitoring of the response to therapy.

Wicker, L. S., G. Chamberlain, et al. (2004). "Fine Mapping, Gene Content, Comparative Sequencing, and Expression Analyses Support Ctla4 and Nramp1 as Candidates for Idd5.1 and Idd5.2 in the Nonobese Diabetic Mouse." *J. Immunol.* **173**(1): 164-173.

<http://www.jimmunol.org/cgi/content/abstract/173/1/164>

At least two loci that determine susceptibility to type 1 diabetes in the NOD mouse have been mapped to chromosome 1, Idd5.1 (insulin-dependent diabetes 5.1) and Idd5.2. In this study, using a series of novel NOD.B10 congenic strains, Idd5.1 has been defined to a 2.1-Mb region containing only four genes, Ctla4, Icos, Als2cr19, and Nrp2 (neuropilin-2), thereby excluding a major candidate gene, Cd28. Genomic sequence comparison of the two functional candidate genes, Ctla4 and Icos, from the B6 (resistant at Idd5.1) and the NOD (susceptible at Idd5.1) strains revealed 62 single nucleotide polymorphisms (SNPs), only two of which were in coding regions. One of these coding SNPs, base 77 of Ctla4 exon 2, is a synonymous SNP and has been correlated previously with type 1 diabetes susceptibility and differential expression of a

CTLA-4 isoform. Additional expression studies in this work support the hypothesis that this SNP in exon 2 is the genetic variation causing the biological effects of Idd5.1. Analysis of additional congenic strains has also localized Idd5.2 to a small region (1.52 Mb) of chromosome 1, but in contrast to the Idd5.1 interval, Idd5.2 contains at least 45 genes. Notably, the Idd5.2 region still includes the functionally polymorphic Nramp1 gene. Future experiments to test the identity of Idd5.1 and Idd5.2 as Ctla4 and Nramp1, respectively, can now be justified using approaches to specifically alter or mimic the candidate causative SNPs.

Workalemahu, G., M. Foerster, et al. (2003). "Human $\gamma\delta$ -T Lymphocytes Express and Synthesize Connective Tissue Growth Factor: Effect of IL-15 and TGF- β 1 and Comparison with $\alpha\beta$ -T Lymphocytes." *J. Immunol.* **170**(1): 153-157.

<http://www.jimmunol.org/cgi/content/abstract/170/1/153>

T lymphocytes bearing the $\gamma\delta$ -TCR accumulate during wound healing and inflammation. However, the role of $\gamma\delta$ -T lymphocytes in fibrogenic tissue reactions is not well understood. Therefore, we addressed the question of whether human $\gamma\delta$ -T cells express and synthesize connective tissue growth factor (CTGF), a factor known to regulate fibrogenesis and wound healing. In addition, the lymphoblastic leukemia T cell line (Loucy) that possesses characteristics typical of $\gamma\delta$ -T cells was used as a model to evaluate the regulation of CTGF gene expression. Blood $\gamma\delta$ -T cells isolated from healthy donors were grown in the presence of IL-15/TGF- β 1 for 48 h and assessed for the expression and synthesis of CTGF. Nonstimulated human blood $\gamma\delta$ -T cells and Loucy $\gamma\delta$ -T cells expressed low levels of CTGF mRNA. Costimulation of the cells with IL-15 and TGF- β 1 resulted in a substantially increased level of CTGF mRNA expression within 4-8 h, and it remained elevated for at least 48 h. In contrast, no CTGF mRNA was detected when nonstimulated and stimulated human CD4+ $\alpha\beta$ -T cells were analyzed. In addition, Western blot analysis of human $\gamma\delta$ -T cell lysates prepared 4 days following stimulation with IL-15 and TGF- β 1 revealed a 38-kDa CTGF protein in cell lysates of human $\gamma\delta$ -T cells. Detection was confirmed using Colo 849 fibroblasts, which can constitutively express high levels of CTGF. In conclusion, we herein present novel evidence that in contrast to CD4+ $\alpha\beta$ -T cells human $\gamma\delta$ -T cells are capable of expressing CTGF mRNA and synthesizing its corresponding protein, which supports the concept that $\gamma\delta$ -T cells may contribute to wound healing or tissue fibrotic processes.

Wright, G. J., H. Cherwinski, et al. (2003). "Characterization of the CD200 Receptor Family in Mice and Humans and Their Interactions with CD200." *J. Immunol.* **171**(6): 3034-3046.

<http://www.jimmunol.org/cgi/content/abstract/171/6/3034>

CD200 (OX2) is a broadly distributed cell surface glycoprotein that interacts with a structurally related receptor (CD200R) expressed on rodent myeloid cells and is involved in regulation of macrophage function. We report the first characterization of human CD200R (hCD200R) and define its binding characteristics to hCD200. We also report the identification of a closely related gene to hCD200R, designated hCD200RLa, and four mouse CD200R-related genes (termed mCD200RLa-d). CD200, CD200R, and CD200R-related genes were closely linked in humans and mice, suggesting that these genes arose by gene duplication. The distributions of the receptor genes were determined by quantitative RT-PCR, and protein expression was confirmed by a set of novel mAbs. The distribution of mouse and human CD200R was similar, with strongest labeling of macrophages and neutrophils, but also other leukocytes, including monocytes, mast cells, and T lymphocytes. Two mCD200 receptor-like family members, designated mCD200RLa and mCD200RLb, were shown to pair with the activatory adaptor

protein, DAP12, suggesting that these receptors would transmit strong activating signals in contrast to the apparent inhibitory signal delivered by triggering the CD200R. Despite substantial sequence homology with mCD200R, mCD200RLa and mCD200RLb did not bind mCD200, and presently have unknown ligands. The CD200 receptor gene family resembles the signal regulatory proteins and killer Ig-related receptors in having receptor family members with potential activatory and inhibitory functions that may play important roles in immune regulation and balance. Because manipulation of the CD200-CD200R interaction affects the outcome of rodent disease models, targeting of this pathway may have therapeutic utility.

Yamanishi, Y., D. L. Boyle, et al. (2002). "Expression and Regulation of Aggrecanase in Arthritis: The Role of TGF- β ." *J. Immunol.* **168**(3): 1405-1412.

<http://www.jimmunol.org/cgi/content/abstract/168/3/1405>

Aggrecanases are key matrix-degrading enzymes that act by cleaving aggrecan at the Glu373-Ala374 site. While these fragments have been detected in osteoarthritis (OA) and rheumatoid arthritis (RA) cartilage and synovial fluid, no information is available on the regulation or expression of the two key aggrecanases (aggrecanase-1 and aggrecanase-2) in synovial tissue (ST) or fibroblast-like synoviocytes (FLS). The aggrecanase-1 gene was constitutively expressed by both RA and OA FLS. Real-time PCR demonstrated that TGF- β significantly increased aggrecanase-1 gene expression in FLS. Aggrecanase-1 induction peaked after 24 h of TGF- β stimulation. The expression of aggrecanase-1 mRNA was significantly greater in RA ST than in OA or nonarthritis ST. Aggrecanase-2 mRNA and protein were constitutively produced by nonarthritis, OA, and RA FLS but were not increased by IL-1, TNF- α , or TGF- β . Furthermore, OA, RA, and nonarthritis ST contained similar amounts of immunoreactive aggrecanase-2. The major form of the aggrecanase-2 enzyme was 70 kDa in nonarthritis ST, whereas a processed 53-kDa form was abundant in RA ST. Therefore, aggrecanase-1 and -2 are differentially regulated in FLS. Both are constitutively expressed, but aggrecanase-1 is induced by cytokines, especially TGF- β . In contrast, aggrecanase-2 protein may be regulated by a post-translational mechanism in OA and RA ST. Synovial and FLS production of aggrecanase can contribute to cartilage degradation in RA and OA.

Yasuoka, H., H. Ihn, et al. (2003). "A Novel Protein Highly Expressed in Testis Is Overexpressed in Systemic Sclerosis Fibroblasts and Targeted by Autoantibodies." *J. Immunol.* **171**(12): 6883-6890.

<http://www.jimmunol.org/cgi/content/abstract/171/12/6883>

Nearly all autoantibody specificities in sera from patients with systemic sclerosis (SSc) target proteins distributed ubiquitously, and Abs against proteins whose expression is restricted to the affected sites have not been identified. In this study we describe SSc-specific autoantibody to a novel testicular Ag, termed protein highly expressed in testis (PHET), which is ectopically overexpressed in SSc dermal fibroblasts. A partial cDNA encoding PHET was isolated by immunoscreening of a HepG2 cDNA library with an SSc serum. PHET appeared to be a member of the UniGene cluster Hs.129872, but had a unique exon composition and a characteristic mRNA expression profile restricted to the testis. Serum Abs to a recombinant PHET fragment were detected in nine (8.4%) of 107 SSc patients, but in none of 50 systemic lupus erythematosus patients or 77 healthy controls. In SSc patients, the presence of anti-PHET Abs was associated with diffuse cutaneous SSc and lung involvement ($p = 0.02$ and 0.01 , respectively). PCR-based quantitative analysis of PHET mRNA expression in cultured dermal fibroblasts showed increased expression of PHET mRNA in SSc fibroblasts compared with control fibroblasts. PHET-reactive Abs purified from SSc sera stained the cytoplasm of SSc

dermal fibroblasts, and the staining intensity tended to be more prominent on SSc compared with control fibroblasts. These findings suggest that the autoantibody response to PHET can be induced by ectopic overexpression of PHET in dermal fibroblasts in SSc patients.

Yu, C.-R., R. M. Mahdi, et al. (2004). "Cell Proliferation and STAT6 Pathways Are Negatively Regulated in T Cells by STAT1 and Suppressors of Cytokine Signaling." *J. Immunol.* **173**(2): 737-746.

<http://www.jimmunol.org/cgi/content/abstract/173/2/737>

Suppressor of cytokine signaling (SOCS) proteins have emerged as important regulators of cytokine signals in lymphocytes. In this study, we have investigated regulation of SOCS expression and their role in Th cell growth and differentiation. We show that SOCS genes are constitutively expressed in naive Th cells, albeit at low levels, and are differentially induced by Ag and Th-polarizing cytokines. Whereas cytokines up-regulate expression of SOCS1, SOCS2, SOCS3, and cytokine-induced Src homology 2 protein, Ags induce down-regulation of SOCS3 within 48 h of Th cell activation and concomitantly up-regulate SOCS1, SOCS2, and cytokine-induced Src homology 2 protein expression. We further show that STAT1 signals play major roles in inducing SOCS expression in Th cells and that induction of SOCS expression by IL-4, IL-12, or IFN- γ is compromised in STAT1-deficient primary Th cells. Surprisingly, IL-4 is a potent inducer of STAT1 activation in Th2 but not Th1 cells, and SOCS1 or SOCS3 expression is dramatically reduced in STAT1-/- Th2 cells. To our knowledge, this is the first report of IL-4-induced STAT1 activation in Th cells, and suggests that its induction of SOCS, may in part, regulate IL-4 functions in Th2 cells. In fact, overexpression of SOCS1 in Th2 cells represses STAT6 activation and profoundly inhibits IL-4-induced proliferation, while depletion of SOCS1 by an anti-sense SOCS1 cDNA construct enhances cell proliferation and induces constitutive activation of STAT6 in Th2 cells. These results are consistent with a model where IL-4 has dual effects on differentiating T cells: it simulates proliferation/differentiation through STAT6 and autoregulates its effects on Th2 growth and effector functions via STAT1-dependent up-regulation of SOCS proteins.

Zarembek, K. A. and P. J. Godowski (2002). "Tissue Expression of Human Toll-Like Receptors and Differential Regulation of Toll-Like Receptor mRNAs in Leukocytes in Response to Microbes, Their Products, and Cytokines." *J. Immunol.* **168**(2): 554-561.

<http://www.jimmunol.org/cgi/content/abstract/168/2/554>

Members of the Toll-like receptor (TLR) family mediate dorsoventral patterning and cellular adhesion in insects as well as immune responses to microbial products in both insects and mammals. TLRs are characterized by extracellular leucine-rich repeat domains and an intracellular signaling domain that shares homology with cytoplasmic sequences of the mammalian IL-1 receptor and plant disease resistance genes. Ten human TLRs have been cloned as well as RP105, a protein similar to TLR4 but lacking the intracellular signaling domain. However, only five TLRs have described functions as receptors for bacterial products (e.g., LPS, lipoproteins). To identify potential sites of action, we used quantitative real-time RT-PCR to examine systematically the expression of mRNAs encoding all known human TLRs, RP105, and several other proteins important in TLR functions (e.g., MD-1, MD-2, CD14, MyD88). Most tissues tested expressed at least one TLR, and several expressed all (spleen, peripheral blood leukocytes). Analysis of TLR expression in fractionated primary human leukocytes (CD4+, CD8+, CD19+, monocytes, and granulocytes) indicates that professional phagocytes express the greatest variety of TLR mRNAs although several TLRs appear more restricted to B cells, suggesting additional roles for TLRs in adaptive immunity. Monocyte-like THP-1 cells regulate TLR mRNA levels in response to a variety of stimuli including phorbol esters, LPS, bacterial

lipoproteins, live bacteria, and cytokines. Furthermore, addition of *Escherichia coli* to human blood ex vivo caused distinct changes in TLR expression, suggesting that important roles exist for these receptors in the establishment and resolution of infections and inflammation.

Zegarra-Moran, O., C. Folli, et al. (2004). "Double Mechanism for Apical Tryptophan Depletion in Polarized Human Bronchial Epithelium." *J. Immunol.* **173**(1): 542-549.

<http://www.jimmunol.org/cgi/content/abstract/173/1/542>

Indoleamine 2,3-dioxygenase is an enzyme that catabolizes tryptophan to kynurenine. We investigated the consequences of IDO induction by IFN- γ in polarized human bronchial epithelium. IDO mRNA expression was undetectable in resting conditions, but strongly induced by IFN- γ . We determined the concentration of tryptophan and kynurenine in the extracellular medium, and we found that apical tryptophan concentration was lower than the basolateral in resting cells. IFN- γ caused a decrease in tryptophan concentration on both sides of the epithelium. Kynurenine was absent in control conditions, but increased in the basolateral medium after IFN- γ treatment. The asymmetric distribution of tryptophan and kynurenine suggested the presence of a transepithelial amino acid transport. Uptake experiments with radiolabeled amino acids demonstrated the presence of a Na⁺-dependent amino acid transporter with broad specificity that was responsible for the tryptophan/kynurenine transport. We confirmed these data by measuring the short-circuit currents elicited by direct application of tryptophan or kynurenine to the apical surface. The rate of amino acid transport was dependent on the transepithelial potential, and we established that in cystic fibrosis epithelia, in which the transepithelial potential is significantly more negative than in noncystic fibrosis epithelia, amino acid uptake was reduced. This work suggests that human airway epithelial cells maintain low apical tryptophan concentrations by two mechanisms, a removal through a Na⁺-dependent amino acid transporter and an IFN- γ -inducible degradation by IDO.

Zhou, H., E. Bengten, et al. (2003). "The T Cell Receptor β Locus of the Channel Catfish, *Ictalurus punctatus*, Reveals Unique Features." *J. Immunol.* **170**(5): 2573-2581.

<http://www.jimmunol.org/cgi/content/abstract/170/5/2573>

Previously, a series of clonal alloantigen-dependent T cell lines established from the channel catfish revealed distinctly different TCR β rearrangements. Here, a follow-up study of the junctional diversity of these TCR gene rearrangements focuses on characterization of the genomic organization of the TCRB locus. Surprisingly, a total of 29 JB genes and two substantially different CB genes were identified downstream of a single DB gene. This is in contrast to the situation in mammals, where two clusters of a DB gene, six or seven JB genes, and a CB gene are found in tandem. The catfish CB genes are [~]36% identical at the amino acid level. All 29 catfish JB gene segments appear functional. Thirteen were used in the 19 cDNAs analyzed, of these eight were used by the 11 catfish clonal alloantigen-dependent T cell lines. As might be expected, CDR3 diversity is enhanced by N-nucleotide additions as well as nucleotide deletions at the V-D and D-J junctions. Taken together, compared with that in mammals, genomic sequencing of the catfish TCR DB-JB-CB region reveals a unique locus containing a greater number of JB genes and two distinct CB genes.

Zwijnenburg, P. J. G., T. van der Poll, et al. (2003). "IL-1 Receptor Type 1 Gene-Deficient Mice Demonstrate an Impaired Host Defense Against Pneumococcal Meningitis." *J. Immunol.* **170**(9):

4724-4730.

<http://www.jimmunol.org/cgi/content/abstract/170/9/4724>

The fatality rate associated with *Streptococcus pneumoniae* meningitis remains high despite adequate antibiotic treatment. IL-1 is an important proinflammatory cytokine, which is up-regulated in brain tissue after the induction of meningitis. To determine the role of IL-1 in pneumococcal meningitis we induced meningitis by intranasal inoculation with 8×10^4 CFU of *S. pneumoniae* and 180 U of hyaluronidase in IL-1R type I gene-deficient (IL-1R^{-/-}) mice and wild-type mice. Meningitis resulted in elevated IL-1{alpha} and IL-1{beta} mRNA and protein levels in the brain. The absence of an intact IL-1 signal was associated with a higher susceptibility to develop meningitis. Furthermore, the lack of IL-1 impaired bacterial clearance, as reflected by an increased number of CFU in cerebrospinal fluid of IL-1R^{-/-} mice. The characteristic pleocytosis of meningitis was not significantly altered in IL-1R^{-/-} mice, but meningitis was associated with lower brain levels of cytokines. The mortality was significantly higher and earlier in the course of the disease in IL-1R^{-/-} mice. These results demonstrate that endogenous IL-1 is required for an adequate host defense in pneumococcal meningitis.

J. Leukoc. Biol. (20)

Ahmad, N., L. C. Chen, et al. (2002). "Regulation of cyclooxygenase-2 by nitric oxide in activated hepatic macrophages during acute endotoxemia." *J. Leukoc. Biol.* **71**(6): 1005-1011.

<http://www.jleukbio.org/cgi/content/abstract/71/6/1005>

Eicosanoids generated via cyclooxygenase-2 (COX-2) and nitric oxide produced from inducible nitric oxide synthase (NOSII) have been implicated in endotoxin-induced tissue injury. In the present studies, we characterized COX-2 and NOSII activity in rat hepatic macrophages and their interaction during acute endotoxemia. Kupffer cells from control animals were found to constitutively express COX-2 and NOSII mRNA and protein. Whereas treatment of the cells with lipopolysaccharide (LPS) and/or interferon- γ (IFN- γ) had no major effect on COX-2, NOSII expression increased. Induction of acute endotoxemia resulted in a rapid and transient increase in constitutive COX-2 expression and prostaglandin E₂ (PGE₂) production by liver macrophages as well as NOSII expression and nitric oxide release. Cells from endotoxin-treated rats were also sensitized to generate more nitric oxide and express increased NOSII in response to LPS and IFN- γ . Inhibition of NOSII with aminoguanidine reduced COX-2 mRNA and protein expression as well as PGE₂ production by activated macrophages from endotoxemic, but not control animals. In contrast, SC236, a specific COX-2 inhibitor, had no effect on NOSII mRNA or protein levels or on nitric oxide production by hepatic macrophages, even after endotoxin administration. These data suggest that activation of COX-2 may be important in the pathophysiological response of hepatic macrophages to endotoxin. Moreover, nitric oxide is involved in regulating COX-2 in activated liver macrophages during acute endotoxemia.

Bajer, A. A., D. Garcia-Tapia, et al. (2003). "Peripheral blood-derived bovine dendritic cells promote IgG1-restricted B cell responses in vitro." *J. Leukoc. Biol.* **73**(1): 100-106.

<http://www.jleukbio.org/cgi/content/abstract/73/1/100>

Regulation of humoral responses involves multiple cell types including the requirements for cognate interactions between T and B cells to drive CD40-dependent responses to T-dependent antigens. A third cell type has also been shown to play an essential role, the dendritic cell (DC). We demonstrate that bovine peripheral blood-derived (PB)-DC are similar in function to features described for human interstitial DC including the production of signature type 2 cytokines [interleukin (IL)-13, IL-10]. PB-DC express moderate-to-high costimulatory molecule expression, and major histocompatibility complex class II is negative for CD14 expression and has low or no expression of CD11c. Consistent with the interstitial phenotype is the ability of PB-DC to influence B cell activation and differentiation via direct expression of CD40L and type 2 cytokines. Collectively, these results suggest that direct B cell-DC interactions may promote an immunoglobulin-isotype expression pattern consistent with type 2 responses, independent of direct T cell involvement.

Catania, A., G. Colombo, et al. (2004). "Autocrine inhibitory influences of {alpha}-melanocyte-stimulating hormone in malignant pleural mesothelioma." *J. Leukoc. Biol.* **75**(2): 253-259.

<http://www.jleukbio.org/cgi/content/abstract/75/2/253>

Malignant pleural mesothelioma is a highly aggressive tumor arising from the mesothelial cells that line the pleural cavities. This tumor is resistant to most conventional anticancer treatments and appears to be very sensitive to growth-promoting influences of cytokines and growth factors. Identification of natural inhibitory pathways that control growth should aid discovery of novel therapeutic approaches. We hypothesized that {alpha}-melanocyte-stimulating hormone ({alpha}-MSH), which is produced by many cell types and antagonizes cytokines and growth factors, could be an endogenous inhibitory molecule in mesothelioma. Twelve mesothelioma cell lines were established from pleural effusions of patients with malignant mesothelioma. Mesothelioma cells were found to express mRNA for proopiomelanocortin and its processing enzymes; release {alpha}-MSH peptide into supernatants; and express melanocortin 1 receptor (MC1R), the high-affinity receptor for {alpha}-MSH. Immunoneutralization of MC1R in the cell lines enhanced expression of interleukin-8 (IL-8), IL-6, and transforming growth factor- β . These molecules promote mesothelioma proliferation and are considered therapeutic targets in this tumor. Coincubation of mesothelioma cells with synthetic {alpha}-MSH significantly reduced cell proliferation. The present research shows an autocrine-inhibitory circuit based on {alpha}-MSH and its receptor MC1R. Activation of MC1R by selective peptides or peptidomimetics might provide a novel strategy to reduce mesothelioma cell proliferation by taking advantage of this endogenous inhibitory circuit.

Collins, L. V., S. Hajizadeh, et al. (2004). "Endogenously oxidized mitochondrial DNA induces in vivo and in vitro inflammatory responses." *J. Leukoc. Biol.* **75**(6): 995-1000.

<http://www.jleukbio.org/cgi/content/abstract/75/6/995>

We report that mitochondrial DNA (mtDNA) is inflammatogenic in vitro and in vivo as a result of the presence of unmethylated CpG sequences and its oxidative status. Purified human and murine mtDNAs induced arthritis when injected intra-articularly (i.a.) in mice. Importantly, oligodeoxynucleotide that contained a single oxidatively damaged base also induced arthritis when injected i.a. in mice. In contrast, neither human nor murine nuclear DNA induced inflammation. mtDNA-induced arthritis was neither B cell- nor T cell-dependent but was mediated by monocytes/macrophages. mtDNA-induced nuclear factor- κ B stimulation resulted in the production of tumor necrosis factor {alpha}, a potent, arthritogenic factor. Finally, extracellular mtDNA was detected in the synovial fluids of rheumatoid arthritis patients but not of control subjects. We conclude that endogenous mtDNA displays inflammatogenic properties as a result

of its content of unmethylated CpG motifs and oxidatively damaged adducts.

Cowland, J. B., D. Carter, et al. (2003). "Tissue expression of copines and isolation of copines I and III from the cytosol of human neutrophils." *J. Leukoc. Biol.* **74**(3): 379-388.

<http://www.jleukbio.org/cgi/content/abstract/74/3/379>

Copines are a recently identified group of proteins characterized by two Ca²⁺-binding C2-domains at the N terminus and an A-domain at the C terminus. Although pEST sequences indicate the existence of at least seven copines in man, only copines I, III, and VI have been identified at protein level. Here, we describe the isolation of copines I and III in the cytosol of human neutrophils by use of Ca²⁺-induced hydrophobic chromatography. This is the first demonstration that copines are coexpressed in the same cell. We found that copine III exists in the cytosol of human neutrophils as a monomer with a blocked N terminus. Copines I and III undergo conformational changes upon Ca²⁺ binding that lead to exposure of hydrophobic patches. Examination of RNA from 68 human tissues demonstrated that copines I-III are ubiquitously expressed whereas copines IV-VII each has a more restricted and individual expression profile. Expression of copines I-III was also demonstrated in neutrophil precursors from bone marrow. Copine I was uniformly expressed at all stages of neutrophil differentiation, whereas copine II and even more so, copine III were expressed in the more immature neutrophil precursors, which indicates an individual function of these copines.

Goepel, F., P. Weinmann, et al. (2004). "Identification of caspase-10 in human neutrophils and its role in spontaneous apoptosis." *J. Leukoc. Biol.* **75**(5): 836-843.

<http://www.jleukbio.org/cgi/content/abstract/75/5/836>

In the present study, we investigated the molecular mechanisms of spontaneous and tumor necrosis factor {alpha} (TNF-{alpha})-mediated apoptosis of human polymorphonuclear neutrophils (PMN). Whereas TNF-{alpha}-mediated apoptosis was almost absent in the presence of the caspase-8 inhibitor Z-Ac-Ala-Glu-Val-Asp-7-fluoromethyl ketone (Z-AEVD-FMK), the inhibitor had no effect on spontaneous apoptosis, suggesting that spontaneous apoptosis was independent of caspase-8. Subsequently, we identified different isoforms of caspase-10 in human PMN and found high expression of caspase-10/b and/or -10/d and low expression of caspase-10/a and -10/c at the mRNA level. At the protein level, freshly isolated PMN showed high expression of caspase-10/b and -10/d as well as moderate expression of caspase-10/a and -10/c. Upon spontaneous apoptosis, caspase-10/b was down-regulated, which was accompanied by the appearance of a specific 47-kDa caspase-10/b cleavage product and an increased caspase-10 activity. In contrast, no down-regulation of caspase-10/a, -10/c, or -10/d was observed, suggesting that spontaneous apoptosis was associated with a differential activation of caspase-10/b. This was confirmed by the finding that spontaneous apoptosis was inhibited in the presence of Z-Ile-Glu-Thr-Asp (Z-IETD)-FMK, which blocks caspase-10. However, no down-regulation of caspase-10 isoforms was observed in the presence of TNF-{alpha}, suggesting that caspase-10 was not involved in TNF-{alpha}-induced apoptosis. Taken together, our study demonstrates that spontaneous and TNF-{alpha}-mediated apoptosis of PMN have different molecular requirements. Whereas TNF-{alpha}-mediated apoptosis depends on the activation of caspase-8, spontaneous apoptosis requires the activation of caspase-10/b. This finding may reveal that PMN apoptosis in different (patho-) physiological settings results from distinct molecular mechanisms.

Iwamoto, S., M. Ishida, et al. (2005). "Lipopolysaccharide stimulation converts vigorously washed dendritic cells (DCs) to nonexhausted DCs expressing CD70 and evoking long-lasting type 1 T cell responses." *J. Leukoc. Biol.*: jlb.1104654.

<http://www.jleukbio.org/cgi/content/abstract/jlb.1104654v1>

A great variety of in vitro culture protocols for human monocyte-derived dendritic cells (mo-DCs) has been used to generate DCs suitable for use in immunotherapy. It is thought that activated DCs undergo one-way differentiation into "exhausted" DCs. In the present study, we contrived an in vitro method for facilitating expression of CD70 by mature DCs. This was achieved by vigorous washing of mo-DCs before exposure to lipopolysaccharide (LPS). Unexpectedly, these mature DCs retain expression of some interleukin (IL)-12 family members after extended periods and maintain their ability to stimulate type 1 T cell responses. In contrast, DCs exposed to IL-4 before LPS stimulation or LPS-stimulated DCs not exposed to washing stress before activation failed to express CD70 and did differentiate into exhausted DCs. It is interesting that DCs expressing CD70 (CD70+ DCs) induced interferon- γ production from purified, allogeneic CD8+ T cells through a direct CD27-CD70 interaction. This is evidence for a pathway resulting in generation of CD8 T effectors by B7-independent mechanisms. These data suggest that exposure of immature DCs to LPS stimulation contributes to their terminal differentiation into CD70+ DCs, which have potent ability to prolong type 1 T cell responses through alternative pathways.

Kiani, A., I. Habermann, et al. (2004). "Expression and regulation of NFAT (nuclear factors of activated T cells) in human CD34+ cells: down-regulation upon myeloid differentiation." *J. Leukoc. Biol.* **76**(5): 1057-1065.

<http://www.jleukbio.org/cgi/content/abstract/76/5/1057>

The calcineurin-dependent, cyclosporin A (CsA)-sensitive transcription factor nuclear factor of activated T cells (NFAT) represents a group of proteins, which is well-characterized as a central regulatory element of cytokine expression in activated T cells. In contrast, little is known about the expression or function of NFAT family members in myeloid cells; moreover, it is unclear whether they are expressed by hematopoietic stem/progenitor cells. Here, we show that NFATc2 (NFAT1) is expressed at high levels in CD34+ cells and megakaryocytes but not in cells committed to the neutrophilic, monocytic, or erythroid lineages. Cytokine-induced in vitro differentiation of CD34+ cells into neutrophil granulocytes results in the rapid suppression of NFATc2 RNA and protein. NFATc2 dephosphorylation/rephosphorylation as well as nuclear/cytoplasmic translocation in CD34+ cells follow the same calcineurin-dependent pattern as in T lymphocytes, suggesting that NFATc2 activation in these cells is equally sensitive to inhibition with CsA. Finally, in vitro proliferation, but not differentiation, of CD34+ cells cultured in the presence of fms-like tyrosine kinase 3 ligand (FLT3L), stem cell factor, granulocyte macrophage-colony stimulating factor (GM-CSF), interleukin-3, and G-CSF is profoundly inhibited by treatment with CsA in a dose-dependent manner. These results suggest a novel and unexpected role for members of the NFAT transcription factor family in the hematopoietic system.

Klegeris, A. and P. L. McGeer (2003). "Toxicity of human monocytic THP-1 cells and microglia toward SH-SY5Y neuroblastoma cells is reduced by inhibitors of 5-lipoxygenase and its activating protein FLAP." *J. Leukoc. Biol.* **73**(3): 369-378.

<http://www.jleukbio.org/cgi/content/abstract/73/3/369>

To explore whether the proinflammatory products of the 5-lipoxygenase (5-LOX) pathway are involved in microglia-mediated toxicity toward neuronal cells, we evaluated the effects of 5-LOX inhibitors using an in vitro assay system where human neuronal SH-SY5Y cells are exposed to toxic secretions from THP-1 monocytic cells or human microglia. The specific 5-LOX inhibitors, REV 5901, zileuton, and 5-hydroxyeicosatetraenoic acid lactone; the nonselective LOX inhibitors, phenidone and dapsone; the dual 5-LOX/cyclooxygenase inhibitor, tepoxalin; and the selective inhibitor of the 5-LOX-activating protein (FLAP), MK-886, inhibited such toxicity. The toxicity was enhanced by the 5-LOX product leukotriene (LT)D₄ and reduced by the selective cysteinyl LT receptor (CysLT₁) antagonist MK-571. The mRNAs for 5-LOX and FLAP were detected in THP-1 cells and human microglia but not in SH-SY5Y cells. The data suggest that inhibition of proinflammatory LT production by 5-LOX inhibition could selectively reduce toxicity of microglial cells and thus be beneficial in neuroinflammatory diseases.

Lahat, N., M. A. Rahat, et al. (2003). "Hypoxia reduces CD80 expression on monocytes but enhances their LPS-stimulated TNF- α secretion." *J. Leukoc. Biol.* **74**(2): 197-205.

<http://www.jleukbio.org/cgi/content/abstract/74/2/197>

Monocytes/macrophages in ischemic tissues are involved in inflammation and suppression of adaptive immunity via secretion of proinflammatory cytokines and reduced ability to trigger T cells, respectively. We subjected human mononuclear cells and mouse macrophages to hypoxia and reoxygenation, the main constituents of ischemia and reperfusion, and added lipopolysaccharide (LPS) to simulate bacterial translocation, which frequently accompanies ischemia. We monitored the secretion of tumor necrosis factor α (TNF- α) and the surface expression of human leukocyte antigen-DR and the costimulatory molecules CD80 and CD86 on monocytes/macrophages. Hypoxia selectively reduced the surface expression of CD80 ($P < 0.01$), and synergistically with LPS, it enhanced TNF- α secretion ($P < 0.003$). Reoxygenation reversed both phenomena. In the mouse macrophage cell line RAW 264.7, hypoxia reduced the surface expression of CD80 and increased its concentrations in the supernatants ($P < 0.01$). Down-regulation of the mRNA coding for the membrane-anchored CD80 was observed, suggesting that hypoxia triggers alternative splicing to generate soluble CD80. Cumulatively, these results suggest that hypoxia simultaneously affects monocytes/macrophages to enhance inflammation and reduce their ability to initiate adaptive-immunity responses associated with ischemic injury.

Nagase, H., M. Miyamasu, et al. (2002). "Cytokine-mediated regulation of CXCR4 expression in human neutrophils." *J. Leukoc. Biol.* **71**(4): 711-717.

<http://www.jleukbio.org/cgi/content/abstract/71/4/711>

Several lines of evidence have suggested that a CXC chemokine receptor 4 (CXCR4)/stromal cell-derived factor-1 [SDF-1; CXC chemokine ligand 12 (CXCL12)] pair is involved in baseline trafficking of leukocytes into extravascular tissues and that modulation of surface CXCR4 expression may represent an alternative mechanism for control of cell-specific biological responses to SDF-1/CXCL12. We explored the regulation of CXCR4 expression by cytokines in polymorphonuclear neutrophils (PMNs). No significant surface expression of CXCR4 in freshly isolated PMNs was detected, but expression became apparent gradually during incubation. SDF-1 α /CXCL12 initiated Ca²⁺ mobilization and migratory responses in 20 h cultured PMNs. The surface CXCR4 expression was suppressed most potently by interferon- γ (IFN- γ). IFN- α , granulocyte-macrophage colony-stimulating factor (GM-CSF), and G-CSF also inhibited spontaneous CXCR4 expression. Real-time, quantitative PCR experiments revealed that a spontaneous increase and an IFN- γ -mediated decrease in surface

CXCR4 paralleled changes in the CXCR4 mRNA level. These results on PMNs support the argument that the SDF-1 (CXCL12)/CXCR4 system is regulated by cell type-specific mechanisms.

Ng, Y. Y., B. van Kessel, et al. (2004). "Gene-expression profiling of CD34+ cells from various hematopoietic stem-cell sources reveals functional differences in stem-cell activity." J. Leukoc. Biol. **75**(2): 314-323.

<http://www.jleukbio.org/cgi/content/abstract/75/2/314>

The replacement of bone marrow (BM) as a conventional source of stem cell (SC) by umbilical cord blood (UCB) and granulocyte-colony stimulating factor-mobilized peripheral blood SC (PBSC) has brought about clinical advantages. However, several studies have demonstrated that UCB CD34+ cells and PBSC significantly differ from BM CD34+ cells qualitatively and quantitatively. Here, we quantified the number of SC in purified BM, UCB CD34+ cells, and CD34+ PBSC using in vitro and in vivo assays for human hematopoietic SC (HSC) activity. A cobblestone area-forming cell (CAFC) assay showed that UCB CD34+ cells contained the highest frequency of CAFcwk6 (3.6- to tenfold higher than BM CD34+ cells and PBSC, respectively), and the engraftment capacity in vivo by nonobese diabetic/severe combined immunodeficiency repopulation assay was also significantly greater than BM CD34+, with a higher proportion of CD45+ cells detected in the recipients at a lower cell dose. To understand the molecular characteristics underlying these functional differences, we performed several DNA microarray experiments using Affymetrix gene chips, containing 12,600 genes. Comparative analysis of gene-expression profiles showed differential expression of 51 genes between BM and UCB CD34+ SC and 64 genes between BM CD34+ cells and PBSC. These genes are involved in proliferation, differentiation, apoptosis, and engraftment capacity of SC. Thus, the molecular expression profiles reported here confirmed functional differences observed among the SC sources. Moreover, this report provides new insights to describe the molecular phenotype of CD34+ HSC and leads to a better understanding of the discrepancy among the SC sources.

Richard, M., P. Veilleux, et al. (2002). "The expression pattern of the ITIM-bearing lectin CLECSF6 in neutrophils suggests a key role in the control of inflammation." J. Leukoc. Biol. **71**(5): 871-880.

<http://www.jleukbio.org/cgi/content/abstract/71/5/871>

In our study of the modulation of the expression of inflammation-related genes in neutrophils, we have found a gene called CLECSF6 (C-type lectin superfamily 6). CLECSF6 expresses two mRNA species at low levels in resting neutrophils. Here, we describe for the first time the sequence of the short mRNA version. It lacks amino acids that are likely to affect the functionality of its protein product. GM-CSF, IL-3, IL-4, and IL-13 caused an accumulation of the short CLECSF6 mRNA in neutrophils. The surface expression of the CLECSF6 protein was reduced by TNF- α , IL-1 α , LPS, and Matrigel(R). CLECSF6 bears the immunoreceptor tyrosine-based inhibition motif (ITIM) involved in signal transduction resulting in the inhibition of leukocyte activation. We propose that some neutrophil activators modulate the expression of CLECSF6 at the mRNA (GM-CSF, IL-3, IL-4, and IL-13) or protein (TNF- α , IL-1 α , LPS, and Matrigel(R)) levels in ways that block ITIM-based transduction of anti-inflammatory signals and therefore promote inflammation.

Rose, B., C. Herder, et al. (2004). "Combined activation of innate and T cell immunity for recognizing

immunomodulatory properties of therapeutic agents." J. Leukoc. Biol. **75**(4): 624-630.

<http://www.jleukbio.org/cgi/content/abstract/75/4/624>

Complex syndromes such as atherosclerosis and type 2 diabetes are disorders that are associated with inflammatory processes involving innate and adaptive immunity. Emerging knowledge about the pathological consequences of immune imbalances in a wide range of disease settings is expected to help to identify novel therapeutic targets. However, current test systems for immunomodulatory drugs tend to be too simplistic, as they rely only on cells of the innate- or the adaptive-immune system, or they are complex, in vivo models, which are not suitable for screening purposes. Using a modified mixed lymphocyte culture (MMLC) assay for combined analysis of innate and adaptive immunity, we show that this assay is very sensitive for the presence of low concentrations of immunomodulatory agents. Low-dose lipopolysaccharide stimulation of cells from two unrelated donors yields a strong cytokine response including interleukin (IL)-12 and IL-18, which induce interferon- γ as a potential analysis parameter. As the MMLC assay is based on the mutual interaction of cells of the innate and adaptive immunity, it enables the monitoring of cytokine release under almost physiological conditions and might be of interest for the characterization of known and novel drugs concerning their immunomodulatory potency.

Takauji, R., S. Iho, et al. (2002). "CpG-DNA-induced IFN- α production involves p38 MAPK-dependent STAT1 phosphorylation in human plasmacytoid dendritic cell precursors." J. Leukoc. Biol. **72**(5): 1011-1019.

<http://www.jleukbio.org/cgi/content/abstract/72/5/1011>

Human plasmacytoid or CD4⁺CD11c⁻ type 2 dendritic cell precursors (PDC) were identified as natural type I interferon (IFN)-producing cells in response to viral and bacterial infection. They represent effector cells of innate immunity and link it to the distinct adaptive immunity by differentiating into mature DC. It has been reported that oligodeoxyribonucleotides containing unmethylated CpG motifs (CpG DNA) stimulate PDC to produce IFN- α , but the molecular mechanisms involved remain unknown. We found that CpG-DNA-induced IFN- α production in PDC was completely impaired by the inhibitor of the p38 mitogen-activated protein kinase (MAPK) pathway. Expression of IFN regulatory factor (IRF)-7 was enhanced by CpG-DNA treatment, which was preceded by the phosphorylation of signal transducer and activator of transcription (STAT)1 on Tyr-701, as well as its enhanced phosphorylation on Ser-727. All of these events were also suppressed by the p38 MAPK inhibitor. STAT1, STAT2, and IRF-9, components of IFN-stimulated gene factor 3 (ISGF3), were recognized in the nuclear fraction of CpG-DNA-treated cells. Neither anti-IFN- α/β antibodies (Ab) nor anti-IFNAR Ab suppressed STAT1 phosphorylation, enhancement of IRF-7 expression, or IFN- α production in the early phase of the culture. These results suggest that CpG DNA induces p38 MAPK-dependent phosphorylation of STAT1 in a manner independent of IFN- α/β , which may cause ISGF3 formation to increase the transcription of the IRF-7 gene, thereby leading to IFN- α production in human PDC.

Tomasinsig, L., M. Scocchi, et al. (2002). "Inducible expression of an antimicrobial peptide of the innate immunity in polymorphonuclear leukocytes." J. Leukoc. Biol. **72**(5): 1003-1010.

<http://www.jleukbio.org/cgi/content/abstract/72/5/1003>

Epithelia- and leukocyte-associated antimicrobial peptides provide immediate protection against

microbial infections by rapidly inactivating potential pathogens. Bac5 is a member of the cathelicidin family of antimicrobial peptides and is stored in the cytoplasmic granules of bovine neutrophils. We investigated the expression of this gene in airway and intestine, and although the gene was not found to be locally expressed in these tissues, a strong Bac5 induction signal was detected by in situ hybridization in neutrophils infiltrating infected lung, consistent with expression of this gene in activated neutrophils. The Bac5 gene was also induced in bovine peripheral neutrophils stimulated with *Escherichia coli* or purified lipopolysaccharide (LPS) but not in other blood cells and in resting neutrophils. The levels of Bac5 mRNA increased at 12-24 h post-stimulation, and a dose-dependent increase in Bac5 expression was determined in the presence of increasing amounts of LPS. A metabolically labeled product with a molecular weight compatible with that of proBac5 was immunoprecipitated from cell-free media of stimulated neutrophils, suggesting that the newly synthesized polypeptide is released extracellularly. Collectively, these results provide the first evidence that fully differentiated neutrophils are capable of de novo synthesis and secretion of a granule-associated antimicrobial peptide.

van de Geijn, G.-J. M., J. Gits, et al. (2004). "Distinct activities of suppressor of cytokine signaling (SOCS) proteins and involvement of the SOCS box in controlling G-CSF signaling." *J. Leukoc. Biol.* **76**(1): 237-244.

<http://www.jleukbio.org/cgi/content/abstract/76/1/237>

Granulocyte-colony stimulating factor (G-CSF) induces proliferation of myeloid progenitor cells and controls their differentiation into mature neutrophils. Signal transducer and activator of transcription (STAT) proteins STAT3 and STAT5 are activated by G-CSF and play distinct roles in neutrophil development. Suppressor of cytokine signaling (SOCS) proteins are induced by STATs and inhibit signaling through various negative-feedback mechanisms. SOCS proteins can compete with docking of signaling substrates to receptors, interfere with Janus tyrosine kinase activity, and target proteins for proteasomal degradation. The latter process is mediated through the conserved C-terminal SOCS box. We determined the role of various SOCS proteins in controlling G-CSF responses and investigated the involvement of the SOCS box therein. We show that SOCS1 and SOCS3, but not CIS and SOCS2, inhibited G-CSF-induced STAT activation in human embryo kidney 293 cells. In myeloid 32D cells, SOCS1 and SOCS3 are induced by G-CSF. However, relative to interleukin-3-containing cultures, during G-CSF-induced neutrophilic differentiation, SOCS3 expression was further elevated, while SOCS1 levels remained constant. SOCS box deletion mutants of SOCS1 and SOCS3 were severely hampered in their abilities to inhibit STAT activation and to efficiently suppress colony formation by primary myeloid progenitors in response to G-CSF. These data demonstrate the importance of the SOCS box for the inhibitory effects of SOCS proteins on G-CSF signaling and show that among the different SOCS family members, SOCS3 is the major negative regulator of G-CSF responses during neutrophilic differentiation.

Wang, J., R. Charboneau, et al. (2002). "The immunosuppressive effects of chronic morphine treatment are partially dependent on corticosterone and mediated by the {micro}-opioid receptor." *J. Leukoc. Biol.* **71**(5): 782-790.

<http://www.jleukbio.org/cgi/content/abstract/71/5/782>

Wild-type and {micro}-opioid receptor knockout (MORKO) mice were used to investigate the role of corticosterone (CORT) and the {micro}-opioid receptor (MOR) in chronic morphine-mediated immunosuppression. We found that although plasma CORT concentrations in CORT infusion (10 mg/kg/day) and morphine-pellet implantation (75 mg) mice were similar (400-450 ng/ml), chronic morphine treatment resulted in a significantly higher (two- to threefold) inhibition of thymic,

splenic, and lymph node cellularity; inhibition of thymic-lymphocyte proliferation; inhibition of IL-2 synthesis; and activation of macrophage nitric oxide (NO) production when compared with CORT infusion. In addition, results show that the inhibition of IFN- γ synthesis and splenic- and lymph node-lymphocyte proliferation and activation of macrophage TNF- α and IL-1 β synthesis occurred only with chronic morphine treatment but not with CORT infusion. These morphine effects were abolished in MORKO mice. The role of the sympathetic nervous system on morphine-mediated effects was investigated by using the ganglionic blocker chlorisondamine. Our results show that chlorisondamine was able to only partially reverse morphine's inhibitory effects. The results clearly show that morphine-induced immunosuppression is mediated by the MOR and that although some functions are amplified in the presence of CORT or sympathetic activation, the inhibition of IFN- γ synthesis and activation of macrophage-cytokine synthesis is CORT-independent and only partially dependent on sympathetic activation.

Workalemahu, G., M. Foerster, et al. (2004). "Expression and synthesis of fibroblast growth factor-9 in human $\gamma\delta$ T-lymphocytes. Response to isopentenyl pyrophosphate and TGF- β 1/IL-15." *J. Leukoc. Biol.* **75**(4): 657-663.

<http://www.jleukbio.org/cgi/content/abstract/75/4/657>

$\gamma\delta$ T-lymphocytes are believed to play a role in maintaining the normal configuration of epithelial tissue. As little is known about the factors mediating this function, we addressed the question of whether $\gamma\delta$ T-lymphocytes produce fibroblast growth factor (FGF)-9 as well as two other growth factors associated with epithelial tissue reconstitution. Blood $\gamma\delta$ T cells isolated from healthy donors were grown in the presence of isopentenyl pyrophosphate (IPP) or transforming growth factor- β 1 (TGF- β 1)/interleukin-15 (IL-15) for 24 h and were assessed for the expression and synthesis of FGF-9, keratinocyte growth factor (KGF), and epidermal growth factor (EGF). Resting human $\gamma\delta$ T cells constitutively expressed KGF and FGF-9 mRNA but no EGF mRNA. In the presence of IPP, FGF-9 mRNA expression significantly increased in a dose-dependent manner, expression of KGF remained unaltered, and EGF mRNA could not be detected. In contrast to IPP, stimulation of the cells with TGF- β 1/IL-15 did not alter FGF-9 expression. Moreover, stimulation with anti-CD3 does not induce FGF-9 expression but triggers a high signal of interferon- γ mRNA. Western blot analysis of $\gamma\delta$ T cell lysates, prepared 4 days following stimulation with IPP, showed an increase of FGF-9 protein as compared with control cells. In conclusion, the results demonstrate for the first time that human blood and bronchoalveolar lavage $\gamma\delta$ T-lymphocytes are capable of expressing FGF-9. The data also provide novel evidence that immunoregulatory cells can synthesize FGF-9.

Wykes, M., K. P. A. MacDonald, et al. (2002). "MUC1 epithelial mucin (CD227) is expressed by activated dendritic cells." *J. Leukoc. Biol.* **72**(4): 692-701.

<http://www.jleukbio.org/cgi/content/abstract/72/4/692>

The MUC1 mucin (CD227) is a cell surface mucin originally thought to be restricted to epithelial tissues. We report that CD227 is expressed on human blood dendritic cells (DC) and monocyte-derived DC following in vitro activation. Freshly isolated murine splenic DC had very low levels of CD227; however, all DC expressed CD227 following in vitro culture. In the mouse spleen, CD227 was seen on clusters within the red pulp and surrounding the marginal zone in the white pulp. Additionally, we confirm CD227 expression by activated human T cells and show for the first time that the CD227 cytoplasmic domain is tyrosine-phosphorylated in activated T cells and DC and is associated with other phosphoproteins, indicating a role in signaling. The function of CD227 on DC and T cells requires further elucidation.

J. Lipid Res. (6)

Berthier, M.-T., A. Houde, et al. (2005). "Impact of adiponectin gene polymorphisms on plasma lipoprotein and adiponectin concentrations of viscerally obese men." J. Lipid Res. **46**(2): 237-244.

<http://www.jlr.org/cgi/content/abstract/46/2/237>

The aim of this study was first to examine the relationships between adiponectin gene (Apm1) polymorphisms and anthropometric indices as well as plasma adiponectin and lipoprotein/lipid levels, and then to investigate whether the presence of visceral obesity or insulin resistance may modulate the impact of these polymorphisms on metabolic risk variables. Molecular screening of the Apm1 gene was achieved, and a sample of 270 unrelated men recruited from the greater Quebec City area and selected to cover a wide range of body fatness values was genotyped. Sequencing of the Apm1 gene revealed two previously reported polymorphisms (c.45T>G and c.276G>T) as well as two newly identified genetic variations (-13752delT and -13702G>C). Carriers of the c.276T allele had higher LDL-cholesterol and lower HDL-triglyceride concentrations than did 276G/G homozygotes ($P = 0.02$ and $P = 0.01$, respectively). Carriers of the c.45G allele exhibited higher plasma adiponectin concentrations than did 45T/T homozygotes ($P = 0.04$). After dividing each genotype group into subgroups for visceral AT, homozygotes for the normal allele at position -13752delT, carriers of the c.45G allele, and carriers of the c.276T allele had similar total apolipoprotein B (apoB) concentrations, whether they were viscerally obese or not. These results suggest that some Apm1 gene polymorphisms influence plasma adiponectin concentrations and lipoprotein/lipid levels. In addition, the impact of these polymorphisms is modulated by the presence of visceral obesity.

Huang, W., V. Mishra, et al. (2004). "Phorbol ester promotes histone H3-Ser10 phosphorylation at the LDL receptor promoter in a protein kinase C-dependent manner." J. Lipid Res. **45**(8): 1519-1527.

<http://www.jlr.org/cgi/content/abstract/45/8/1519>

Histone modification is emerging as a major regulatory mechanism for modulating gene expression by altering the accessibility of transcription factors to DNA. This study unravels the relationship between histone H3 modifications and LDL receptor induction, focusing also on routes by which phosphorylation is mediated in human hepatoma HepG2 cells. We show that while histone H3 is constitutively acetylated at LDL receptor chromatin, 12-O - tetradecanoylphorbol-13-acetate (TPA) causes rapid hyperphosphorylation of histone H3 on serine 10 (histone H3-Ser10), despite global reduction in its phosphorylation levels. Ser10 hyperphosphorylation precedes LDL receptor induction and is independent of the p42/44MAPK, p38MAPK, pp90RSK, or MSK-1 cascade. Interestingly, inhibition of protein kinase C (PKC) blocks Ser10 hyperphosphorylation and also compromises LDL receptor induction by TPA. Consistent with its role, recombinant purified PKC phosphorylate purified histone H3-Ser10. Collectively, our findings highlight a novel role for PKC in regulating histone H3-Ser10 phosphorylation and suggest that histone modification provides numerous regulatory opportunities to set the overall range of control attainable for LDL receptor gene induction.

Jones, D. R., R. J. Schmidt, et al. (2002). "Estrogen receptor-mediated repression of human hepatic lipase gene transcription." J. Lipid Res. **43**(3): 383-391.

<http://www.jlr.org/cgi/content/abstract/43/3/383>

Estrogen replacement therapy in women decreases hepatic lipase (HL) activity, which may account for the associated increase in HDL cholesterol. To investigate whether estrogen decreases HL transcription, transient cotransfection assays with HL promoter and estrogen receptor- α (ER α) expression constructs were performed in HepG2 cells. 17 β -estradiol (E2) decreased transcription driven by the -1557/+41 human HL promoter by up to 50% at 10⁻⁷ M. Mutation of ER α by deletion of its transactivation domains or ligand-binding domain eliminated E2-induced repression of the promoter, whereas deletion of the DNA-binding domain of ER α resulted in a 7-fold activation by E2. The E2-induced repression was maintained after mutation of a potential estrogen-response element in the promoter. The region of estrogen responsiveness was localized to -1557/-1175 of the HL promoter by deletion analysis. Mutation of an AP-1 site at -1493 resulted in a partial loss of E2-induced repression, similar to that caused by deletion of nucleotides -1557 to -1366. Gel shift assays with nuclear extracts from E2-treated HepG2 cells stably expressing ER α demonstrated an increase in binding to an AP-1 consensus oligonucleotide. The AP-1 activator, phorbol 12-myristate 13-acetate, inhibited the HL promoter by greater than 50%. Collectively, the data suggest that estrogen represses the transcription of the HL gene, possibly through an AP-1 pathway. -- Jones, D. R., R. J. Schmidt, R. T. Pickard, P. S. Foxworthy, and P. I. Eacho. Estrogen receptor-mediated repression of human hepatic lipase gene transcription. *J. Lipid Res.* 2002. 43: 383-391.

Lu, S., M. Huffman, et al. (2002). "Regulation of MTP expression in developing swine." J. Lipid Res. **43**(8): 1303-1311.

<http://www.jlr.org/cgi/content/abstract/43/8/1303>

To define the developmental expression of microsomal triglyceride transfer protein (MTP) large subunit mRNA and protein, samples of small intestine and liver were collected from 40-day gestation fetal, 2-day-old newborn, 3-week-old suckling, and 2-month-old weanling swine. In fetal animals, MTP mRNA expression was high in intestine and liver. Postnatally, jejunal expression paralleled the intake of a high-fat breast milk diet and declined after weaning. Ileal expression was comparable with that of jejunum in 2-day-old animals, but declined to low levels afterward. Hepatic expression declined postnatally and remained low. MTP protein expression generally paralleled mRNA expression, except in fetal intestine in which no 97 kDa protein was detected. In 2-day-old piglets, a high-triacylglycerol diet increased jejunal and ileal MTP mRNA levels, as compared to a low-triacylglycerol diet. To test the roles of glucocorticoids and fatty acids in MTP regulation, a newborn swine enterocyte cell line (IPEC-1) was used. Except at day 2 of differentiation, dexamethasone did not influence MTP expression. Fatty acids either up-regulated or down-regulated MTP expression, depending on the specific fatty acid and duration of exposure. Although programmed genetic cues regulate MTP expression during development, clearly the amount and fatty acid composition of dietary lipid also play regulatory roles.

Nwankwo, J. O., A. A. Spector, et al. (2003). "A nucleotide insertion in the transcriptional regulatory region of FADS2 gives rise to human fatty acid delta-6-desaturase deficiency." J. Lipid Res. **44**(12): 2311-2319.

<http://www.jlr.org/cgi/content/abstract/44/12/2311>

Fatty acid delta-6-desaturase (FADS2) is the rate-limiting enzyme in mammalian synthesis of long-chain polyunsaturated fatty acids. We investigated the molecular mechanism of FADS2 deficiency in skin fibroblasts from a patient deficient in this enzyme. Expression analyses demonstrated an 80% to 90% decrease in the steady-state level of FADS2 mRNA in patient-derived cells compared with normal controls that was consistent with previous metabolic biochemical studies. In vitro transcription assays indicated an 80% decrease in the rate of transcriptional initiation in patient-derived cells, thus implicating transcriptional regulation as the mechanism for the decreased transcript levels. Sequence analysis of the 5' end of the gene revealed the insertion of a thymidine between positions -941 and -942 upstream of the translation start site in patient-derived cells compared with normal cells and published sequences. Promoter-reporter assays demonstrated a 6-fold decrease in promoter activity in the polymorphic variant FADS2 regulatory region compared with the normal gene, confirming the functional relevance of the insertion mutation to the decreased expression of the gene in the patient-derived cells. These findings indicate that fatty acid delta-6-desaturase deficiency and decreased FADS2 transcription are caused by a nucleotide insertion in the transcriptional regulatory region of the human FADS2 gene.

Paradis, M.-E., P. Couture, et al. (2003). "The T111I mutation in the EL gene modulates the impact of dietary fat on the HDL profile in women." J. Lipid Res. **44**(10): 1902-1908.

<http://www.jlr.org/cgi/content/abstract/44/10/1902>

The objective of the present study was to examine the impact of the T111I missense mutation in exon 3 of the endothelial lipase (EL) gene on HDL and its potential interaction effect with dietary fat. The study sample included 281 women and 216 men aged between 17 and 76 years from the Quebec Family Study. Plasma HDL3-C levels of I111I homozygote women were higher compared with those of women carrying the wild-type allele ($P = 0.03$). These differences were not attenuated when adjusted for levels of obesity and were not observed among men. Dietary PUFA interacted with the T111I mutation to modulate apolipoprotein A-I (apoA-I) and HDL3-C levels among women. Specifically, a diet rich in PUFA was associated with increased apoA-I levels among women carriers of the I111 allele and with decreased apoA-I among women homozygotes for the wild-type allele ($P = 0.002$). A similar interaction was observed with plasma HDL3-C levels ($P = 0.003$). These interactions were not observed among men. In conclusion, the EL T111I mutation appears to have a modest effect on plasma HDL levels. The gene-diet interaction among women, however, suggests that the T111I missense mutation may confer protection against the lowering effect of a high dietary PUFA intake on plasma apoA-I and HDL3-C levels.

J. Med. Genet. (26)

Aucan, C., A. J. Walley, et al. (2004). "Common apolipoprotein E polymorphisms and risk of clinical malaria in the Gambia." J. Med. Genet. **41**(1): 21-24.

<http://jmg.bmjournals.com>

Baysal, B. E., J. E. Willett-Brozick, et al. (2004). "An Alu-mediated partial SDHC deletion causes familial and sporadic paraganglioma." J. Med. Genet. **41**(9): 703-709.

<http://jmg.bmjjournals.com>

Brancati, F., E. M. Valente, et al. (2004). "A locus for autosomal dominant keratoconus maps to human chromosome 3p14-q13." J. Med. Genet. **41**(3): 188-192.

<http://jmg.bmjjournals.com>

Breedveld, G. J., B. van Wetten, et al. (2004). "A new locus for a childhood onset, slowly progressive autosomal recessive spinocerebellar ataxia maps to chromosome 11p15." J. Med. Genet. **41**(11): 858-866.

<http://jmg.bmjjournals.com>

De Bustos, C., A. Smits, et al. (2005). "A PDGFRA promoter polymorphism, which disrupts the binding of ZNF148, is associated with primitive neuroectodermal tumours and ependymomas." J. Med. Genet. **42**(1): 31-37.

<http://jmg.bmjjournals.com/cgi/content/abstract/42/1/31>

Background: Platelet derived growth factor receptor {alpha} (PDGFR{alpha}) expression is typical for a variety of brain tumours, while in normal adult brain PDGFR{alpha} expression is limited to a small number of neural progenitor cells. The molecular mechanisms responsible for the PDGFR{alpha} expression in tumours are not known, but in the absence of amplification, changes in transcriptional regulation might be an important factor in this process. Methods and results: We have investigated the link between single nucleotide polymorphisms (SNPs) within the PDGFR{alpha} gene promoter and the occurrence of brain tumours (medulloblastomas, supratentorial primitive neuroectodermal tumours (PNETs), ependymal tumours, astrocytomas, oligodendrogliomas, and mixed gliomas). These SNPs give rise to five different promoter haplotypes named H1 and H2{alpha}-{delta}. It is apparent from the haplotype frequency distribution that both PNET (10-fold) and ependymoma (6.5-fold) patient groups display a significant over-representation of the H2{delta} haplotype. The precise functional role in PDGFR{alpha} gene transcription for the H2{delta} haplotype is not known yet, but we can show that the H2{delta} haplotype specifically disrupts binding of the transcription factor ZNF148 as compared to the other promoter haplotypes. Conclusions: The specific over-representation of the H2{delta} haplotype in both patients with PNETs and ependymomas suggests a functional role for the ZNF148/PDGFR{alpha} pathway in the pathogenesis of these tumours.

Edwards, R. H., M. R. Ward, et al. (2004). "Absence of BRAF mutations in UV-protected mucosal melanomas." J. Med. Genet. **41**(4): 270-272.

<http://jmg.bmjjournals.com/cgi/content/abstract/41/4/270>

Background: Mutations in BRAF have recently been identified in a significant percentage of primary and metastatic cutaneous malignant melanomas. As ultraviolet (UV) exposure may play a

role in the development of cutaneous melanoma lesions with BRAF mutations, BRAF mutation frequency in melanomas arising in sites protected from sun exposure may be lower than those from sun-exposed areas. Thus, we determined the BRAF mutation frequency in a panel of 13 mucosal melanomas and compared those data with data from all currently published series of cutaneous melanomas. Methods: BRAF exon 15 DNA from 13 archival primary mucosal melanomas (eight vulvar, four anorectal, and one laryngeal) was sequenced using intron-based primers. As archival DNA occasionally produces poor-quality template, results were confirmed with a TspRI restriction fragment length polymorphism (RFLP) that distinguishes wild-type BRAF from the common mutant form V599E. A binomial test was used to compare the mutation frequency in the mucosal melanomas with the published mutation frequency in cutaneous melanomas. Results: None of the 13 mucosal melanomas in this series had an exon 15 BRAF mutation, as compared to 54/165 (33%) primary cutaneous melanomas with BRAF mutations in a compilation of all current published studies ($p = 0.006$). Discussion: These data suggest that UV exposure, plays a role in the genesis of BRAF mutations in cutaneous melanoma, despite the absence of the characteristic C>T or CC>TT mutation signature associated with UV exposure, and suggests mechanisms other than pyrimidine dimer formation are important in UV-induced mutagenesis.

Font-Llitjos, M., M. Jimenez-Vidal, et al. (2005). "New insights into cystinuria: 40 new mutations, genotype-phenotype correlation, and digenic inheritance causing partial phenotype." J. Med. Genet. **42**(1): 58-68.

<http://jmg.bmjjournals.com/cgi/content/abstract/42/1/58>

Objective: To clarify the genotype-phenotype correlation and elucidate the role of digenic inheritance in cystinuria. Methods: 164 probands from the International Cystinuria Consortium were screened for mutations in SLC3A1 (type A) and SLC7A9 (type B) and classified on the basis of urine excretion of cystine and dibasic amino acids by obligate heterozygotes into 37 type I (silent heterozygotes), 46 type non-I (hyperexcretor heterozygotes), 14 mixed, and 67 untyped probands. Results: Mutations were identified in 97% of the probands, representing 282 alleles (86.8%). Forty new mutations were identified: 24 in SLC3A1 and 16 in SLC7A9. Type A heterozygotes showed phenotype I, but mutation DupE5-E9 showed phenotype non-I in some heterozygotes. Type B heterozygotes showed phenotype non-I, with the exception of 10 type B mutations which showed phenotype I in some heterozygotes. Thus most type I probands carried type A mutations and all type non-I probands carried type B mutations. Types B and A mutations contributed to mixed type, BB being the most representative genotype. Two mixed cystinuria families transmitted mutations in both genes: double compound heterozygotes (type AB) had greater aminoaciduria than single heterozygotes in their family. Conclusions: Digenic inheritance is an exception (two of 164 families), with a limited contribution to the aminoaciduria values (partial phenotype) in cystinuria. Further mutational analysis could focus on one of the two genes (SLC3A1 preferentially for type I and SLC7A9 for type non-I probands), while for mixed probands analysis of both genes might be required, with priority given to SLC7A9.

Fukuchi, K., T. Katsuya, et al. (2004). "LMNA mutation in a 45 year old Japanese subject with Hutchinson-Gilford progeria syndrome." J. Med. Genet. **41**(5): e67-.

<http://jmg.bmjjournals.com>

Halangk, J., T. Berg, et al. (2004). "Keratin 8 Y54H and G62C mutations are not associated with liver

disease." J. Med. Genet. **41**(7): e92-.

<http://jmg.bmjjournals.com>

Halliday, D. J., S. Hutchinson, et al. (2002). "Twelve novel FBN1 mutations in Marfan syndrome and Marfan related phenotypes test the feasibility of FBN1 mutation testing in clinical practice." J. Med. Genet. **39**(8): 589-593.

<http://jmg.bmjjournals.com>

Howell, V. M., C. J. Haven, et al. (2003). "HRPT2 mutations are associated with malignancy in sporadic parathyroid tumours." J. Med. Genet. **40**(9): 657-663.

<http://jmg.bmjjournals.com/cgi/content/abstract/40/9/657>

Background: Hyperparathyroidism is a common endocrinopathy characterised by the formation of parathyroid tumours. In this study, we determine the role of the recently identified gene, HRPT2, in parathyroid tumorigenesis. Methods: Mutation analysis of HRPT2 was undertaken in 60 parathyroid tumours: five HPT-JT, three FIHP, three MEN 1, one MEN 2A, 25 sporadic adenomas, 17 hyperplastic glands, two lithium associated tumours, and four sporadic carcinomas. Loss of heterozygosity at 1q24-32 was performed on a subset of these tumours. Results: HRPT2 somatic mutations were detected in four of four sporadic parathyroid carcinoma samples, and germline mutations were found in five of five HPT-JT parathyroid tumours (two families) and two parathyroid tumours from one FIHP family. One HPT-JT tumour with germline mutation also harboured a somatic mutation. In total, seven novel and one previously reported mutation were identified. "Two-hits" (double mutations or one mutation and loss of heterozygosity at 1q24-32) affecting HRPT2 were found in two sporadic carcinomas, two HPT-JT-related and two FIHP related tumours. Conclusions: The results in this study support the role of HRPT2 as a tumour suppressor gene in sporadic parathyroid carcinoma, and provide further evidence for HRPT2 as the causative gene in HPT-JT, and a subset of FIHP. In light of the strong association between mutations of HRPT2 and sporadic parathyroid carcinoma demonstrated in this study, it is hypothesised that HRPT2 mutation is an early event that may lead to parathyroid malignancy and suggest intragenic mutation of HRPT2 as a marker of malignant potential in both familial and sporadic parathyroid tumours.

Hutchings, H., H. Ginisty, et al. (2005). "Identification of a new locus for isolated familial keratoconus at 2p24." J. Med. Genet. **42**(1): 88-94.

<http://jmg.bmjjournals.com>

Ishihara, N., K. Yamada, et al. (2004). "Clinical and molecular analysis of Mowat-Wilson syndrome associated with ZFX1B mutations and deletions at 2q22-q24.1." J. Med. Genet. **41**(5): 387-393.

<http://jmg.bmjjournals.com>

Jakkula, E., J. Lohiniva, et al. (2003). "A recurrent R718W mutation in COMP results in multiple epiphyseal dysplasia with mild myopathy: clinical and pathogenetic overlap with collagen IX mutations." J. Med. Genet. **40**(12): 942-948.

<http://jmg.bmjjournals.com>

Jezewski, P. A., A. R. Vieira, et al. (2003). "Complete sequencing shows a role for MSX1 in non-syndromic cleft lip and palate." J. Med. Genet. **40**(6): 399-407.

<http://jmg.bmjjournals.com/cgi/content/abstract/40/6/399>

MSX1 has been proposed as a gene in which mutations may contribute to non-syndromic forms of cleft lip and/or cleft palate. Support for this comes from human linkage and linkage disequilibrium studies, chromosomal deletions resulting in haploinsufficiency, a large family with a stop codon mutation that includes clefting as a phenotype, and the Msx1 phenotype in a knockout mouse. This report describes a population based scan for mutations encompassing the sense and antisense transcribed sequence of MSX1 (two exons, one intron). We compare the completed genomic sequence of MSX1 to the mouse Msx1 sequence to identify non-coding homology regions, and sequence highly conserved elements. The samples studied were drawn from a panethnic collection including people of European, Asian, and native South American ancestry. The gene was sequenced in 917 people and potentially aetiological mutations were identified in 16. These included missense mutations in conserved amino acids and point mutations in conserved regions not identified in any of 500 controls sequenced. Five different missense mutations in seven unrelated subjects with clefting are described. Evolutionary sequence comparisons of all known Msx1 orthologues placed the amino acid substitutions in context. Four rare mutations were found in non-coding regions that are highly conserved and disrupt probable regulatory regions. In addition, a panel of 18 population specific polymorphic variants were identified that will be useful in future haplotype analyses of MSX1. MSX1 mutations are found in 2% of cases of clefting and should be considered for genetic counselling implications, particularly in those families in which autosomal dominant inheritance patterns or dental anomalies appear to be cosegregating with the clefting phenotype.

Koillinen, H., P. Lahermo, et al. (2005). "A genome-wide scan of non-syndromic cleft palate only (CPO) in Finnish multiplex families." J. Med. Genet. **42**(2): 177-184.

<http://jmg.bmjjournals.com>

Koskenmies, S., P. Lahermo, et al. (2004). "Linkage mapping of systemic lupus erythematosus (SLE) in Finnish families multiply affected by SLE." J. Med. Genet. **41**(1): 2e-5.

<http://jmg.bmjjournals.com>

Matichard, E., P. Verpillat, et al. (2004). "Melanocortin 1 receptor (MC1R) gene variants may increase the risk of melanoma in France independently of clinical risk factors and UV exposure." J. Med. Genet. **41**(2): 13e-.

<http://jmg.bmjjournals.com>

Nakabayashi, K., S. Makino, et al. (2004). "Genomic imprinting of PPP1R9A encoding neurabin I in skeletal muscle and extra-embryonic tissues." J. Med. Genet. **41**(8): 601-608.

<http://jmg.bmjjournals.com>

Nelis, E., J. Berciano, et al. (2004). "Autosomal dominant axonal Charcot-Marie-Tooth disease type 2 (CMT2G) maps to chromosome 12q12-q13.3." J. Med. Genet. **41**(3): 193-197.

<http://jmg.bmjjournals.com>

Ohmori, H., Y. Ando, et al. (2004). "Common origin of the Val30Met mutation responsible for the amyloidogenic transthyretin type of familial amyloidotic polyneuropathy." J. Med. Genet. **41**(4): e51-.

<http://jmg.bmjjournals.com>

Roberts, P. S., S. Dabora, et al. (2004). "Somatic mosaicism is rare in unaffected parents of patients with sporadic tuberous sclerosis." J. Med. Genet. **41**(5): e69-.

<http://jmg.bmjjournals.com>

Stover, E. H., K. J. Borthwick, et al. (2002). "Novel ATP6V1B1 and ATP6V0A4 mutations in autosomal recessive distal renal tubular acidosis with new evidence for hearing loss." J. Med. Genet. **39**(11): 796-803.

<http://jmg.bmjjournals.com/cgi/content/abstract/39/11/796>

Autosomal recessive distal renal tubular acidosis (rdRTA) is characterised by severe hyperchloraemic metabolic acidosis in childhood, hypokalaemia, decreased urinary calcium solubility, and impaired bone physiology and growth. Two types of rdRTA have been differentiated by the presence or absence of sensorineural hearing loss, but appear otherwise clinically similar. Recently, we identified mutations in genes encoding two different subunits of the renal {alpha}-intercalated cell's apical H⁺-ATPase that cause rdRTA. Defects in the B1 subunit gene ATP6V1B1, and the a4 subunit gene ATP6V0A4, cause rdRTA with deafness and with preserved hearing, respectively. We have investigated 26 new rdRTA kindreds, of which 23 are consanguineous. Linkage analysis of seven novel SNPs and five polymorphic markers in, and tightly linked to, ATP6V1B1 and ATP6V0A4 suggested that four families do not link to either locus, providing strong evidence for additional genetic heterogeneity. In ATP6V1B1, one novel and five previously reported mutations were found in 10 kindreds. In 12 ATP6V0A4 kindreds, seven of 10 mutations were novel. A further nine novel ATP6V0A4 mutations were found in "sporadic" cases. The previously reported association between ATP6V1B1 defects and severe hearing loss in childhood was maintained. However, several patients with ATP6V0A4 mutations have developed hearing loss, usually in young adulthood. We show here that ATP6V0A4 is expressed within the human inner ear. These findings provide further evidence for genetic heterogeneity in rdRTA, extend the spectrum of disease causing mutations in ATP6V1B1 and

ATP6V0A4, and show ATP6V0A4 expression within the cochlea for the first time.

Tukel, T., A. Uzumcu, et al. (2005). "A new syndrome, congenital extraocular muscle fibrosis with ulnar hand anomalies, maps to chromosome 21qter." *J. Med. Genet.* **42**(5): 408-415.

<http://jmg.bmjjournals.com/cgi/content/abstract/42/5/408>

Background: Congenital fibrosis of the extraocular muscles (CFEOM) is a heterogeneous group of disorders that may be associated with other anomalies. The association of a CFEOM syndrome with ulnar hand abnormalities (CFEOM/U) has not been reported to date. Objective: To describe a new autosomal recessive syndrome of CFEOM and ulnar hand abnormalities, and localise the disease causing gene. Methods: Clinical evaluation of the affected members and positional mapping. Results: Six affected patients with CFEOM/U (aged 2 to 29 years) from a large consanguineous Turkish family were studied. Ophthalmological involvement was characterised by non-progressive restrictive ophthalmoplegia with blepharoptosis of the right eye. The postaxial oligodactyly/oligosyndactyly of the hands was more severe on the right side. A genome-wide scan established linkage of this new autosomal recessive syndrome to a locus on chromosome 21qter. The multipoint LOD score was 4.53 at microsatellite marker D21S1259, and fine mapping defined a [~]1.5 Mb critical region between microsatellite marker D21S1897 and the telomere of the long arm. Conclusions: CFEOM/U maps to a 1.5 Mb region at chromosome 21qter. Future identification of the disease causing gene may provide insights into the development of the extraocular muscles and brain stem {alpha} motor neurones, as well as anteroposterior limb development.

Warner, J., M. Epstein, et al. (2004). "Genetic testing in familial isolated hyperparathyroidism: unexpected results and their implications." *J. Med. Genet.* **41**(3): 155-160.

<http://jmg.bmjjournals.com/cgi/content/abstract/41/3/155>

Familial hyperparathyroidism is not uncommon in clinical endocrine practice. It encompasses a spectrum of disorders including multiple endocrine neoplasia types 1 (MEN1) and 2A, hyperparathyroidism-jaw tumour syndrome (HPT-JT), familial hypocalciuric hypercalcaemia (FHH), and familial isolated hyperparathyroidism (FIHP). Distinguishing among the five syndromes is often difficult but has profound implications for the management of patient and family. The availability of specific genetic testing for four of the syndromes has improved diagnostic accuracy and simplified family monitoring in many cases but its current cost and limited accessibility require rationalisation of its use. No gene has yet been associated exclusively with FIHP. FIHP phenotypes have been associated with mutant MEN1 and calcium-sensing receptor (CASR) genotypes and, very recently, with mutation in the newly identified HRPT2 gene. The relative proportions of these are not yet clear. We report results of MEN1, CASR, and HRPT2 genotyping of 22 unrelated subjects with FIHP phenotypes. We found 5 (23%) with MEN1 mutations, four (18%) with CASR mutations, and none with an HRPT2 mutation. All those with mutations had multiglandular hyperparathyroidism. Of the subjects with CASR mutations, none were of the typical FHH phenotype. These findings strongly favour a recommendation for MEN1 and CASR genotyping of patients with multiglandular FIHP, irrespective of urinary calcium excretion. However, it appears that HRPT2 genotyping should be reserved for cases in which other features of the HPT-JT phenotype have occurred in the kindred. Also apparent is the need for further investigation to identify additional genes associated with FIHP.

Yarden, J., D. Radojkovic, et al. (2004). "Polymorphisms in the mannose binding lectin gene affect the cystic fibrosis pulmonary phenotype." *J. Med. Genet.* **41**(8): 629-633.

<http://jmg.bmjjournals.com>

J. Med. Microbiol. (24)

Afset, J. E., K. Bergh, et al. (2003). "High prevalence of atypical enteropathogenic *Escherichia coli* (EPEC) in Norwegian children with diarrhoea." *J. Med. Microbiol.* **52**(11): 1015-1019.

<http://jmm.sgmjournals.org/cgi/content/abstract/52/11/1015>

The aim of the present study was to investigate the relative contribution of enteropathogenic *Escherichia coli* (EPEC) as a cause of infectious diarrhoea in Norwegian children. Data from faecal specimens from children <2 years old with diarrhoea during the year 2001 were analysed. *E. coli* isolates with the attaching and effacing genotype (eae+) were examined for the presence of the bundle-forming pilus (bfpA) and Shiga toxin genes by PCR, and for genetic relatedness by PFGE. During the 1-year period, 598 specimens from 440 patients <2 years old were analysed. Potential enteric pathogens were identified in 124 patients (28.2 %). EPEC was the most frequently identified agent (44 patients), followed by rotavirus (41 patients), *Campylobacter jejuni* (17 patients) and adenovirus (17 patients). All other agents were detected in five patients or less. Only one of the eae+ *E. coli* isolates was classified as typical EPEC (bfpA+). Among the 43 isolates that were classified as atypical EPEC (bfpA-), eight strains belonged to EPEC serogroups, whereas the majority of strains (n = 35) were not agglutinated by EPEC antisera. None of the EPEC isolates were genetically related. This study demonstrates that atypical EPEC of non-EPEC serogroups is highly prevalent among Norwegian children with diarrhoea.

Afset, J. E., L. Bevanger, et al. (2004). "Association of atypical enteropathogenic *Escherichia coli* (EPEC) with prolonged diarrhoea." *J. Med. Microbiol.* **53**(11): 1137-1144.

<http://jmm.sgmjournals.org/cgi/content/abstract/53/11/1137>

The aim of the present case control study was to investigate the prevalence of atypical enteropathogenic *Escherichia coli* (EPEC) and its possible role in causing diarrhoea among children < 5 years of age in Norway. Stool specimens received in the laboratory from children with suspected gastroenteritis (n = 251) were, in addition to routine testing, analysed for the presence of EPEC by PCR of the eae, bfpA and stx genes. Specimens from healthy children (n = 210) recruited from Maternal and Child Health Centres were analysed for EPEC only. EPEC isolates (eae+, stx-) were classified as typical (bfpA+) or atypical (bfpA-), and were tested for O: K serogroup. Information on duration of diarrhoea was recorded in a questionnaire and from referral forms. Atypical EPEC was diagnosed in 37 patients (14.7 %) compared to 21 (10.0 %) of the healthy controls [Odds ratio (OR) = 1.4, P = 0.3]. Only three isolates, all from patients, belonged to EPEC serogroups. One patient had typical EPEC. Twenty (22.5 %) of 89 patients with diarrhoea lasting $\geq$ 14 days had atypical EPEC. The association between atypical EPEC and prolonged diarrhoea (OR = 2.1, P = 0.04) was caused by a high prevalence among female patients (40.6 %). In conclusion, atypical EPEC was found to be slightly more prevalent in patients than controls, without any overall significant association with diarrhoea. However, a

significant association was observed with diarrhoea lasting 14 days or more, a finding that may indicate a role for atypical EPEC in prolonged disease.

Ahmad, S., Z. Khan, et al. (2004). "Isolation and molecular identification of *Candida dubliniensis* from non-human immunodeficiency virus-infected patients in Kuwait." *J. Med. Microbiol.* **53**(7): 633-637.

<http://jmm.sgmjournals.org/cgi/content/abstract/53/7/633>

Candida dubliniensis is an emerging pathogen capable of causing oropharyngeal, vaginal and bloodstream infections. Although *C. dubliniensis* is similar to *Candida albicans* in several phenotypic characteristics, it differs from it with respect to epidemiology, certain virulence factors and the ability to develop resistance to fluconazole rapidly. In this study, the first seven isolations of *C. dubliniensis* from Kuwait are described, all originating from non-human immunodeficiency virus (HIV)-infected patients. The isolates were initially identified by the Vitek 2 yeast identification system, positive germ tube test, production of rough colonies and chlamydospores on Staib agar and by their inability to assimilate xylose, trehalose or methyl $\{\alpha\}$ -D-glucoside. The species identity of the isolates was subsequently confirmed by specific amplification of rDNA targeting the internally transcribed spacer 2 (ITS2), restriction endonuclease digestion of the amplified DNA and direct DNA sequencing of the ITS2. Using the E-test method, the MICs of *C. dubliniensis* test isolates were in the range 0.125-0.75 $\{\mu\}$ g ml⁻¹ for fluconazole, 0.002-0.75 $\{\mu\}$ g ml⁻¹ for itraconazole, 0.006-0.125 $\{\mu\}$ g ml⁻¹ for ketoconazole, 0.002-0.5 $\{\mu\}$ g ml⁻¹ for amphotericin B and 0.002-0.016 $\{\mu\}$ g ml⁻¹ for voriconazole. Two of the isolates were resistant to 5-flucytosine (>32 $\{\mu\}$ g ml⁻¹), but none against fluconazole. The study reinforces the current view that *C. dubliniensis* has a much wider geographical and epidemiological distribution.

Al-Soud, W. A., M. Bennedsen, et al. (2003). "Assessment of PCR-DGGE for the identification of diverse *Helicobacter* species, and application to faecal samples from zoo animals to determine *Helicobacter* prevalence." *J. Med. Microbiol.* **52**(9): 765-771.

<http://jmm.sgmjournals.org/cgi/content/abstract/52/9/765>

Helicobacter species are fastidious bacterial pathogens that are difficult to culture by standard methods. A PCR-denaturing gradient gel electrophoresis (PCR-DGGE) technique for detection and identification of different *Helicobacter* species was developed and evaluated. The method involves PCR detection of *Helicobacter* DNA by genus-specific primers that target 16S rDNA and subsequent differentiation of *Helicobacter* PCR products by use of DGGE. Strains are identified by comparing mobilities of unknown samples to those determined for reference strains; sequence analysis can also be performed on purified amplicons. Sixteen DGGE profiles were derived from 44 type and reference strains of 20 *Helicobacter* species, indicating the potential of this approach for resolving infection of a single host by multiple *Helicobacter* species. Some more highly related species were not differentiated whereas in highly heterogeneous species, sequence divergence was observed and more than one PCR-DGGE profile was obtained. Application of the PCR-DGGE method to DNA extracted from faeces of zoo animals revealed the presence of *Helicobacter* DNA in 13 of 16 samples; a correlation was seen between the mobility of PCR products in DGGE analysis and DNA sequencing. In combination, this indicated that zoo animals are colonized by a wide range of different *Helicobacter* species; seven animals appeared to be colonized by multiple *Helicobacter* species. By this approach, presumptive identifications were made of *Helicobacter bilis* and *Helicobacter hepaticus* in a Nile crocodile, *Helicobacter cinaedi* in a baboon and a red panda, and *Helicobacter felis* in a wolf and a Taiwan beauty snake. All of these PCR products ($\sim$ 400 bp) showed 100 % sequence similarity to 16S rDNA sequences of the mentioned species. These results demonstrate the potential of PCR-DGGE-based analysis

for identification of *Helicobacter* species in complex ecosystems, such as the gastrointestinal tract, and could contribute to a better understanding of the ecology of helicobacters and other pathogens with a complex aetiology.

Clarke, L., J. E. Moore, et al. (2003). "Development of a diagnostic PCR assay that targets a heat-shock protein gene (*groES*) for detection of *Pseudomonas* spp. in cystic fibrosis patients." J. Med. Microbiol. **52**(9): 759-763.

<http://jmm.sgmjournals.org/cgi/content/abstract/52/9/759>

Laboratory detection of *Pseudomonas* spp., in particular *Pseudomonas aeruginosa*, remains an important assay in the management of patients with cystic fibrosis (CF). As the *groES* and *groEL* genes of *P. aeruginosa* have now been cloned and their nucleotide sequences determined, the aim of this study was to develop a novel PCR assay for the detection of *Pseudomonas* spp. from patients with CF by employing conserved primer regions of the *groE* heat-shock protein domain gene. A PCR assay was designed that targeted a 536 bp region of the *groE* gene to detect *Pseudomonas* spp. PCR amplification of genomic DNA from extracted organisms generated an amplicon of the expected size (approx. 536 bp) for all *P. aeruginosa* (n = 60), *Pseudomonas putida*, *Pseudomonas fluorescens* and *Pseudomonas stutzeri* isolates examined, but did not produce a positive amplicon for several other genera and species that are commonly isolated from the sputum of CF patients. RFLP analysis of the amplicons of all *P. aeruginosa* isolates demonstrated a single RFLP type that consisted of three bands at approximately 80, 190 and 250 bp; direct sequencing of the amplicons demonstrated the presence of a single sequence type, indicating the highly conserved nature of this region. In addition, the assay successfully produced a positive signal from primary non-selective plates of three known *P. aeruginosa* culture-positive CF patients, but was unable to generate a signal in a further six CF patients who had no history of infection with *P. aeruginosa* or other *Pseudomonas* spp. This assay is recommended to detect the presence of *Pseudomonas* spp., including *P. aeruginosa*, from primary culture plates that originate from laboratory analysis of CF patients' sputum, particularly at review, in those patients with no previous history of *Pseudomonas* infection or those who appear to be transiently colonized by this organism. Employment of such molecular methodologies, in conjunction with routine clinical sputum cultures, may provide improved information on the microbial status of CF patients, which will aid clinicians in both optimum patient management in terms of antibiotic regimes and CF centre infection-control practices.

Fry, N. K., O. Tzivra, et al. (2004). "Laboratory diagnosis of pertussis infections: the role of PCR and serology." J. Med. Microbiol. **53**(6): 519-525.

<http://jmm.sgmjournals.org/cgi/content/abstract/53/6/519>

This study reports on practical laboratory aspects of pertussis diagnosis. PCR assays were applied to respiratory specimens obtained during a large study of infants (less than 5 months old) admitted to paediatric intensive care units (n = 122), children (less than 15 years old) admitted to paediatric wards (n = 16) and their household contacts (n = 320). Estimation of antibodies to pertussis toxin and culture for *Bordetella pertussis* were attempted on specimens from the same patients, where available, and the overall utility of the diagnostic PCR assays was assessed by comparison to these results. A PCR assay for the human mitochondrial cytochrome oxidase (HMC) gene was used for quality control of the extracted samples and an internal process control (IPC) was included in each sample to test for PCR inhibition. Four of 458 samples were considered unsuitable (three HMC negative, one IPC negative) and excluded from further analyses. Positive PCR results were considered valid if they were either (i) positive for both of two *B. pertussis* gene targets (pertussis toxin S1 promoter and the insertion element IS481), i.e.

consensus PCR positive, or (ii) repeatably positive in only one assay. Using these criteria, 52 of 454 (11.5 %) samples were considered as PCR positive for *B. pertussis*. Six of 356 samples were culture-positive for *B. pertussis*, 1/88 infants, 3/14 children and 2/254 contacts, giving an overall isolation rate of 1.7 %. Using these data, PCR gave an almost fivefold increase in diagnostic yield compared with culture (McNemar's test; $P < 0.0001$). Sera from 9/111 infants, 5/10 children and 14/210 contacts were positive. Serology and PCR results showed a high level of agreement (113/121) for infants and children. PCR demonstrated a significant improvement in diagnostic yield over culture. Serological testing also resulted in a significant increase in diagnostic yield compared to culture alone. PCR is a useful technique, but validity of results must be assured by careful control. Rapid diagnosis of *B. pertussis* infection particularly in infants by PCR, together with serological assays, can enhance surveillance systems for pertussis in all age groups.

Goto, T., H. Nagamune, et al. (2002). "Rapid identification of *Streptococcus intermedius* by PCR with the *ily* gene as a species marker gene." *J. Med. Microbiol.* **51**(2): 178-186.

<http://jmm.sgmjournals.org/cgi/content/abstract/51/2/178>

Streptococcus intermedius belongs to the anginosus group of streptococci (AGS) and is associated with endogenous infections leading to abscesses in the oral cavity and at deep-seated sites, such as the brain and liver. Two other species, *S. anginosus* and *S. constellatus*, and some presently unnamed taxa, are also classified as AGS. Recently, *S. constellatus* subsp. *pharyngis*, a new subspecies with biochemical characteristics similar to *S. intermedius*, was described with the potential for causing confusion when trying to identify isolates of these two species routinely with commercial identification kits, such as Rapid ID32 Strep and Fluo-Card Milleri. To correctly identify *S. intermedius*, this study attempted to develop an accurate PCR identification system with the *ily* gene as a species marker. This approach relies on amplification of an 819-bp fragment of the *ily* gene and its 3'-flanking region and is shown here to be specific for *S. intermedius* strains among all other streptococcal species. Moreover, this PCR system was applicable in direct rapid PCR with whole bacterial cells and TaKaRa Z-Taq™ (TaKaRa), a highly efficient DNA polymerase, as the template and DNA amplification enzyme, respectively.

Jurstrand, M., J. S. Jensen, et al. (2005). "Detection of *Mycoplasma genitalium* in urogenital specimens by real-time PCR and by conventional PCR assay." *J. Med. Microbiol.* **54**(1): 23-29.

<http://jmm.sgmjournals.org/cgi/content/abstract/54/1/23>

A real-time LightCycler PCR (LC-PCR) with hybridization probes for detection of *Mycoplasma genitalium* in endocervical and first void urine specimens was developed and compared to a conventional PCR. The primers for both assays were identical and designed to amplify a 427 bp fragment of the 16S rRNA gene of *M. genitalium*. The LC-PCR assay had a detection limit of < 5 bacterial genomes per reaction when dilutions of genomic DNA from a type strain of *M. genitalium* were tested. First void urine from 398 men and first void urine and endocervical specimens from 301 women attending an STD clinic were analysed by LC-PCR and by the conventional PCR. Using the conventional PCR as reference, the LC-PCR had a specificity of 99.7 % and a sensitivity of 72.2 % for the detection of *M. genitalium* in first void urine samples from men. There was no significant difference in the performance of the LC-PCR assay compared to the conventional PCR when endocervical swabs were considered (58 and 65 %, respectively) or with a set of endocervical swab/urine specimens for which the LC-PCR assay detected 73 % of the infections (specificity = 98.6 % and sensitivity = 68.2 %) while the conventional PCR detected 85 % of the infections. With female urine specimens there was a significant difference between the two assays (38 and 73 %, respectively; $P = 0.01$ McNemar's test). This illustrates the need to analyse both endocervical and urine specimens, because *M.*

genitalium DNA was detected in only one of the two specimens in a great number of the M. genitalium-infected women. The lower sensitivity of the LC-PCR assay was probably caused by a combination of inhibition and limitations regarding the amount of template DNA. The LC-PCR assay was easy to perform and the simultaneous amplification and detection eliminated the need for further handling of PCR products. With improvement in sample preparation methods and increased volumes of the template DNA, the LC-PCR assay could be a useful routine diagnostic method.

Kobayashi, D., Y. Eishi, et al. (2002). "Gastric mucosal density of *Helicobacter pylori* estimated by real-time PCR compared with results of urea breath test and histological grading." J. Med. Microbiol. **51**(4): 305-311.

<http://jmm.sgmjournals.org/cgi/content/abstract/51/4/305>

The accuracy of the urea breath test (UBT) and histological grading for estimation of the density of *Helicobacter pylori* in gastric mucosa is not known. Real-time (TaqMan) PCR was used to estimate the total number of *H. pylori* genomes in biopsy samples. These values were compared with those obtained by the UBT and the histological grade obtained by the Sydney system. The UBT and endoscopy with antral and corporal biopsies were performed in 88 consecutive untreated patients with dyspepsia. Bacterial culture and the rapid urease test were done with fresh biopsy materials. TaqMan PCR and histological examination were done on serial paraffin sections of the biopsy samples. Of the five methods tested, TaqMan PCR had the highest sensitivity and specificity (both 100%) in the diagnosis of *H. pylori* infection. The mean density of *H. pylori* genomes for pairs of biopsy samples from individual patients was compared with the individual values obtained by the UBT; correlation between the results was significant. The density of *H. pylori* genomes was higher in histological grades 1, 2 and 3 than in grade 0, without significant differences between adjacent grades from 1 to 3. These results suggest that the severity of *H. pylori* infection of the stomach can be estimated by the UBT and that histopathologists might state whether the organism is present or absent, rather than making a quantitative statement as recommended in the Sydney system.

Kotlowski, R., A. Martin, et al. (2004). "One-tube cell lysis and DNA extraction procedure for PCR-based detection of *Mycobacterium ulcerans* in aquatic insects, molluscs and fish." J. Med. Microbiol. **53**(9): 927-933.

<http://jmm.sgmjournals.org/cgi/content/abstract/53/9/927>

The purpose of this study was to develop a simple procedure for cell lysis and DNA extraction for direct detection of *Mycobacterium ulcerans* in aquatic insects, gills and intestinal contents of fish, molluscs and human tissue samples using a nested PCR method specific for the insertion sequence IS2404. The simultaneous action of sodium N-lauroyl sarcosine, guanidinium isothiocyanate, chloroform and Tris-saturated phenol on mycobacteria, followed by a DNA purification method using mini-columns fitted with silica-cellulose membranes was successfully employed to extract DNA from cultured bacteria, environmental and human tissue samples. All specimens were collected from Buruli ulcer endemic regions. *M. ulcerans* DNA was detected in 11 of 57 aquatic insects, one of six molluscs and three of 15 fish, supporting the hypothesis that the fauna of major Buruli ulcer endemic foci in swampy terrain of tropical and subtropical regions can be a source of *M. ulcerans* infection.

Kulkarni, S. P., M. A. Jaleel, et al. (2005). "Evaluation of an in-house-developed PCR for the diagnosis of tuberculous meningitis in Indian children." J. Med. Microbiol. **54**(4): 369-373.

<http://jmm.sgmjournals.org/cgi/content/abstract/54/4/369>

Early and rapid detection of the causative organism is necessary in tuberculosis, particularly tuberculous meningitis, as the disease affects mainly children and if untreated or improperly treated can cause severe central nervous system disorders and can often be fatal. An in-house-developed PCR technique was developed for the detection of *Mycobacterium tuberculosis* DNA, in which the target for amplification was a 340 bp nucleotide sequence located within the 38 kDa protein gene. The test can detect as small an amount of DNA as 10 fg, which is equivalent to two to three organisms, and is highly specific. Amplified product was detected by ethidium bromide staining after electrophoresis and Southern hybridization. Evaluation of test sensitivity and specificity was carried out using acid-fast bacilli-positive sputum samples from patients with pulmonary tuberculosis and an equal number of non-tuberculosis patient samples as negative controls. In a double-masked study 30 cerebrospinal fluid samples from tuberculous meningitis patients and 30 samples from non-tuberculous meningitis patients were investigated. Out of the 30 samples 22 were positive by ethidium bromide-stained gel electrophoresis and 27 gave positive results by Southern hybridization. All of the 30 control samples showed negative results. The sensitivity of this PCR was 90 % and specificity, 100 %.

Leclercq, S., J. S. Harms, et al. (2002). "Induction of a Th1-type of immune response but not protective immunity by intramuscular DNA immunisation with *Brucella abortus* GroEL heat-shock gene." J. Med. Microbiol. **51**(1): 20-26.

<http://jmm.sgmjournals.org/cgi/content/abstract/51/1/20>

The immunogenicity and protective efficacy of a DNA vaccine encoding the GroEL heat-shock gene from *Brucella abortus* was tested in BALB/c mice immunised by intramuscular (i.m.) needle injection or epidermally by gene gun. The *Brucella* GroEL gene was amplified by PCR and cloned into two different mammalian expression vectors pCMV-link and pCMV-tPA. The D17 cell line was transfected with both constructs and GroEL transcripts were detected by Northern blot. To determine the level of protein synthesised, transfected cell lysates were then submitted to Western blot. The non-secreted form of the recombinant GroEL produced by the pCMV-link construct was detected in much greater amount than the secreted form of the protein produced by the pCMV-tPA construct. After immunisation, a strong anti-GroEL IgG response was detected in mice vaccinated by i.m. injection or gene gun only when the pCMV-link/GroEL plasmid was used. Regarding the pattern of immune response induced, i.m. needle injection raised a predominantly Th1 response with mostly IgG2a-specific anti-GroEL and high levels of IFN- γ produced by splenic T cells. Gene gun immunisation induced a Th0 type of immune response in mice characterised by a high IgG1/IgG2a ratio, and IL-4 and interferon (IFN)- γ production. Even though a distinct pattern of immune response was generated depending upon the immunisation route used, neither method engendered a significant level of protection with the GroEL DNA vaccine.

Leung, E. T.-Y., K.-M. Kam, et al. (2003). "Detection of katG Ser315Thr substitution in respiratory specimens from patients with isoniazid-resistant *Mycobacterium tuberculosis* using PCR-RFLP." J. Med. Microbiol. **52**(11): 999-1003.

<http://jmm.sgmjournals.org/cgi/content/abstract/52/11/999>

Mutations in the *katG* locus of catalase peroxidase in *Mycobacterium tuberculosis* (MTB) account for major isoniazid (INH) resistance. In the South China region, a collection of 906 respiratory specimens and 142 MTB isolates was used to evaluate the sensitivity and specificity of a PCR-RFLP method for the detection of INH resistance-associated mutations. Except for four catalase-negative MTB isolates, *katG* PCR for a 620-bp amplicon was successful for all purified MTB isolates. For respiratory specimens, diagnostic sensitivity and specificity of *katG* PCR was 85 and 100 %. Subsequent RFLP of the *katG* amplicons by *MspI* digestion identified that 51 % of INH-resistant MTB were associated with the Thr315 phenotype, and that codon 463 was a polymorphic site with no linkage to INH resistance. The Arg463 wild-type MTB isolates predominant in the Western world were replaced by isolates carrying Leu463 in the South China region. RFLP patterns of *katG* amplicons from respiratory specimens were identical to those of the corresponding MTB cultured colonies. This method has potential application for rapid diagnosis of INH resistance due to *katG* Ser315Thr mutation.

Liu, D., L. Pearce, et al. (2002). "PCR identification of dermatophyte fungi *Trichophyton rubrum*, *T. soudanense* and *T. gourvilii*." *J. Med. Microbiol.* **51**(2): 117-122.

<http://jmm.sgmjournals.org/cgi/content/abstract/51/2/117>

Diagnosis of dermatophytosis employing conventional laboratory procedures has been complicated by the slow growth and varied morphological features shown by dermatophytes. After analysis of the nucleotide base sequences of a 1.2-kb fragment amplified from a dermatophyte fungus *Trichophyton rubrum* by arbitrarily primed PCR with random primer OPD18, a pair of primers (TR1F and TR1R) was designed and evaluated for specific identification of *T. rubrum*. The sensitivity of the primers TR1F and TR1R was high, as a specific PCR band of c. 600 bp was detected from as little as 7 pg of *T. rubrum* DNA. By examining 92 dermatophyte strains and clinical isolates, it was found that this pair of primers reacted in PCR with *T. rubrum*, *T. soudanense* and *T. gourvilii* through formation of the specific fragment of 600 bp, but not with any other of the dermatophyte species or varieties, fungi, yeasts or bacteria tested. As *T. rubrum* is one of the most frequently isolated dermatophyte fungi, and *T. soudanense* and *T. gourvilii* are relatively uncommon in many parts of the world, these primers can be used for rapid, sensitive and specific identification and differentiation of *T. rubrum* from other fungi and micro-organisms.

Ma, L., G. Kutty, et al. (2003). "Characterization of variants of the gene encoding the p55 antigen in *Pneumocystis* from rats and mice." *J. Med. Microbiol.* **52**(11): 955-960.

<http://jmm.sgmjournals.org/cgi/content/abstract/52/11/955>

Variants of the p55 gene in rat-derived *Pneumocystis carinii* have been identified and its counterpart in mouse-derived *P. carinii* f. sp. *muris* has been cloned. By PCR amplification of *P. carinii* genomic DNA, five variants were identified that differed from each other in size and sequence, primarily in the number and size of encoded amino acid repeats. For *P. carinii* f. sp. *muris*, a single PCR fragment (471 bp) was obtained, which contained an incomplete ORF encoding a 157 aa protein that was most similar to a p55 variant in *P. carinii*, with nucleotide and amino acid sequence identity of 79 and 68 %, respectively. Southern blot analysis revealed the presence of more than one copy of the p55 gene in both *Pneumocystis* species. Thus, like other *Pneumocystis* antigens, p55 exhibits polymorphism that could potentially benefit the organism in host interactions.

Maegawa, T., T. Karasawa, et al. (2002). "Linkage between toxin production and purine biosynthesis in *Clostridium difficile*." *J. Med. Microbiol.* **51**(1): 34-41.

<http://jmm.sgmjournals.org/cgi/content/abstract/51/1/34>

The production of toxins A and B by *Clostridium difficile* was greatly enhanced under biotin-limited conditions, in which a 140-kDa protein was expressed strongly. Gene cloning revealed that this protein was a homologue of formylglycinamide ribonucleotide synthetase (FGAM synthetase, EC 6.3.5.3), which is known as PurL in *Escherichia coli* and catalyses the fourth step of the de novo purine biosynthesis pathway. This enzyme consisted of a single polypeptide, although FGAM synthetases of gram-positive bacteria usually consist of two subunits. Inhibition of the enzymic activity of *C. difficile* PurL by O-diazoacetyl-L-serine (azaserine) resulted in enhanced toxin B production even in biotin-sufficient conditions. In contrast, blockade of the preceding step of the PurL catalysing step by sulfamethoxazole inhibited toxin B production almost completely. These results suggest that accumulation of formylglycinamide ribonucleotide (FGAR), a substrate of FGAM synthetase, enhances toxin production by *C. difficile* and depletion of FGAR reduces toxin production.

Paciorek, J. (2002). "Virulence properties of *Escherichia coli* faecal strains isolated in Poland from healthy children and strains belonging to serogroups O18, O26, O44, O86, O126 and O127 isolated from children with diarrhoea." *J. Med. Microbiol.* **51**(7): 548-571.

<http://jmm.sgmjournals.org/cgi/content/abstract/51/7/548>

Four hundred and twenty-seven *Escherichia coli* isolates from 427 cases of infantile diarrhoea in Poland, belonging to serogroups O18, O26, O44, O86, O126 and O127 and 150 *E. coli* isolates from 52 healthy children were examined for selected virulence properties. The presence of the plasmid pAA, a plasmid encoding enterohaemolysin, the genes encoding intimin (eae), bundle-forming pili (bfp), Shiga toxins I and II (stxI, stxII) and cytotoxic necrotising factor types 1 and 2 (cnf1, cnf2) was investigated by PCR. Adhesion to HEP-2 cell monolayers was also tested and selected strains were investigated for the presence of P-fimbriae and haemolytic activity. Typical enteropathogenic *E. coli* isolates (typical EPEC, strains possessing eae and bfp, but not stx) were not found. The particular classes of *E. coli* among 427 isolates from ill children were: atypical EPEC (eae+ bfp, stx-), 21.3%; Shiga toxin-producing *E. coli* (STEC), 0.7%; diffusely adherent *E. coli* (DAEC), 4%; enteroaggregative *E. coli* (EAEC), 16.9%; necrotoxic *E. coli* type 1 (NTEC1), 0.2%; and cell-detaching *E. coli* (CDEC), 29%. With the exception of STEC, all the above classes of *E. coli* were found among the isolates from healthy children which comprised: atypical EPEC 8.0%, DAEC 6.7%, EAEC 17.3%, NTEC1 14.0% and CDEC 40.0%. Cell detachment (CD) was significantly associated with 3-h haemolytic activity. There was also strong correlation between haemolytic activity (Hly) and the presence of P-fimbriae. No correlation was found between the presence of the cnf1 gene and CD, Hly or P-fimbriae.

Panelius, J., P. Lahdenne, et al. (2002). "Recombinant OspC from *Borrelia burgdorferi sensu stricto*, *B. afzelii* and *B. garinii* in the serodiagnosis of Lyme borreliosis." *J. Med. Microbiol.* **51**(9): 731-739.

<http://jmm.sgmjournals.org/cgi/content/abstract/51/9/731>

Genes for the outer-surface protein C (OspC) from three north European human isolates of *Borrelia burgdorferi sensu stricto*, *B. afzelii* and *B. garinii* were cloned and sequenced. Polyhistidine-tagged recombinant OspC (rOspC) proteins were produced in *Escherichia coli* and used, after biotinylation, as antigens on streptavidin-coated plates in enzyme-linked

immunosorbent assays (ELISA). In IgM ELISA, 30% (5/17) and 35% (6/17) of patients with erythema migrans (EM) in the acute or convalescent phase, respectively, reacted with one to three rOspCs. Of the patients, 53% (8/15) with neuroborreliosis (NB) and 53% (8/15) with Lyme arthritis (LA) had IgM antibodies to OspC. The immunoreactivity was stronger against rOspC from *B. afzelii* and *B. garinii* than against rOspC from *B. burgdorferi sensu stricto*. In early Lyme borreliosis (LB), rOspC and flagella performed equally well in detecting IgM antibodies. Cross-reactive antibodies to rOspC were observed in serum samples from patients with rheumatoid factor positivity and with syphilis or Epstein-Barr virus (EBV) infection. In IgM ELISA, thiocyanate in the serum dilution buffer reduced EBV-associated non-specific positive reactions. Of the patient sera examined in IgG ELISA, 30% (5/17) with EM in the acute phase, 35% (6/17) with EM in the convalescent phase, 33% (5/15) with NB and 60% (9/15) with LA were positive. Because of the heterogeneity of OspC, a polyvalent antigen with several OspC variants from at least *B. afzelii* and *B. garinii* is needed to improve the sensitivity of OspC ELISA in the serodiagnosis of LB in Europe.

Robey, M., E. Morgan, et al. (2002). "A new chromosomal locus associated with gut-modulated phenotypes in *Salmonella enterica* serotype Typhimurium." *J. Med. Microbiol.* **51**(3): 247-263.

<http://jmm.sgmjournals.org/cgi/content/abstract/51/3/247>

A cosmid DNA library had been constructed previously from 40-kb fragments of genomic DNA from a virulent invasive strain of *Salmonella enterica* serotype Typhimurium (TML) in an avirulent hypo-invasive Typhimurium strain (LT7). Selection of invasive clones from the library was attempted by iterative passage through a rabbit ileal organ culture. After the fourth passage, a clone, designated LT7(pHC20uu.2), was isolated. Exposure to both gut tissue and Caco-2 cells enhanced the growth, invasiveness for gut and Caco-2 cells, and flagellin expression of LT7(pHC20uu.2) although its invasiveness was less than that of strain TML. Expression of appendages (surface structures c. 60-70 nm diameter) was shown to play a role in but not to confer invasiveness, and was demonstrated in the absence of direct contact with eukaryotic cells. Exposure to gut tissue also affected the expression of several outer-membrane proteins (OMPs) in all four *Salmonella* strains - TML, LT7, LT7(pHC79), LT7(pHC20uu.2) - used in this work. As the genes involved in flagella, invasins and porin expression are distributed around the *Salmonella* chromosome, it is possible that pHC20uu.2 encodes a pleiotropic regulator of genes involved in gastro-enteric virulence and adaptation to the in-vivo gut environment. pHC20uu.2 mapped at c. centisome 25 on the *Salmonella* chromosome close to, but distinct from, SPI-5.

Schmidt, D. and P.-M. Rath (2003). "Faster genetic identification of medically important aspergilli by using gellan gum as gelling agent in mycological media." *J. Med. Microbiol.* **52**(8): 653-655.

<http://jmm.sgmjournals.org/cgi/content/abstract/52/8/653>

Using gellan gum as a substitute for agar-agar in a mycological medium and sequencing of the ITS 1 and 2 regions resulted in an accurate identification of *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus terreus* and *Aspergillus ustus* within 24 h of subculture.

Smith, S. I., C. Kirsch, et al. (2002). "Prevalence of *Helicobacter pylori* vacA, cagA and iceA genotypes in Nigerian patients with duodenal ulcer disease." *J. Med. Microbiol.* **51**(10): 851-854.

<http://jmm.sgmjournals.org/cgi/content/abstract/51/10/851>

Distinct virulence factors of *Helicobacter pylori* have been associated with clinical outcome of the infection; however, considerable variations have been reported from different geographic regions. Data on genotypes of African *H. pylori* isolates are sparse. The aim of this study was to determine the prevalence of specific genotypes of *H. pylori* in Nigerian patients with duodenal ulcer and non-ulcer dyspepsia. *H. pylori* was cultured from endoscopic biopsies obtained from 41 Nigerian patients (19 with duodenal ulcer, 22 with non-ulcer dyspepsia). The *vacA* alleles, *cagA* and *iceA* genotypes were determined by PCR. The *vacA* s1,m1 and s1,m2 genotypes were found in 26.3% and 22.7%, and in 73.7% and 72.7% of *H. pylori* isolates from patients with duodenal ulcer and non-ulcer dyspepsia, respectively. The *iceA1* genotype was present in 94.7% and 86.4% of isolates from duodenal ulcer and non-ulcer dyspepsia patients, respectively. *cagA*⁺ infection was found predominantly (>90%) in Nigerian *H. pylori* isolates irrespective of the clinical diagnosis. In conclusion, *vacA* s1,m2, *iceA1* and *cagA*⁺ are common genotypes of *H. pylori* isolated from Nigerian patients. As in several other developing countries there seems to be no association between these genotypes and duodenal ulcer disease.

Soki, J., E. Fodor, et al. (2004). "Molecular characterization of imipenem-resistant, *cfiA*-positive *Bacteroides fragilis* isolates from the USA, Hungary and Kuwait." *J. Med. Microbiol.* **53**(5): 413-419.

<http://jmm.sgmjournals.org/cgi/content/abstract/53/5/413>

Fifteen *Bacteroides fragilis* isolates from the USA, Hungary and Kuwait were examined for carbapenem resistance, for carbapenemase activity and, with the use of various PCR-based methods and nucleotide sequencing, for *cfiA* genes and activating insertion sequence (IS) elements. All the *B. fragilis* isolates were *cfiA*-positive, 10 of the *cfiA* genes being upregulated by IS elements that are already known. Of these 10, one was of a novel type (designated IS943) and two further ones (IS614B and IS614C) were suspected hybrids of IS612, IS614 and IS942. There were five *cfiA*-positive imipenem-resistant *B. fragilis* isolates with elevated imipenem MICs (minimal inhibitory concentration) that harboured no IS insertion upstream of the *cfiA* gene, but produced carbapenemase; these isolates might possess a novel activation mechanism. On the basis of the available phenotypic and genotypic evidence, the present data suggest that there are at least two *cfiA* activation mechanisms among *B. fragilis* isolates.

Uehara, N., A. Yagihashi, et al. (2003). "Human {beta}-defensin-2 induction in *Helicobacter pylori*-infected gastric mucosal tissues: antimicrobial effect of overexpression." *J. Med. Microbiol.* **52**(1): 41-45.

<http://jmm.sgmjournals.org/cgi/content/abstract/52/1/41>

The objective of this study was to understand more of the innate immune response to *Helicobacter pylori* by determining the expression of human {beta}-defensin-2 (hBD-2) in various gastric mucosal tissues and MKN45 gastric cancer cells with or without *H. pylori*. Semi-quantitative TaqMan RT-PCR and immunohistochemistry were carried out. The antimicrobial effects of a transfected hBD-2 gene against *H. pylori* were also evaluated. The results showed that hBD-2 was expressed in inflamed gastric mucosal tissues with *H. pylori* infection, but not in the absence of *H. pylori* infection. Expression was also detected in gastric cancers in patients with *H. pylori* infection. Expression was induced in the MKN45 gastric cancer cell line by *H. pylori* in a manner dependent on the abundance of bacteria. hBD-2-transfected 3T3J2-1 cells secreted hBD-2 protein into the culture medium and this protein inhibited growth of *H. pylori* completely. The results suggest that hBD-2 may be involved in the pathophysiology of *H. pylori*-induced gastritis.

Xu, J., J. R. Rao, et al. (2002). "Improved molecular identification of *Thermoactinomyces* spp. associated with mushroom worker's lung by 16S rDNA sequence typing." J. Med. Microbiol. **51**(12): 1117-1127.

<http://jmm.sgmjournals.org/cgi/content/abstract/51/12/1117>

Mushroom worker's lung (MWL) is a hypersensitivity pneumonitis or allergic alveolitis caused by a type III IgG-mediated immunopathogenic inflammatory reaction in the host due to the inhalation of several thermophilic organisms, including *Thermoactinomyces* spp. It is difficult to distinguish phenotypically the eight species of this genus; therefore, this study sought to develop an improved molecular means of identifying *Thermoactinomyces* spp. associated with MWL by partial 16S rDNA PCR amplification and direct sequencing. Hypervariable regions within the 16S rRNA gene, which could be employed as signature sequences of the eight individual species, were identified and employed with highly conserved flanking primers to allow initial PCR amplification, before direct DNA sequencing of the 16S rDNA amplicons. A novel 24-mer 16S rDNA oligonucleotide upstream primer was designed from in silico alignments of all *Thermoactinomyces* spp. and was employed in combination with downstream (reverse) 16S rDNA primers. This permitted the successful identification of all four isolates associated with mushroom workers' lung. The method may be useful in the identification of *Thermoactinomyces* spp. associated with allergic alveolitis or pneumonitis associated with occupational exposure in agricultural and horticultural environments.

J. Mol. Endocrinol. (2)

Gadal, F., A. Starzec, et al. (2005). "Integrative analysis of gene expression patterns predicts specific modulations of defined cell functions by estrogen and tamoxifen in MCF7 breast cancer cells." J. Mol. Endocrinol. **34**(1): 61-75.

<http://jme.endocrinology-journals.org/cgi/content/abstract/34/1/61>

To explore the mechanisms whereby estrogen and antiestrogen (tamoxifen (TAM)) can regulate breast cancer cell growth, we investigated gene expression changes in MCF7 cells treated with 17{beta}-estradiol (E2) and/or with 4-OH-TAM. The patterns of differential expression were determined by the ValiGen Gene Identification (VGID) process, a subtractive hybridization approach combined with microarray validation screening. Their possible biologic consequences were evaluated by integrative data analysis. Over 1000 cDNA inserts were isolated and subsequently cloned, sequenced and analyzed against nucleotide and protein databases (NT/NR/EST) with BLAST software. We revealed that E2 induced differential expression of 279 known and 28 unknown sequences, whereas TAM affected the expression of 286 known and 14 unknown sequences. Integrative data analysis singled out a set of 32 differentially expressed genes apparently involved in broad cellular mechanisms. The presence of E2 modulated the expression patterns of 23 genes involved in anchors and junction remodeling; extracellular matrix (ECM) degradation; cell cycle progression, including G1/S check point and S-phase regulation; and synthesis of genotoxic metabolites. In tumor cells, these four mechanisms are associated with the acquisition of a motile and invasive phenotype. TAM partly reversed the E2-induced differential expression patterns and consequently restored most of the biologic functions deregulated by E2, except the mechanisms associated with cell cycle progression. Furthermore,

we found that TAM affects the expression of nine additional genes associated with cytoskeletal remodeling, DNA repair, active estrogen receptor formation and growth factor synthesis, and mitogenic pathways. These modulatory effects of E2 and TAM upon the gene expression patterns identified here could explain some of the mechanisms associated with the acquisition of a more aggressive phenotype by breast cancer cells, such as E2-independent growth and TAM resistance.

Kim, J., L. Jia, et al. (2005). "The role of protein kinase A pathway and cAMP responsive element-binding protein in androgen receptor-mediated transcription at the prostate-specific antigen locus." J. Mol. Endocrinol. **34**(1): 107-118.

<http://jme.endocrinology-journals.org/cgi/content/abstract/34/1/107>

Androgen-independent prostate cancer is a lethal form of the disease that is marked by metastasis and rapid proliferation in its final stages. As no effective therapy for this aggressive tumor currently exists, it is imperative to elucidate and target the mechanisms involved in the progression to androgen independence. Accumulating evidence indicates that aberrant activation of androgen receptor (AR) via signal transduction pathways, AR gene mutation and/or amplification, and/or coregulator alterations may contribute to the progression of prostate cancer. In the present study, the effects of protein kinase A (PKA) signaling and its downstream factors on AR activity at the prostate-specific antigen (PSA) gene were tested. Activation of PKA by forskolin resulted in enhanced androgen-induced expression of the PSA gene, an effect that was blocked by the AR antagonist, bicalutamide. Interestingly, when either p300 or CBP was overexpressed, PKA activation was sufficient to stimulate PSA promoter-driven transcription in the absence of androgen, which was not inhibited by bicalutamide. PKA activation did not significantly alter AR protein levels but significantly increased the phosphorylated form of its downstream effector, cAMP responsive element-binding protein (CREB) in the presence of androgen. Furthermore, chromatin immunoprecipitation showed that the combination of androgen and forskolin increased phosphorylated CREB occupancy, which was accompanied by histone acetylation, at the putative cAMP responsive element located in the 5' upstream regulatory region of the PSA gene. Remarkably, mammalian two-hybrid assay indicated that p300/CBP may bridge the interaction between AR and CREB, suggesting a novel enhanceosomal cooperation. These results demonstrate an intriguing interplay between a signal transduction pathway, coactivator overexpression and AR signaling as a possible combined mechanism of progression to androgen-independent prostate cancer.

J. Mollus. Stud. (4)

Adema, C. M. (2002). "COMPARATIVE STUDY OF CYTOPLASMIC ACTIN DNA SEQUENCES FROM SIX SPECIES OF PLANORBIDAE (GASTROPODA: BASOMMATOPHORA)." J. Mollus. Stud. **68**(1): 17-23.

<http://mollus.oupjournals.org/cgi/content/abstract/68/1/17>

Full-length actin-encoding sequences were PCR-amplified from genomic DNA of six planorbid species; *Biomphalaria glabrata* (Say; M-line strain), *B. alexandrina* (Ehrenberg), *B. pfeifferi* (Krauss), *B. tenagophila* (Orbigny), *B. obstructa* (Morelet) and *Helisoma trivolvis* (Say), using

primers designed from a previously reported *B. glabrata* cytoplasmic (β) actin cDNA. The amplified sequences contained two conserved exons (126 nt and 1005 nt, respectively), separated by an intron that varied in size between snail species (ranging from 671 to 794 nt). Sequence similarities occurred between the introns of the actin genes from *B. glabrata*, *B. alexandrina* and *B. pfeifferi* and between those from *B. tenagophila* and *B. obstructa*, yet considerable differences were evident between these two groups and the intron derived from *H. trivolvis*. Analysis of exons for sequence similarities, the presence of conserved residues (deduced amino acids), and construction of gene trees indicated that these planorbid genes encode cytoplasmic (β) actins rather than muscular (α) actins. Southern blotting and hybridisation experiments suggested that *B. glabrata* and *H. trivolvis* may have multiple (up to 5) actin genes, and it can not be ruled out that actin sequences obtained from different planorbid species were derived from paralogous genes. Interestingly however, the gene trees resolved actins derived from gastropod, cephalopod and bivalve molluscs. The sequences presented add to the growing body of information on the molecular biology of planorbid snails.

Huff, S. W., D. Campbell, et al. (2004). "INVESTIGATIONS INTO THE PHYLOGENETIC RELATIONSHIPS OF FRESHWATER PEARL MUSSELS (BIVALVIA: MARGARITIFERIDAE) BASED ON MOLECULAR DATA: IMPLICATIONS FOR THEIR TAXONOMY AND BIOGEOGRAPHY." *J. Mollus. Stud.* **70**(4): 379-388.

<http://mollus.oupjournals.org/cgi/content/abstract/70/4/379>

The phylogenetic relationships among selected members of the family Margaritiferidae are investigated using sequence data from five molecular markers. Parsimony analyses of the data support the recognition of those nominal species for which multiple samples were included in the study (*Margaritifera margaritifera*, *M. laevis*, *M. falcata* and *M. auricularia*). Although not always strongly supported, the following relationships were consistently recovered: (*Cumberlandia* + *Margaritifera auricularia*), (*M. falcata* (*M. marrianae* + *M. laevis*)) and to a lesser degree (*Dahurinaia dahurica* + *M. margaritifera*). The molecular phylogeny indicates that the taxonomy of the group is in need of revision since the genus *Margaritifera* is not monophyletic and a new taxonomy by Smith (2001) is not supported. A complicated pattern of biogeography was suggested by the three clades of Old World + New World species. It is difficult to determine whether this pattern is a reflection of extinction and contraction of an ancient, formerly widespread margaritiferid fauna, peripheral isolation of formerly widespread taxa, fish host dispersal, or even host switching.

Okusu, A. and G. Giribet (2003). "New 18S rRNA sequences from neomenioid aplacophorans and the possible origin of persistent exogenous contamination." *J. Mollus. Stud.* **69**(4): 385-387.

<http://mollus.oupjournals.org>

Zaslavskaya, N. I. and N. K. Kolotuchina (2003). "Genetic and morphological differentiation between two species of *Nucella* (Gastropoda: Muricidae) in the northwestern Pacific." *J. Mollus. Stud.* **69**(4): 381-385.

<http://mollus.oupjournals.org>

J. Neurol. Neurosurg. Psychiatry (1)

Cagli, S., N. Oktar, et al. (2003). "Failure to detect Chlamydia pneumoniae DNA in cerebral aneurysmal sac tissue with two different polymerase chain reaction methods." J. Neurol. Neurosurg. Psychiatry **74**(6): 756-759.

<http://jnnp.bmjournals.com/cgi/content/abstract/74/6/756>

Objective: Chlamydia pneumoniae (C pneumoniae) is a common cause of a usually mild, community acquired pneumonia. This organism, however, can spread from the respiratory tract into other parts of the body and has been detected in up to 70% of atheromatous lesions in blood vessels. Although the exact mechanism of the C Pneumoniae contribution to the pathogenesis of atherosclerosis remains unknown, prophylactic antibiotic trials are planned for people at high risk for coronary disease. Method: In this study the authors aimed to investigate C pneumoniae DNA content in the cerebral aneurysmal sac tissue with the aid of polymerase chain reaction (PCR) method. C pneumoniae DNA was searched in 15 surgically clipped and removed aneurysmal sac tissue and in two tumour (an ependymoma of the fourth ventricle and a craniofaringoma) samples by touchdown enzyme time release PCR (TETR PCR) targeting 16S rRNA gene and by nested PCR targeting ompA gene. Results: Both PCR methods were sensitive to detect in C pneumoniae 4x10⁻² genomes. C pneumoniae DNA was not detected in any of the 17 sample tissues of these patients. Conclusion: The contribution of C pneumoniae in the development of intracranial aneurysms cannot be excluded despite the results of this study. Further studies on the possible role of C pneumoniae or any other micro-organisms in the pathogenesis of aneurysms should be performed.

J. Neurosci. (32)

Akama, K. T. and B. S. McEwen (2003). "Estrogen Stimulates Postsynaptic Density-95 Rapid Protein Synthesis via the Akt/Protein Kinase B Pathway." J. Neurosci. **23**(6): 2333-2339.

<http://www.jneurosci.org/cgi/content/abstract/23/6/2333>

Estrogens induce synaptogenesis in the CA1 region of the dorsal hippocampus during the estrous cycle of the female rat. Functional consequences of such estrogen-mediated synaptogenesis include cyclic changes in neurotransmission and memory. At the molecular level, estrogen stimulates the rapid activation of specific signal transduction pathways, and of particular interest is the activation of Akt (protein kinase B), a key signal transduction intermediate that initiates protein translation by alleviating the downstream translational repression of eukaryotic initiation factor 4E-binding protein 1 (4E-BP1). Using a well established in vitro model system of differentiated NG108-15 neurons to investigate such rapid signaling effects of estrogen, we show that estrogen stimulates the phosphorylation of Akt, an indication of kinase activation, as well as the phosphorylation of 4E-BP1. In turn, the activation of these signaling intermediates suggests a non-genomic mechanism by which estrogen might likewise lead to protein translation of dendrite-localized mRNA transcripts in the hippocampus in vivo. We therefore considered the translation of the dendritic spine scaffolding protein postsynaptic density-95 (PSD-95). Although estrogen

does not stimulate a rapid increase in PSD-95 mRNA levels in NG108-15 neurons, we show here that estrogen does however stimulate a rapid increase in PSD-95 new protein synthesis in vitro and that this new protein synthesis is Akt dependent. These results demonstrate an essential role for Akt in estrogen-stimulated dendritic spine protein expression, describe for the first time a signal transduction pathway in PSD-95 expression, and delineate a novel, molecular mechanism by which ovarian hormones might translationally regulate synaptogenesis via activating protein synthesis for dendritic function.

Bark, C., F. P. Bellinger, et al. (2004). "Developmentally Regulated Switch in Alternatively Spliced SNAP-25 Isoforms Alters Facilitation of Synaptic Transmission." *J. Neurosci.* **24**(40): 8796-8805.

<http://www.jneurosci.org/cgi/content/abstract/24/40/8796>

Although the basic molecular components that promote regulated neurotransmitter release are well established, the contribution of these proteins as regulators of the plasticity of neurotransmission and refinement of synaptic connectivity during development is elaborated less fully. For example, during the period of synaptic growth and maturation in brain, the expression of synaptosomal protein 25 kDa (SNAP-25), a neuronal t-SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) essential for action potential-dependent neuroexocytosis, is altered through alternative splicing of pre-mRNA transcripts. We addressed the role of the two splice-variant isoforms of SNAP-25 with a targeted mouse mutation that impairs the shift from SNAP-25a to SNAP-25b. Most of these mutant mice die between 3 and 5 weeks of age, which coincides with the time when SNAP-25b expression normally reaches mature levels in brain and synapse formation is essentially completed. The altered expression of these SNAP-25 isoforms influences short-term synaptic function by affecting facilitation but not the initial probability of release. This suggests that mechanisms controlling alternative splicing between SNAP-25 isoforms contribute to a molecular switch important for survival that helps to guide the transition from immature to mature synaptic connections, as well as synapse regrowth and remodeling after neural injury.

Besong, G., G. Battaglia, et al. (2002). "Activation of Group III Metabotropic Glutamate Receptors Inhibits the Production of RANTES in Glial Cell Cultures." *J. Neurosci.* **22**(13): 5403-5411.

<http://www.jneurosci.org/cgi/content/abstract/22/13/5403>

The chemokine RANTES is critically involved in neuroinflammation and has been implicated in the pathophysiology of multiple sclerosis. We examined the possibility that activation of G-protein-coupled metabotropic glutamate (mGlu) receptors regulates the formation of RANTES in glial cells. A 15 hr exposure of cultured astrocytes to tumor necrosis factor-[alpha] and interferon-[gamma] induced a substantial increase in both RANTES mRNA and extracellular RANTES levels. These increases were markedly reduced when astrocytes were coincubated with L-2-amino-4-phosphonobutanoate (L-AP-4), 4-phosphonophenylglycine, or L-serine-O-phosphate, which selectively activate group III mGlu receptor subtypes (i.e., mGlu4, -6, -7, and -8 receptors). Agonists of mGlu1/5 or mGlu2/3 receptors were virtually inactive. Inhibition of RANTES release produced by L-AP-4 was attenuated by the selective group III mGlu receptor antagonist (R,S)-[alpha]-methylserine-O-phosphate or by pretreatment of the cultures with pertussis toxin. Cultured astrocytes expressed mGlu4 receptors, and the ability of L-AP-4 to inhibit RANTES release was markedly reduced in cultures prepared from mGlu4 knock-out mice. This suggests that activation of mGlu4 receptors negatively modulates the production of RANTES in glial cells. We also examined the effect of L-AP-4 on the development of experimental allergic encephalomyelitis (EAE) in Lewis rats. L-AP-4 was subcutaneously infused for 28 d by an osmotic minipump that released 250 nl/hr of a solution of 250 mM of the drug. Detectable levels of L-AP-4 (~100 nM)

were found in the brain dialysate of EAE rats. Infusion of L-AP-4 did not affect the time at onset and the severity of neurological symptoms but significantly increased the rate of recovery from EAE. In addition, lower levels of RANTES mRNA were found in the cerebellum and spinal cord of EAE rats infused with L-AP-4. These results suggest that pharmacological activation of group III mGlu receptors may be useful in the experimental treatment of neuroinflammatory CNS disorders.

Cirelli, C. and G. Tononi (2004). "Locus Ceruleus Control of State-Dependent Gene Expression." J. Neurosci. **24**(23): 5410-5419.

<http://www.jneurosci.org/cgi/content/abstract/24/23/5410>

Wakefulness and sleep are accompanied by changes in behavior and neural activity, as well as by the upregulation of different functional categories of genes. However, the mechanisms responsible for such state-dependent changes in gene expression are unknown. Here we investigate to what extent state-dependent changes in gene expression depend on the central noradrenergic (NA) system, which is active in wakefulness and reduces its firing during sleep. We measured the levels of [~]5000 transcripts expressed in the cerebral cortex of control rats and in rats pretreated with DSP-4 [N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine], a neurotoxin that removes the noradrenergic innervation of the cortex. We found that NA depletion reduces the expression of [~]20% of known wakefulness-related transcripts. Most of these transcripts are involved in synaptic plasticity and in the cellular response to stress. In contrast, NA depletion increased the expression of the sleep-related gene encoding the translation elongation factor 2. These results indicate that the activity of the central NA system during wakefulness modulates neuronal transcription to favor synaptic potentiation and counteract cellular stress, whereas its inactivity during sleep may play a permissive role to enhance brain protein synthesis.

Dahl, J. P., J. Wang-Dunlop, et al. (2003). "Characterization of the WAVE1 Knock-Out Mouse: Implications for CNS Development." J. Neurosci. **23**(8): 3343-3352.

<http://www.jneurosci.org/cgi/content/abstract/23/8/3343>

Developing neurons must respond to a wide range of extracellular signals during the process of brain morphogenesis. One mechanism through which immature neurons respond to such signals is by altering cellular actin dynamics. A recently discovered link between extracellular signaling events and the actin cytoskeleton is the WASP/WAVE (Wiscott-Aldrich Syndrome protein/WASP-family verprolin-homologous protein) family of proteins. Through a direct interaction with the Arp2/3 (actin-related protein) complex, this family functions to regulate the actin cytoskeleton by mediating signals from cdc42 as well as other small GTPases. To evaluate the role of WASP/WAVE proteins in the process of neuronal morphogenesis, we used a retroviral gene trap to generate a line of mice bearing a disruption in the WAVE1 gene. Using a heterologous reporter gene, we found that WAVE1 expression becomes increasingly restricted to the CNS over the course of development. Homozygous disruption of the WAVE1 gene results in postnatal lethality. In addition, these animals have severe limb weakness, a resting tremor, and notable neuroanatomical malformations without overt histopathology of peripheral organs. We did not detect any alterations in neuronal morphology in vivo or the ability of embryonic neurons to form processes in vitro. Our data indicate that WAVE1, although important for the general development of the CNS, is not essential for the formation and extension of neuritic processes.

De Zeeuw, C. I., E. Chorev, et al. (2003). "Deformation of Network Connectivity in the Inferior Olive of Connexin 36-Deficient Mice Is Compensated by Morphological and Electrophysiological Changes at the Single Neuron Level." J. Neurosci. **23**(11): 4700-4711.

<http://www.jneurosci.org/cgi/content/abstract/23/11/4700>

Compensatory mechanisms after genetic manipulations have been documented extensively for the nervous system. In many cases, these mechanisms involve genetic regulation at the transcription or expression level of existing isoforms. We report a novel mechanism by which single neurons compensate for changes in network connectivity by retuning their intrinsic electrical properties. We demonstrate this mechanism in the inferior olive, in which widespread electrical coupling is mediated by abundant gap junctions formed by connexin 36 (Cx36). It has been shown in various mammals that this electrical coupling supports the generation of subthreshold oscillations, but recent work revealed that rhythmic activity is sustained in knock-outs of Cx36. Thus, these results raise the question of whether the olivary oscillations in Cx36 knock-outs simply reflect the status of wild-type neurons without gap junctions or the outcome of compensatory mechanisms. Here, we demonstrate that the absence of Cx36 results in thicker dendrites with gap-junction-like structures with an abnormally wide interneuronal gap that prevents electrotonic coupling. The mutant olivary neurons show unusual voltage-dependent oscillations and an increased excitability that is attributable to a combined decrease in leak conductance and an increase in voltage-dependent calcium conductance. Using dynamic-clamp techniques, we demonstrated that these changes are sufficient to transform a wild-type neuron into a knock-out-like neuron. We conclude that the absence of Cx36 in the inferior olive is not compensated by the formation of other gap-junction channels but instead by changes in the cytological and electroresponsive properties of its neurons, such that the capability to produce rhythmic activity is maintained.

Dhingra, A., M. Jiang, et al. (2002). "Light Response of Retinal ON Bipolar Cells Requires a Specific Splice Variant of Galpha o." J. Neurosci. **22**(12): 4878-4884.

<http://www.jneurosci.org/cgi/content/abstract/22/12/4878>

Glutamate released onto retinal ON bipolar neurons binds to a metabotropic receptor to activate a heterotrimeric G-protein (Go) that ultimately closes a nonspecific cation channel. Signaling requires the [alpha] subunit (G[alpha]o), but its effector is unknown. Because G[alpha]o is transcribed into two splice variants ([alpha]o1 and [alpha]o2) that differ in the key GTPase domain, the next step in elucidating this pathway was to determine which splice variant carries the signal. Here we show by reverse transcription-PCR and Western blots that retina expresses both splice variants. Furthermore, in situ hybridization and immunostaining on mouse retina deficient in one splice variant or the other show that both [alpha]o1 and [alpha]o2 are expressed by ON bipolar cells but that [alpha]o1 is much more abundant. Finally, electroretinography performed on mice deficient for one splice variant or the other shows that the positive b-wave (response of ON bipolar cells to rod and cone input) requires [alpha]o1 but not [alpha]o2. Thus, the light response of the ON bipolar cell is probably carried by its strongly expressed splice variant, G[alpha]o1.

Esper, R. M. and J. A. Loeb (2004). "Rapid Axoglial Signaling Mediated by Neuregulin and Neurotrophic Factors." J. Neurosci. **24**(27): 6218-6227.

<http://www.jneurosci.org/cgi/content/abstract/24/27/6218>

During peripheral nervous system development, Schwann cells are precisely matched to the axons that they support. This is mediated by axonal neuregulins that are essential for Schwann cell survival and differentiation. Here, we show that sensory and motor axons rapidly release heparin-binding forms of neuregulin in response to Schwann cell-derived neurotrophic factors in a dose-dependent manner. Neuregulin release occurs within minutes, is saturable, and occurs from axons that were isolated using a newly designed chamber slide apparatus. Although NGF and glial cell line-derived neurotrophic factor (GDNF) were the most potent neurotrophic factors to release neuregulin from sensory neurons, GDNF and BDNF were most potent for motor neurons and were the predominant neuregulin-releasing neurotrophic factors produced by cultured Schwann cells. Comparable levels of neuregulin could be released at a similar rate from neurons after protein kinase C activation with the phorbol ester, phorbol 12-myristate 13-acetate, which has also been shown to promote the cleavage and release of neuregulin from its transmembrane precursor. The rapid release of neuregulin from axons in response to Schwann cell-derived neurotrophic factors may be part of a spatially restricted system of communication at the axoglial interface important for proper peripheral nerve development, function, and repair.

Giacobini, P., A. S. Kopin, et al. (2004). "Cholecystokinin Modulates Migration of Gonadotropin-Releasing Hormone-1 Neurons." J. Neurosci. **24**(20): 4737-4748.

<http://www.jneurosci.org/cgi/content/abstract/24/20/4737>

Expression of the brain-gut peptide cholecystokinin (CCK) in the developing olfactory-gonadotropin-releasing hormone-1 (GnRH-1) neuroendocrine systems was characterized, and the function of CCK in these systems was analyzed both in vivo and in vitro. We present novel data demonstrating that CCK transcript and protein are expressed in sensory cells in the developing olfactory epithelium and vomeronasal organ, with both ligand and receptors (CCK-1R and CCK-2R) found on olfactory axons throughout prenatal development. In addition, migrating GnRH-1 neurons in nasal regions express CCK-1R but not CCK-2R receptors. The role of CCK in olfactory-GnRH-1 system development was evaluated using nasal explants, after assessing that the in vivo expression of both CCK and CCK receptors was mimicked in this in vitro model. Exogenous application of CCK (10^{-7} M) reduced both olfactory axon outgrowth and migration of GnRH-1 cells. This inhibition was mediated by CCK-1R receptors. Moreover, CCK-1R but not CCK-2R antagonism caused a shift in the location of GnRH-1 neurons, increasing the distance that the cells migrated. GnRH-1 neuronal migration in mice carrying a genetic deletion of either CCK-1R or CCK-2R receptor genes was also analyzed. At embryonic day 14.5, the total number of GnRH-1 cells was identical in wild-type and mutant mice; however, the number of GnRH-1 neurons within forebrain was significantly greater in CCK-1R^{-/-} embryos, consistent with an accelerated migratory process. These results indicate that CCK provides an inhibitory influence on GnRH-1 neuronal migration, contributing to the appropriate entrance of these neuroendocrine cells into the brain, and thus represent the first report of a developmental role for CCK.

Hayasaka, N., S. I. LaRue, et al. (2002). "In Vivo Disruption of Xenopus CLOCK in the Retinal Photoreceptor Cells Abolishes Circadian Melatonin Rhythmicity without Affecting Its Production Levels." J. Neurosci. **22**(5): 1600-1607.

<http://www.jneurosci.org/cgi/content/abstract/22/5/1600>

Xenopus laevis retinas, like retinas from all vertebrate classes, have endogenous circadian clocks that control many aspects of normal retinal physiology occurring in cells throughout all layers of the retina. The localization of the clock(s) that controls these various rhythms remains unclear. One of the best studied rhythmic events is the nocturnal release of melatonin. Photoreceptor layers can synthesize rhythmic melatonin when these cells are in isolation. However, within the

intact retina, melatonin is controlled in a complex way, indicating that signals from many parts of the retina may contribute to the production of melatonin rhythmicity. To test this hypothesis, we generated transgenic tadpoles that express different levels of a dominant negative *Xenopus* CLOCK specifically in the retinal photoreceptors. Eyes from these tadpoles continued to produce melatonin at normal levels, but with greatly disrupted rhythmicity, the severity of which correlated with the transgene expression level. These results demonstrate that although many things contribute to melatonin production in vivo, the circadian clock localized in the retinal photoreceptors is necessary for its rhythmicity. Furthermore, these data show that the control of the level of melatonin synthesis is separable from the control of its rhythmicity and may be controlled by different molecular machinery. This type of specific "molecular lesion" allows perturbation of the clock in intact tissues and is valuable for dissection of clock control of tissue-level processes in this and other complex systems.

Kury, P., R. Greiner-Petter, et al. (2002). "Mammalian Achaete Scute Homolog 2 Is Expressed in the Adult Sciatic Nerve and Regulates the Expression of Krox24, Mob-1, CXCR4, and p57kip2 in Schwann Cells." J. Neurosci. **22**(17): 7586-7595.

<http://www.jneurosci.org/cgi/content/abstract/22/17/7586>

The molecular control mechanisms and regulatory molecules involved in nerve repair are not yet well known. Schwann cells have been attributed an important role in peripheral nerve regeneration; therefore, attention has been drawn to regulatory factors expressed by these glial cells. Here, we demonstrate that Mash2, a basic helix-loop-helix (bHLH) transcription factor previously shown to be crucial for placenta development, is expressed by Schwann cells of adult peripheral nerves. We observed that this gene is downregulated after nerve lesion and, using cDNA array hybridization technology, we could demonstrate that Mash2 is a regulator of Krox24, Mob-1, and CXCR4 expression in cultured Schwann cells. In addition, we provide strong evidence that Mash2 is a negative regulator of Schwann cell proliferation. Mash2 represents a first candidate for the missing class B bHLH proteins in peripheral nerves.

Li, Y., A. Ji, et al. (2004). "Cell-Specific Expression and Lipopolysaccharide-Induced Regulation of Tumor Necrosis Factor {alpha} (TNF{alpha}) and TNF Receptors in Rat Dorsal Root Ganglion." J. Neurosci. **24**(43): 9623-9631.

<http://www.jneurosci.org/cgi/content/abstract/24/43/9623>

The proinflammatory and lipopolysaccharide (LPS)-inducible cytokine tumor necrosis factor {alpha} (TNF{alpha}) has been shown to enhance primary sensory nociceptive signaling. However, the precise cellular sites of TNF{alpha} and TNF receptor synthesis are still a matter of controversy. Therefore, we differentiated the neuronal and non-neuronal sites of TNF{alpha}, TNFR1, and TNFR2 mRNA synthesis in dorsal root ganglion (DRG) of control rats and evaluated how their expression is altered under systemic challenge with LPS. In situ hybridization (ISH), RT-PCR analysis of laser-microdissected cells, and immunocytochemistry revealed absence of TNF{alpha} from DRG neurons and LPS-induced expression of TNF{alpha} exclusively in a subpopulation of non-neuronal DRG cells. Using RT-PCR and Northern blotting TNFR1 and TNFR2 mRNAs were found to be constitutively expressed and increased after LPS. TNFR1 mRNA was expressed in virtually all neurons and in non-neuronal cells with increased levels after LPS in both. TNFR2 was exclusively expressed and regulated in non-neuronal cells. RT-PCR analysis of microdissected DRG neurons and of the sensory neuronal cell line F11 confirmed the neuronal expression of TNFR1 and excluded that of TNFR2. Double ISH revealed varying levels of TNFR1 mRNA in virtually all DRG neurons including putative nociceptive neurons coding for calcitonin gene-related peptide, substance P, or vanilloid receptor 1. Taken together, we provide

evidence that non-neuronally synthesized TNF{alpha} may directly act on primary afferent neurons via TNFR1 but not TNFR2. This is likely to be relevant under conditions of inflammatory pain and infections accompanied by widespread TNF{alpha} synthesis and release and may drive sickness behavior.

Marciano, P. G., J. Brettschneider, et al. (2004). "Neuron-Specific mRNA Complexity Responses during Hippocampal Apoptosis after Traumatic Brain Injury." J. Neurosci. **24**(12): 2866-2876.

<http://www.jneurosci.org/cgi/content/abstract/24/12/2866>

In an effort to understand the complexity of genomic responses within selectively vulnerable regions after experimental brain injury, we examined whether single apoptotic neurons from both the CA3 and dentate differed from those in an uninjured brain. The mRNA from individual active caspase 3(+)/terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling [TUNEL(-)] and active caspase 3(+)/TUNEL(+) pyramidal and granule neurons in brain-injured mice were amplified and compared with those from nonlabeled neurons in uninjured brains. Gene analysis revealed that overall expression of mRNAs increased with activation of caspase 3 and decreased to below uninjured levels with TUNEL reactivity. Cell type specificity of the apoptotic response was observed with both regionally distinct expression of mRNAs and differences in those mRNAs that were maximally regulated. Immunohistochemical analysis for two of the most highly differentially expressed genes (prion and Sos2) demonstrated a correlation between the observed differential gene expression after traumatic brain injury and corresponding protein translation.

Meadows, L. S., J. Malhotra, et al. (2002). "Functional and Biochemical Analysis of a Sodium Channel beta 1 Subunit Mutation Responsible for Generalized Epilepsy with Febrile Seizures Plus Type 1." J. Neurosci. **22**(24): 10699-10709.

<http://www.jneurosci.org/cgi/content/abstract/22/24/10699>

Generalized epilepsy with febrile seizures plus type 1 is an inherited human epileptic syndrome, associated with a cysteine-to-tryptophan (C121W) mutation in the extracellular immunoglobulin domain of the auxiliary [beta]1 subunit of the voltage-gated sodium channel. The mutation disrupts [beta]1 function, but how this leads to epilepsy is not understood. In this study, we make several observations that may be relevant for understanding why this [beta]1 mutation results in seizures. First, using electrophysiological recordings from mammalian cell lines, coexpressing sodium channel [alpha] subunits and either wild-type [beta]1 or C121W[beta]1, we show that loss of [beta]1 functional modulation, caused by the C121W mutation, leads to increased sodium channel availability at hyperpolarized membrane potentials and reduced sodium channel rundown during high-frequency channel activity, compared with channels coexpressed with wild-type [beta]1. In contrast, neither wild-type [beta]1 nor C121W[beta]1 significantly affected sodium current time course or the voltage dependence of channel activation. We also show, using a *Drosophila* S2 cell adhesion assay, that the C121W mutation disrupts [beta]1-[beta]1 homophilic cell adhesion, suggesting that the mutation may alter the ability of [beta]1 to mediate protein-protein interactions critical for sodium channel localization. Finally, we demonstrate that neither functional modulation nor cell adhesion mediated by wild-type [beta]1 is occluded by coexpression of C121W[beta]1, arguing against the idea that the mutant [beta]1 acts as a dominant-negative subunit. Together, these data suggest that C121W[beta]1 causes subtle effects on channel function and subcellular distribution that bias neurons toward hyperexcitability and epileptogenesis.

Misawa, H., E. H. Sherr, et al. (2002). "Identification of a Monogenic Locus (jams1) Causing Juvenile Audiogenic Seizures in Mice." J. Neurosci. **22**(23): 10088-10093.

<http://www.jneurosci.org/cgi/content/abstract/22/23/10088>

Epilepsy is a debilitating disease with a strong genetic component. Positional cloning has identified a few genes for rare monogenic epilepsy syndromes; however, the genetics of common human epilepsies are too complex to be analyzed easily by current techniques. Mouse models of epilepsy can further this analysis by eliminating genetic background heterogeneity and enabling the production of sufficient numbers of offspring. Here, we report that Black Swiss mice have a heretofore unrecognized specific susceptibility to audiogenic seizures. These seizures are characterized by wild running, loss of righting reflex, and tonic flexion and extension, and are followed by a postictal period. The susceptibility to these seizures is developmentally regulated, peaking at 21 d of age and nearly disappearing by adulthood. Interestingly, both the susceptibility to seizures and their developmental regulation appear unrelated to hearing thresholds in the Black Swiss strain and backcrossed progeny. Genetic mapping and linkage analysis of hybrid mice localize the seizure gene, jams1 (juvenile audiogenic monogenic seizures), to a 1.6 {+/-} 0.5 centimorgan (cM) region on mouse chromosome 10, delimited by the gene basigin (Bsg) and marker D10Mit140. Interestingly, the majority of the critical region is syntenic to a region on human chromosome 19p13.3 implicated in a familial form of juvenile febrile convulsions. Cloning the gene for audiogenic seizures in these mice may provide important insight into the fundamental mechanisms for developmentally regulated human epilepsy syndromes.

Mittoux, V., S. Ouay, et al. (2002). "Corticostriatopallidal Neuroprotection by Adenovirus-Mediated Ciliary Neurotrophic Factor Gene Transfer in a Rat Model of Progressive Striatal Degeneration." J. Neurosci. **22**(11): 4478-4486.

<http://www.jneurosci.org/cgi/content/abstract/22/11/4478>

Ciliary neurotrophic factor (CNTF) is a potent protective factor for striatal neurons in animal models of Huntington's disease (HD). Clinical application of this potential therapeutic still requires the design and optimization of delivery systems. In the case of HD, spatial spread in the vast volume occupied by the striatum and long-term delivery of the factor are particular challenges for these systems. We explored the potential of adenovirus-mediated gene transfer to fulfill these requirements by studying the functional and anatomical effects of single-site striatal delivery of CNTF recombinant vectors in a rat model of HD. In an initial series of experiments, unilateral injections of CNTF adenovirus were performed in rats 10, 30, or 90 d before a 5 d neurotoxic treatment with systemic 3-nitropropionic acid (3NP). Preservation of striatal neurons was observed at all time points, demonstrating temporally extended neuroprotective effects of the CNTF adenovirus. In a second series of experiments, bilateral injections of CNTF adenovirus were performed in the medial aspect of the striatum 10 d before starting 3NP intoxication. Despite placement of the CNTF-producing vector outside the lateral striatal area susceptible to lesion, massive protection of corticostriatopallidal circuits was observed, associated with significant behavioral benefits. This spatial spread of neuroprotection is discussed with reference to the retrograde transport of the adenovirus vector and the anterograde transport of the transgenic CNTF. Overall, adenovirus-mediated CNTF gene transfer appears to be a potentially useful delivery system for widespread, long-term circuit neuroprotection in HD patients.

Moore, J. P., Jr., E. Shang, et al. (2002). "In Situ GABAergic Modulation of Synchronous Gonadotropin

Releasing Hormone-1 Neuronal Activity." *J. Neurosci.* **22**(20): 8932-8941.

<http://www.jneurosci.org/cgi/content/abstract/22/20/8932>

Evidence indicates that gonadotropin releasing hormone-1 [GnRH-1, also known as luteinizing hormone releasing hormone (LHRH)] neurons can exhibit synchronized neuroendocrine secretory activity before entrance into the CNS. In this study, we used calcium imaging to evaluate patterns of activity in individual, embryonic, GnRH-1 neurons as well as population dynamics of GnRH-1 neurons in mouse nasal explants maintained for 1 versus 3 weeks. Independent of age, GnRH-1 neurons displayed significant calcium peaks that synchronized at an interval of ~20 min across multiple GnRH-1 cells within an explant. Acute tetrodotoxin treatment decreased the amplitude of calcium peaks in individual GnRH-1 neurons and the duration but not the frequency of synchronized activity in the population of GnRH-1 neurons. Acute GABAB receptor antagonism increased the frequency of synchronized neuronal activity at both ages, whereas acute GABAA receptor antagonism decreased calcium oscillations in individual GnRH-1 cells as well as synchronization of the calcium pulses within the GnRH-1 population at the 1 week time point to background non-GnRH-1 cell levels. These results indicate that developing GnRH-1 neurons rely heavily on GABAergic signaling to initiate synchronized bouts of activity but thereafter, possess an innate capacity for synchronized activity patterns that are modulated by, but not completely dependent on GABAergic signaling.

Obata, K., H. Yamanaka, et al. (2004). "Role of Mitogen-Activated Protein Kinase Activation in Injured and Intact Primary Afferent Neurons for Mechanical and Heat Hypersensitivity after Spinal Nerve Ligation." *J. Neurosci.* **24**(45): 10211-10222.

<http://www.jneurosci.org/cgi/content/abstract/24/45/10211>

To investigate whether activation of mitogen-activated protein kinase (MAPK) in damaged and/or undamaged primary afferents participates in neuropathic pain after partial nerve injury, we examined the phosphorylation of extracellular signal-regulated protein kinase (ERK), p38 MAPK, and c-Jun N-terminal kinase (JNK) in the L4 and L5 dorsal root ganglion (DRG) in the L5 spinal nerve ligation (SNL) model. We first confirmed, using activating transcription factor 3 and neuropeptide Y immunoreactivity, that virtually all L4 DRG neurons are spared from axotomy in this model. In the injured L5 DRG, the L5 SNL induced the activation of ERK, p38, and JNK in different populations of DRG neurons. In contrast, in the uninjured L4 DRG, the L5 SNL induced only p38 activation in tyrosine kinase A-expressing small- to medium-diameter neurons. Intrathecal ERK, p38, and JNK inhibitor infusions reversed SNL-induced mechanical allodynia, whereas only p38 inhibitor application attenuated SNL-induced thermal hyperalgesia. Furthermore, the L5 dorsal rhizotomy did not prevent SNL-induced thermal hyperalgesia. We therefore hypothesized that p38 activation in the uninjured L4 DRG might be involved in the development of heat hypersensitivity in the L5 SNL model. In fact, the treatment of the p38 inhibitor and also anti-nerve growth factor reduced SNL-induced upregulation of brain-derived neurotrophic factor and transient receptor potential vanilloid type 1 expression in the L4 DRG. Together, our results demonstrate that the L5 SNL induces differential activation of MAPK in injured and uninjured DRG neurons and, furthermore, that MAPK activation in the primary afferents may participate in generating pain hypersensitivity after partial nerve injury.

Ohira, K., H. Kumanogoh, et al. (2005). "A Truncated Tropo-Myosine-Related Kinase B Receptor, T1, Regulates Glial Cell Morphology via Rho GDP Dissociation Inhibitor 1." *J. Neurosci.* **25**(6): 1343-1353.

<http://www.jneurosci.org/cgi/content/abstract/25/6/1343>

Through tropo-myosine-related kinase B (TrkB) receptors, brain-derived neurotrophic factor (BDNF) performs many biological functions such as neural survival, differentiation, and plasticity. T1, an isoform of TrkB receptors that lacks a tyrosine kinase, predominates in the adult mammalian CNS, yet its role remains controversial. In this study, to examine whether T1 transduces a signal and to determine its function, we first performed an affinity purification of T1-binding protein with the T1-specific C-terminal peptide and identified Rho GDP dissociation inhibitor 1 (GDI1), a GDP dissociation inhibitor of Rho small G-proteins, as a signaling protein directly associated with T1. The binding of BDNF to T1 caused Rho GDI1 to dissociate from the C-terminal tail of T1. Astrocytes cultured for 30 d expressed only endogenous T1 among the BDNF receptors. In 30 d cultured astrocytes, Rho GDI1, when dissociated in a BDNF-dependent manner, controlled the activities of the Rho GTPases, which resulted in rapid changes in astrocytic morphology. Furthermore, using 2 d cultured astrocytes that were transfected with T1, a T1 deletion mutant, or cyan fluorescent protein fusion protein of the T1-specific C-terminal sequence, we demonstrated that T1-Rho GDI1 signaling was indispensable for regulating the activities of Rho GTPases and for the subsequent morphological changes among astrocytes. Therefore, these findings indicate that the T1 signaling cascade can alter astrocytic morphology via regulation of Rho GTPase activity.

Oliver, D., M. Knipper, et al. (2003). "Resting Potential and Submembrane Calcium Concentration of Inner Hair Cells in the Isolated Mouse Cochlea Are Set by KCNQ-Type Potassium Channels." J. Neurosci. **23**(6): 2141-2149.

<http://www.jneurosci.org/cgi/content/abstract/23/6/2141>

Cochlear inner hair cells (IHCs) transduce sound-induced vibrations into a receptor potential (RP) that controls afferent synaptic activity and, consequently, frequency and timing of action potentials in the postsynaptic auditory neurons. The RP is thought to be shaped by the two voltage-dependent K⁺ conductances, IK,f and IK,s, that are carried by large-conductance Ca²⁺- and voltage-dependent K⁺ (BK)- and KV-type K⁺ channels. Using whole-cell voltage-clamp recordings in the acutely isolated mouse cochlea, we show that IHCs display an additional K⁺ current that is active at the resting membrane potential ([-]72 mV) and deactivates on hyperpolarization. It is potently blocked by the KCNQ-channel blockers linopirdine and XE991 but is insensitive to tetraethylammonium and 4-aminopyridine, which inhibit IK,f and IK,s, respectively. Single-cell PCR and immunocytochemistry showed expression of the KCNQ4 subunit in IHCs. In current-clamp experiments, block of the KCNQ current shifted the resting membrane potential by ~7 to [-]65 mV and led to a significant activation of BK channels. Using BK channels as an indicator for submembrane intracellular Ca²⁺ concentration ([Ca²⁺]_i), it is shown that the shift in IHC resting potential observed after block of the KCNQ channels leads to an increase in [Ca²⁺]_i to values [Ca²⁺]_i > 1 μM. In conclusion, KCNQ channels set the resting membrane potential of IHCs in the isolated organ of Corti and thus maintain [Ca²⁺]_i at low levels. Destabilization of the resting potential and increase in [Ca²⁺]_i, as may result from impaired KCNQ4 function in IHCs, provide a novel explanation for the progressive hearing loss (DFNA2) observed in patients with defective KCNQ4 genes.

Ono, T., N. Sekino-Suzuki, et al. (2003). "Alivin 1, a Novel Neuronal Activity-Dependent Gene, Inhibits Apoptosis and Promotes Survival of Cerebellar Granule Neurons." J. Neurosci. **23**(13): 5887-5896.

<http://www.jneurosci.org/cgi/content/abstract/23/13/5887>

Neurons require Ca^{2+} -dependent gene transcription for their activity-dependent survival, the mechanisms of which have not been fully elucidated yet. Here, we demonstrate that a novel primary response gene, *alivin 1* (*ali1*), is an activity-dependent gene and promotes survival of neurons. Sequence analyses reveal that rat, mouse, and human *Ali1* proteins contain seven leucine-rich repeats, one IgC2-like loop and a transmembrane domain, and display homology to *Kek* and *Trk* families. Expression of *ali1* mRNA in cultured cerebellar granule neurons is rigidly regulated by KCl and/or NMDA concentrations in the culture medium and tightly correlated to depolarization-dependent survival and/or NMDA-dependent survival of the granule neuron. *ali1* mRNA expression was regulated at the transcriptional step by the Ca^{2+} influx through voltage-dependent L-type Ca^{2+} channels when the cells were stimulated by 25 mM KCl. Expression of *ali1* mRNA in cultured cortical neurons was inhibited when their spontaneous electrical activity was blocked by tetrodotoxin. Thus, the expression is neuronal activity dependent. Overexpression of *Ali1* in cerebellar granule neurons inhibited apoptosis that was induced by the medium containing 5 mM KCl. The addition of anti-*Ali1* antiserum or the soluble putative extracellular *Ali1* domain to the 25 mM KCl-supported culture inhibited the survival of the granule neuron. These results suggest that expression of *ali1* promotes depolarization-dependent survival of the granule neuron. Mouse *ali1* was mapped to a locus ~ 55.3 cM from the centromere on chromosome 15 that is syntenic to positional candidate loci for familial Alzheimer's disease type 5 and Parkinson's disease 8 on human chromosome 12.

Pla, A. F., D. Maric, et al. (2005). "Canonical Transient Receptor Potential 1 Plays a Role in Basic Fibroblast Growth Factor (bFGF)/FGF Receptor-1-Induced Ca^{2+} Entry and Embryonic Rat Neural Stem Cell Proliferation." *J. Neurosci.* **25**(10): 2687-2701.

<http://www.jneurosci.org/cgi/content/abstract/25/10/2687>

Basic fibroblast growth factor (bFGF) and its major receptor FGF receptor-1 (FGFR-1) play an important role in the development of the cortex. The mechanisms underlying the mitogenic role of bFGF/FGFR-1 signaling have not been elucidated. Intracellular Ca^{2+} concentrations ($[\text{Ca}^{2+}]_i$) in proliferating cortical neuroepithelial cells are markedly dependent on Ca^{2+} entry (Maric et al., 2000a). The absence of voltage-dependent Ca^{2+} entry channels, which emerge later, indicates that other membrane mechanisms regulate $[\text{Ca}^{2+}]_i$ during proliferation. Canonical transient receptor potential (TRPC) family channels are candidates because they are voltage independent and are expressed during CNS development (Strubing et al., 2003). Here, we investigated the involvement of TRPC1 in bFGF-mediated Ca^{2+} entry and proliferation of embryonic rat neural stem cells (NSCs). Both TRPC1 and FGFR-1 are expressed in the embryonic rat telencephalon and coimmunoprecipitate. Quantitative fluorescence-activated cell sorting analyses of phenotyped telencephalic dissociates show that $\sim 80\%$ of NSCs are TRPC1+, proliferating, and express FGFR-1. Like NSCs profiled ex vivo, NSC-derived progeny proliferating in vitro coexpress TRPC1 and FGFR1. Antisense knock-down of TRPC1 significantly decreases bFGF-mediated proliferation of NSC progeny, reduces the Ca^{2+} entry component of the Ca^{2+} response to bFGF without affecting Ca^{2+} release from intracellular stores or 1-oleoyl-2-acetyl-sn-glycerol-induced Ca^{2+} entry, and significantly blocks an inward cation current evoked by bFGF in proliferating NSCs. Both Ca^{2+} influx evoked by bFGF and NSC proliferation are attenuated by Gd^{3+} and SKF96365 two antagonists of agonist-stimulated Ca^{2+} entry. Together, these results show that TRPC1 contributes to bFGF/FGFR-1-induced Ca^{2+} influx, which is involved in self-renewal of embryonic rat NSCs.

Rathee, P. K., C. Distler, et al. (2002). "PKA/AKAP/VR-1 Module: A Common Link of Gs-Mediated Signaling to Thermal Hyperalgesia." *J. Neurosci.* **22**(11): 4740-4745.

<http://www.jneurosci.org/cgi/content/abstract/22/11/4740>

Inflammatory mediators not only activate "pain"-sensing neurons, the nociceptors, to trigger acute pain sensations, more important, they increase nociceptor responsiveness to produce inflammatory hyperalgesia. For example, prostaglandins activate Gs-protein-coupled receptors and initiate cAMP- and protein kinase A (PKA)-mediated processes. We demonstrate for the first time at the cellular level that heat-activated ionic currents were potentiated after exposure to the cAMP activator forskolin in rat nociceptive neurons. The potentiation was prevented in the presence of the selective PKA inhibitor PKI14-22, suggesting PKA-mediated phosphorylation of the heat transducer protein. PKA regulatory subunits were found in close vicinity to the plasma membrane in these neurons, and PKA catalytic subunits only translocated to the cell periphery when activated. The translocation and the current potentiation were abolished in the presence of an A-kinase anchoring protein (AKAP) inhibitor. Similar current changes after PKA activation were obtained from human embryonic kidney 293t cells transfected with the wild-type heat transducer protein vanilloid receptor 1 (VR-1). The forskolin-induced current potentiation was greatly reduced in cells transfected with VR-1 mutants carrying point mutations at the predicted PKA phosphorylation sites. The heat transducer VR-1 is therefore suggested as the molecular target of PKA phosphorylation, and potentiation of current responses to heat depends on phosphorylation at predicted PKA consensus sites. Thus, the PKA/AKAP/VR-1 module presents as the molecular correlate of Gs-mediated inflammatory hyperalgesia.

Rizzi, M., S. Caccia, et al. (2002). "Limbic Seizures Induce P-Glycoprotein in Rodent Brain: Functional Implications for Pharmacoresistance." *J. Neurosci.* **22**(14): 5833-5839.

<http://www.jneurosci.org/cgi/content/abstract/22/14/5833>

The causes and mechanisms underlying multidrug resistance (MDR) in epilepsy are still elusive and may depend on inadequate drug concentration in crucial brain areas. We studied whether limbic seizures or anticonvulsant drug treatments in rodents enhance the brain expression of the MDR gene (*mdr*) encoding a permeability glycoprotein (P-gp) involved in MDR to various cancer chemotherapeutic agents. We also investigated whether changes in P-gp levels affect anticonvulsant drug concentrations in the brain. *Mdr* mRNA measured by RT-PCR increased by 85% on average in the mouse hippocampus 3-24 hr after kainic acid-induced limbic seizures, returning to control levels by 72 hr. Treatment with therapeutic doses of phenytoin or carbamazepine for 7 d did not change *mdr* mRNA expression in the mouse hippocampus 1-72 hr after the last drug administration. Six hours after seizures, the brain/plasma ratio of phenytoin was reduced by 30% and its extracellular concentration estimated by microdialysis was increased by twofold compared with control mice. Knock-out mice (*mdr1a/b* [-]/[-]) lacking P-gp protein showed a 46% increase in phenytoin concentrations in the hippocampus 1 and 4 hr after injection compared with wild-type mice. A significant 23% increase was found in the cerebellum at 1 hr and in the cortex at 4 hr. Carbamazepine concentrations were measurable in the hippocampus at 3 hr in *mdr1a/b* [-]/[-] mice, whereas they were undetectable at the same time interval in wild-type mice. In rats having spontaneous seizures 3 months after electrically induced status epilepticus, *mdr1* mRNA levels were enhanced by 1.8-fold and fivefold on average in the hippocampus and entorhinal cortex, respectively. Thus, changes in P-gp mRNA levels occur in limbic areas after both acute and chronic epileptic activity. P-gp alterations significantly affect antiepileptic drugs concentrations in the brain, suggesting that seizure-induced *mdr* mRNA expression contributes to MDR in epilepsy.

Sedelis, M., K. Hofele, et al. (2003). "Chromosomal Loci Influencing the Susceptibility to the Parkinsonian Neurotoxin 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine." *J. Neurosci.* **23**(23): 8247-8253.

<http://www.jneurosci.org/cgi/content/abstract/23/23/8247>

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the dysfunction of the nigrostriatal dopaminergic pathway. Although its etiology is not yet fully understood, an interaction of genetic predisposition and environmental factors is frequently discussed. The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) can evoke PD-like symptoms and neuropathological changes in various species, including mice. It was found repeatedly that mouse strains differ in their susceptibility to MPTP, which might serve as a model for genetic predisposition to neurodegeneration of the nigrostriatal system. In the present study, F2 intercross mice, derived from parental strains with high (C57BL/6J) versus low (BALB/cJ) MPTP susceptibility, were treated with MPTP and phenotyped for dopamine (DA) loss in the neostriatum, a highly sensitive marker of nigrostriatal dysfunction. A subsequent quantitative trait loci analysis revealed a gender-dependent locus for DA loss on chromosome 15 and a putative locus on chromosome 13. A number of potential candidate genes, including the membrane dopamine transporter, are located in the respective areas. Several mechanisms that are possibly involved in the control of the action of MPTP on the nigrostriatal system are discussed.

Seifert, G., K. Hutmacher, et al. (2004). "Enhanced Relative Expression of Glutamate Receptor 1 Flip AMPA Receptor Subunits in Hippocampal Astrocytes of Epilepsy Patients with Ammon's Horn Sclerosis." J. Neurosci. **24**(8): 1996-2003.

<http://www.jneurosci.org/cgi/content/abstract/24/8/1996>

Astrocytes express ionotropic glutamate receptors (GluRs), and recent evidence suggests that these receptors contribute to direct signaling between neurons and glial cells in vivo. Here, we have used functional and molecular analyses to investigate receptor properties in astrocytes of human hippocampus resected from patients with pharmacoresistant temporal lobe epilepsy (TLE). Histopathological analysis allowed us to distinguish two forms of epilepsy: Ammon's horn sclerosis (AHS) and lesion-associated TLE. Human hippocampal astrocytes selectively expressed the AMPA subtype of ionotropic glutamate receptors. Single-cell RT-PCR found preferential expression of the subunits GluR1 and GluR2 in human astrocytes, and the expression patterns were similar in patients with AHS and lesion-associated epilepsy. The AMPA receptor-specific modulators, cyclothiazide (CTZ) and 4-[2-(phenylsulfonylamino)ethylthio]-2,6-difluoro-phenoxyacetamide (PEPA), were used to investigate splice variant expression. Astrocytes of sclerotic specimens displayed a slower dissociation of CTZ from the receptor and a lower ratio of current potentiation by PEPA to potentiation by CTZ, suggesting enhanced expression of flip receptor variants in AHS versus lesion-associated epilepsy. Real-time PCR and restriction analysis substantiated this presumption by identifying elevated flip-to-flop mRNA ratios of GluR1 in single astrocytes of AHS specimens. These findings imply that in AHS, glutamate may lead to prolonged depolarization of astrocytes, thereby facilitating the generation or spread of seizure activity.

Shankar, S. L., K. O'Guin, et al. (2003). "The Growth Arrest-Specific Gene Product Gas6 Promotes the Survival of Human Oligodendrocytes via a Phosphatidylinositol 3-Kinase-Dependent Pathway." J. Neurosci. **23**(10): 4208-4218.

<http://www.jneurosci.org/cgi/content/abstract/23/10/4208>

Microarray analysis revealed that transcripts for the Axl and Mer receptor tyrosine kinases are expressed at high levels in O4+-immunopanned oligodendrocytes isolated from second trimester human fetal spinal cord. In humans the sole known ligand for the Axl/Rse/Mer kinases is growth arrest-specific gene 6 (Gas6), which in the CNS is secreted by neurons and endothelial cells. We hypothesized that Gas6 is a survival factor for oligodendrocytes and receptor activation signals downstream to the phosphatidylinositol 3 (PI3)-kinase/Akt pathway to increase cell survival in the

absence of cell proliferation. To test this hypothesis, we grew enriched human oligodendrocytes for 6 d on a monolayer of NIH3T3 cells stably expressing Gas6. CNP+ oligodendrocytes on Gas6-secreting 3T3 cells had more primary processes and arborizations than those plated solely on 3T3 cells. Also, a twofold increase in CNP+ and MBP+ oligodendrocytes was observed when they were plated on the Gas6-secreting cells. The effect was abolished in the presence of Axl-Fc but remained unchanged in the presence of the irrelevant receptor fusion molecule TrkA-Fc. A significant decrease in CNP+/TUNEL+ oligodendrocytes was observed when recombinant human Gas6 (rhGas6) was administered to oligodendrocytes plated on poly-L-lysine, supporting a role for Gas6 signaling in oligodendrocyte survival during a period of active myelination in human fetal spinal cord development. PI3-kinase inhibitors blocked the anti-apoptotic effect of rhGas6, whereas a MEK/ERK inhibitor had no effect. Thus Gas6 sustains human fetal oligodendrocyte viability by receptor activation and downstream signaling via the PI3-kinase/Akt pathway.

Sokolowski, B. H. A., Y. Sakai, et al. (2004). "Identification and Localization of an Arachidonic Acid-Sensitive Potassium Channel in the Cochlea." *J. Neurosci.* **24**(28): 6265-6276.

<http://www.jneurosci.org/cgi/content/abstract/24/28/6265>

Receptor cells of the auditory and vestibular end organs of vertebrates acquire various types of potassium channels during development. Their expression and kinetics can differ along the tonotopic axis as well as in different cell types of the sensory epithelium. These variations can play a crucial role in modulating sensory transduction and cochlear tuning. Whole-cell tight-seal recordings of isolated hair cells revealed the presence of an arachidonic acid-sensitive A-type channel in the short (outer) hair cells of the chicken cochlea. This polyunsaturated fatty acid blocked the A-current, thereby increasing the amplitude and duration of the voltage response in these cells. We identified the gene encoding this channel as belonging to a member of the Shal subfamily, Kv4.2. Expression of the recombinant channel shows half-activation and inactivation potentials shifted to more positive values relative to native channels, suggesting that the native channel is coexpressed with an accessory subunit. RT-PCR revealed that transcription begins early in development, whereas in situ hybridization showed mRNA expression limited to the intermediate and short hair cells located in specific regions of the adult cochlea. Additional localization, using immunofluorescent staining, revealed clustering in apical-lateral regions of the receptor cell as well as in the cochlear ganglion. These experiments provide evidence that in addition to membrane proteins modulating excitation in these receptor cells, fatty acids contribute to the coding of auditory stimuli via these channels.

Suzuki, T., I. Hide, et al. (2004). "Production and Release of Neuroprotective Tumor Necrosis Factor by P2X7 Receptor-Activated Microglia." *J. Neurosci.* **24**(1): 1-7.

<http://www.jneurosci.org/cgi/content/abstract/24/1/1>

After a brain insult, ATP is released from injured cells and activates microglia. The microglia that are activated in this way then release a range of bioactive substances, one of which is tumor necrosis factor (TNF). The release of TNF appears to be dependent on the P2X7 receptor. The inhibitors 1,4-diamino-2,3-dicyano-1,4-bis[2-amino-phenylthio]butadiene (U0126), anthra[1,9-cd]pyrazol-6(2H)-one (SP600125), and 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1H-imidazole (SB203580), which target MEK (mitogen-activated protein kinase kinase), JNK (c-Jun N-terminal kinase), and p38, respectively, all potentially suppress the production of TNF in ATP-stimulated microglia, whereas the production of TNF mRNA is strongly inhibited by U0126 and SP600125. SB203580 did not affect the increased levels of TNF mRNA but did prevent TNF mRNA from accumulating in the cytoplasm. The ATP-provoked activation of JNK and p38 [but not extracellular signal-regulated kinase (ERK)] could be inhibited by brilliant blue G, a P2X7 receptor

blocker, and by genistein and 4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo[3,4-D]pyrimidine, which are general and src-family-specific tyrosine kinase inhibitors, respectively. Most important, we found that treatment of the microglia in neuron-microglia cocultures with the P2X7 agonist 2'-3'-O-(benzoyl-benzoyl) ATP led to significant reductions in glutamate-induced neuronal cell death, and that either TNF- α converting enzyme inhibitor or anti-TNF readily suppressed the protective effect implied by this result. Together, these findings indicate that both ERK and JNK are involved in the regulation of TNF mRNA expression, that p38 is involved in the nucleocytoplasmic transport of TNF mRNA, and that a PTK (protein tyrosine kinase), possibly a member of the src family, acts downstream of the P2X7 receptor to activate JNK and p38. Finally, our data suggest that P2X7 receptor-activated microglia protect neurons against glutamate toxicity primarily because they are able to release TNF.

Tabakoff, B., S. V. Bhavé, et al. (2003). "Selective Breeding, Quantitative Trait Locus Analysis, and Gene Arrays Identify Candidate Genes for Complex Drug-Related Behaviors." *J. Neurosci.* **23**(11): 4491-4498.

<http://www.jneurosci.org/cgi/content/abstract/23/11/4491>

Acute functional tolerance to ethanol develops during a single exposure to ethanol; it has been suggested to be a predisposing factor for the development of ethanol dependence. Genetic determinants of acute functional tolerance, as well as of ethanol dependence, have been clearly demonstrated. We describe a novel approach that uses a combination of selective breeding (to segregate genes contributing to the phenotype of interest, i.e., acute functional tolerance to the incoordinating effect of ethanol), quantitative trait locus analysis (to define chromosomal regions associated with acute functional tolerance), and DNA microarray technology (to identify differentially expressed genes in the brains of the selected lines of mice) to identify candidate genes for the complex phenotype of ethanol tolerance. The results indicate the importance of a signal transduction cascade that involves the glutamate receptor δ 2 protein, the Ephrin B3 ligand, and the NMDA receptor, as well as a transcriptional regulatory protein that may be induced by activation of the NMDA receptor (zinc finger protein 179) and a protein that can modulate downstream responses to NMDA receptor activation (peroxiredoxin), in mediating acute tolerance to the incoordinating effect of ethanol.

Trankner, D., H. Jagle, et al. (2004). "Molecular Basis of an Inherited Form of Incomplete Achromatopsia." *J. Neurosci.* **24**(1): 138-147.

<http://www.jneurosci.org/cgi/content/abstract/24/1/138>

Mutations in the genes encoding the CNGA3 and CNGB3 subunits of the cyclic nucleotide-gated (CNG) channel of cone photoreceptors have been associated with autosomal recessive achromatopsia. Here we analyze the molecular basis of achromatopsia in two siblings with residual cone function. Psychophysical and electroretinographic analyses show that the light sensitivity of the cone system is lowered, and the signal transfer from cones to secondary neurons is perturbed. Both siblings carry two mutant CNGA3 alleles that give rise to channel subunits with different single-amino acid substitutions. Heterologous expression revealed that only one mutant forms functional channels, albeit with grossly altered properties, including changes in Ca²⁺ blockage and permeation. Surprisingly, coexpression of this mutant subunit with CNGB3 rescues the channel phenotype, except for the Ca²⁺ interaction. We argue that these alterations are responsible for the perturbations in light sensitivity and synaptic transmission.

Willis, D., K. W. Li, et al. (2005). "Differential Transport and Local Translation of Cytoskeletal, Injury-Response, and Neurodegeneration Protein mRNAs in Axons." *J. Neurosci.* **25**(4): 778-791.

<http://www.jneurosci.org/cgi/content/abstract/25/4/778>

Recent studies have begun to focus on the signals that regulate axonal protein synthesis and the functional significance of localized protein synthesis. However, identification of proteins that are synthesized in mammalian axons has been mainly based on predictions. Here, we used axons purified from cultures of injury-conditioned adult dorsal root ganglion (DRG) neurons and proteomics methodology to identify axonally synthesized proteins. Reverse transcription (RT)-PCR from axonal preparations was used to confirm that the mRNA for each identified protein extended into the DRG axons. Proteins and the encoding mRNAs for the cytoskeletal proteins {beta}-actin, peripherin, vimentin, {gamma}-tropomyosin 3, and cofilin 1 were present in the axonal preparations. In addition to the cytoskeletal elements, several heat shock proteins (HSP27, HSP60, HSP70, grp75, {alpha}B crystallin), resident endoplasmic reticulum (ER) proteins (calreticulin, grp78/BiP, ERp29), proteins associated with neurodegenerative diseases (ubiquitin C-terminal hydrolase L1, rat ortholog of human DJ-1/Park7, {gamma}-synuclein, superoxide dismutase 1), anti-oxidant proteins (peroxiredoxins 1 and 6), and metabolic proteins (e.g., phosphoglycerate kinase 1 (PGK 1), {alpha} enolase, aldolase C/Zebirin II) were included among the axonally synthesized proteins. Detection of the mRNAs encoding each of the axonally synthesized proteins identified by mass spectrometry in the axonal compartment indicates that the DRG axons have the potential to synthesize a complex population of proteins. Local treatment of the DRG axons with NGF or BDNF increased levels of cytoskeletal mRNAs into the axonal compartment by twofold to fivefold but had no effect on levels of the other axonal mRNAs studied. Neurotrophins selectively increased transport of {beta}-actin, peripherin, and vimentin mRNAs from the cell body into the axons rather than changing transcription or mRNA survival in the axonal compartment.

J. Nutr. (9)

Auborn, K. J., S. Fan, et al. (2003). "Indole-3-Carbinol Is a Negative Regulator of Estrogen." *J. Nutr.* **133**(7): 2470S-2475.

<http://www.nutrition.org/cgi/content/abstract/133/7/2470S>

Studies increasingly indicate that dietary indole-3-carbinol (I3C) prevents the development of estrogen-enhanced cancers including breast, endometrial and cervical cancers. Epidemiological, laboratory, animal and translational studies support the efficacy of I3C. Whereas estrogen increases the growth and survival of tumors, I3C causes growth arrest and increased apoptosis and ameliorates the effects of estrogen. Our long-range goal is to best use I3C together with other nutrients to achieve maximum benefits for cancer prevention. This study examines the possibility that induction of growth arrest in response to DNA damage (GADD) in genes by diindolylmethane (DIM), which is the acid-catalyzed condensation product of I3C, promotes metabolically stressed cancer cells to undergo apoptosis. We evaluated whether genistein, which is the major isoflavonoid in soy, would alter the ability of I3C/DIM to cause apoptosis and decrease expression driven by the estrogen receptor (ER)-{alpha}. Expression of GADD was evaluated by real-time reverse transcription-polymerase chain reaction. Proliferation and apoptosis were measured by a mitochondrial function assay and by fluorescence-activated cell sorting analysis. The luciferase reporter assay was used to specifically evaluate expression

driven by ER- α . The estrogen-sensitive MCF-7 breast cancer cell line was used for these studies. We show a synergistic effect of I3C and genistein for induction of GADD expression, thus increasing apoptosis, and for decrease of expression driven by ER- α . Because of the synergistic effect of I3C and genistein, the potential exists for prophylactic or therapeutic efficacy of lower concentrations of each phytochemical when used in combination.

Cooney, C. A., A. A. Dave, et al. (2002). "Maternal Methyl Supplements in Mice Affect Epigenetic Variation and DNA Methylation of Offspring." *J. Nutr.* **132**(8): 2393S-2400.

<http://www.nutrition.org/cgi/content/abstract/132/8/2393S>

This study was designed to determine if maternal dietary methyl supplements increase DNA methylation and methylation-dependent epigenetic phenotypes in mammalian offspring. Female mice of two strains were fed two levels of dietary methyl supplement or control diet prior to and during pregnancy. Offspring of these mice vary in phenotype, which is epigenetically determined and affects health and 2-y survival. Phenotype and DNA methylation of a long terminal repeat (LTR) controlling expression of the agouti gene were assayed in the resulting offspring. Methyl supplements increase the level of DNA methylation in the agouti LTR and change the phenotype of offspring in the healthy, longer-lived direction. This shows that methyl supplements have strong effects on DNA methylation and phenotype and are likely to affect long-term health. Optimum dietary supplements for the health and longevity of offspring should be intensively investigated. This should lead to public policy guidance that teaches optimal, rather than minimal, dose levels of maternal supplements.

Finnell, R. H., O. Spiegelstein, et al. (2002). "DNA Methylation in Folbp1 Knockout Mice Supplemented with Folic Acid during Gestation." *J. Nutr.* **132**(8): 2457S-2461.

<http://www.nutrition.org/cgi/content/abstract/132/8/2457S>

Periconceptional folic acid supplementation has been shown to prevent up to 70% of neural tube and other birth defects in humans; however, the mechanism is still unknown. In this study, we tested whether defective intracellular folate transport, as achieved by inactivation of the murine folate-binding protein 1 (Folbp1), affects global DNA methylation in the liver and brain from gestational day (GD) 15 [IMG]f1.gif" BORDER="0"> embryos. Complete Folbp1 inactivation is embryolethal but can be reversed by maternal folinic acid (FA) supplementation, and thus we also tested the effect of FA supplementation on DNA methylation in Folbp1 fetuses. Overall, the extent of global DNA methylation seems to be similar across all genotypes in unsupplemented control Folbp1 mice; however, explicit conclusions regarding Folbp1^{-/-} fetuses were not possible because only a single living unsupplemented fetus was viable at GD 15 [IMG]f1.gif" BORDER="0">. FA supplementation induced global DNA hypomethylation across all genotypes. FA-induced hypomethylation is most likely due to its ability to inhibit the enzyme glycine hydroxymethyltransferase, thereby inhibiting the homocysteine remethylation cycle necessary to regenerate S-adenosylmethionine, the methyl donor for DNA methyltransferases. Our hypothesis was that due to defective folate transport in Folbp1^{-/-} embryos and fetuses, DNA would be hypomethylated, thereby altering the temporal expression of critical genes necessary for normal embryonic development. However, these results suggest that an extended examination of changes in DNA methylation prior to GD 15 [IMG]f1.gif" BORDER="0"> is required to unequivocally prove or disprove the hypothesis.

Kim, S., I. Sohn, et al. (2005). "Hepatic Gene Expression Profiles Are Altered by Genistein Supplementation in Mice with Diet-Induced Obesity." *J. Nutr.* **135**(1): 33-41.

<http://www.nutrition.org/cgi/content/abstract/135/1/33>

We reported previously that genistein enhances the expression of genes involved in fatty acid catabolism through activation of peroxisome proliferator-activated receptor (PPAR) {alpha} in HepG2 cells, suggesting that genistein holds great promise for therapeutic applications to lipid abnormalities such as obesity and hyperlipidemia in humans. In this study, we examined the changes in hepatic transcriptional profiles using cDNA microarrays in mice with high-fat diet (HFD)-induced obesity supplemented with genistein. C57BL/6J male mice (n = 10/group) were fed a low-fat diet (LFD), a HFD, or a HFD supplemented with 2 g/kg genistein (HFD+GEN) for 12 wk. Mice fed the HFD had abnormal lipid profiles and significantly greater body weight and visceral fat accumulation than the LFD-fed group. Genistein supplementation improved lipid profiles and hepatic steatosis and attenuated the increases in body weight and visceral fat in HFD-fed mice. The cDNA microarrays revealed marked alterations in the expression of 107 genes in the mice fed the HFD and/or the HFD+GEN. Of 97 transcripts altered in the HFD-fed group, 84 genes were normalized by genistein supplementation. However, several genes involved in fatty acid catabolism were not normalized but were still upregulated in the HFD+GEN-fed group, relative to the LFD-fed group. Furthermore, carnitine O-octanoyltransferase, which accelerates fatty acid oxidation, was not affected by the HFD, but was induced by genistein supplementation. These results are consistent with our previous study showing that genistein is an activator of PPAR {alpha} in vitro. This study showed beneficial effects of genistein supplementation in preventing the development of obesity and metabolic abnormalities in mice with diet-induced obesity. Our results also provide interesting information about the genes associated with the beneficial effects of genistein as well as the mechanisms underlying the development and maintenance of the obesity phenotype in vivo.

Liu, L. and Y.-Y. Yeh (2002). "S-Alk(en)yl Cysteines of Garlic Inhibit Cholesterol Synthesis by Deactivating HMG-CoA Reductase in Cultured Rat Hepatocytes." *J. Nutr.* **132**(6): 1129-1134.

<http://www.nutrition.org/cgi/content/abstract/132/6/1129>

The effects of water-soluble organosulfur compounds of garlic on hepatic cholesterol biosynthesis in cultured rat hepatocytes were studied. S-Alk(en)yl cysteines, i.e., S-allyl cysteine (SAC), S-ethyl cysteine (SEC) and S-propyl cysteine (SPC) inhibited cholesterol synthesis from [14C]acetate but not from [14C]mevalonate. The activity of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase in the cells treated with SAC, SEC and SPC was 30-40% lower than that of the untreated cells. S-Alk(en)yl cysteines did not alter abundance of mRNA coded for HMG-CoA reductase or protein concentration of the enzyme. The ratio of expressed to total activity (E/T) of HMG-CoA reductase was then determined as an index of phosphorylation status of the enzyme. The E/T ratio was reduced 18-29% by SAC, SEC and SPC, resulting primarily from decreased expressed activity. The results suggest that S-alk(en)yl cysteines inhibit cholesterol synthesis by deactivating HMG-CoA reductase via enhanced phosphorylation, but not changing levels of mRNA or the amount of the enzyme. Additionally, of the three S-alk(en)yl cysteines tested, only SAC appears to further decrease the activity of HMG-CoA reductase by increasing sulfhydryl oxidation of the enzyme.

McCann, S. E., K. B. Moysich, et al. (2002). "The Risk of Breast Cancer Associated with Dietary Lignans Differs by CYP17 Genotype in Women." *J. Nutr.* **132**(10): 3036-3041.

<http://www.nutrition.org/cgi/content/abstract/132/10/3036>

Lignans are plant compounds metabolized in the gut to produce the phytoestrogens enterolactone and enterodiol. Reduced breast cancer risks associated with higher urinary lignan excretion may be related to competitive inhibition of endogenous estrogens. Evidence exists that associations with reproductive risk factors for breast cancer differ according to cytochrome P450c17{alpha} (CYP17) genotype. Genetic variability in estrogen metabolism could affect lignan metabolism thereby modifying risk associations. We examined breast cancer risk, dietary lignans and CYP17 genotype among 207 women with primary, incident, histologically confirmed breast cancer and 188 controls frequency matched to cases by age and county of residence. Self-reported frequency of intake of 170 foods and beverages during the 2 y before the interview and other relevant data were collected by detailed in-person interviews. Dietary lignan intake was expressed as the sum of enterolactone and enterodiol production from foods. Odds ratios (OR) and 95% confidence intervals (CI) were estimated by unconditional logistic regression, adjusting for age, education and other breast cancer risk factors. Women in the highest tertile of dietary lignans tended to have reduced breast cancer risk (OR 0.45, 95% CI 0.20-1.01 and OR 0.59, 95% CI 0.28-1.27, pre- and postmenopausal women, respectively). Substantially reduced risks in the highest tertile of lignans were observed for premenopausal women with at least one A2 allele (OR 0.12, 95% CI 0.03-0.50). Our results suggest that CYP17 genotype may be important in modifying the effect on breast cancer risk of exogenous estrogens, particularly for premenopausal women.

Perez, M. J., A. Suarez, et al. (2002). "Dietary Nucleotide Supplementation Reduces Thioacetamide-Induced Liver Fibrosis in Rats." *J. Nutr.* **132**(4): 652-657.

<http://www.nutrition.org/cgi/content/abstract/132/4/652>

Dietary nucleotides reportedly promote functionality and repair in fibrotic liver. Liver fibrosis is characterized by an excessive accumulation of extracellular matrix components, which lead to the impairment of the hepatic function. The aim of this work was to evaluate the influence of dietary nucleotides on liver fibrosis induced by thioacetamide and to elucidate the mechanism by which nucleotides exert their protective effects. Rats consumed ad libitum 300 mg/L thioacetamide in drinking water and were pair-fed diets with (group TN) or without nucleotides (group TS) for 4 mo. Liver histology and extracellular matrix components, liver collagenase and prolyl 4-hydroxylase activities, and tissue inhibitor of metalloproteinases-1 were assessed. The degree of fibrosis was lower in group TN than in group TS. Group TN had lower hepatic concentration of hydroxyproline ($P < 0.05$), collagen type I ($P = 0.12$) and type III ($P = 0.20$), fibronectin ($P = 0.05$), laminin ($P = 0.11$) and desmin ($P = 0.07$), higher collagenolytic activity ($P < 0.05$), lower prolyl 4-hydroxylase activity ($P < 0.05$) and lower prolyl 4-hydroxylase ($P = 0.10$) and tissue inhibitor of metalloproteinase-1 ($P = 0.06$) expression than group TS. Moreover, expression of tissue inhibitor of the metalloproteinases-1 gene was lower in group TN than in group TS ($P < 0.05$). These data indicate that the reduction of liver fibrosis in nucleotide-supplemented rats may rely on the enhancement of collagenase activity and the reduction of collagen content and maturation.

Ten Bruggencate, S. J. M., I. M. J. Bovee-Oudenhoven, et al. (2005). "Dietary Fructooligosaccharides Increase Intestinal Permeability in Rats." *J. Nutr.* **135**(4): 837-842.

<http://www.nutrition.org/cgi/content/abstract/135/4/837>

We showed previously that fructooligosaccharides (FOS) decrease the resistance to salmonella infection in rats. However, the mechanism responsible for this effect is unclear. Therefore, we

examined whether dietary FOS affects intestinal permeability before and after infection with *Salmonella enterica* serovar Enteritidis. Male Wistar rats were fed restricted quantities of a purified diet that mimicked the composition of a Western human diet. The diet was supplemented with 60 g/kg cellulose (control) or 60 g/kg FOS and with 4 mmol/kg of the intestinal permeability marker chromium EDTA (CrEDTA) (n = 8 or 10). After an adaptation period of 2 wk, rats were orally infected with 10⁸ colony-forming units (cfu) of *S. enteritidis*. Mucin concentrations in intestinal contents and mucosa were measured fluorimetrically, as markers of mucosal irritation. Intestinal permeability was determined by measuring urinary CrEDTA excretion. Translocation of salmonella was quantified by analysis of urinary nitric oxide metabolites with time. Before infection, FOS increased mucosal lactobacilli and enterobacteria in cecum and colon, but not in the ileum. However, FOS increased cytotoxicity of fecal water and intestinal permeability. Moreover, FOS increased fecal mucin excretion and mucin concentrations in cecal and colonic contents, and in cecal mucosa before infection. After infection, mucin excretion and intestinal permeability in the FOS groups increased even further in contrast to the control group. In addition, FOS increased translocation of salmonella to extraintestinal sites. Thus, FOS impairs the intestinal barrier in rats, as indicated by higher intestinal permeability. Whether these results can be extrapolated to humans requires further investigation.

Tryfonidou, M. A., J. J. Stevenhagen, et al. (2002). "Moderate Cholecalciferol Supplementation Depresses Intestinal Calcium Absorption in Growing Dogs." *J. Nutr.* **132**(9): 2644-2650.

<http://www.nutrition.org/cgi/content/abstract/132/9/2644>

Hormonal regulation of calcium (Ca) absorption was investigated in a cholecalciferol (vitamin D₃)-supplemented group (hVitD) vs. a control group (cVitD) of growing Great Danes (100 vs. 12.5 {micro}g vitamin D₃/kg diet). Although Ca intakes did not differ, fractional Ca absorption was significantly lower in the hVitD group than in the cVitD group. There were no differences in plasma concentrations of Ca, inorganic phosphate, parathyroid hormone, growth hormone or insulin-like growth factor I between groups. Plasma 25-hydroxycholecalciferol [25(OH)D₃] concentrations were maintained in the hVitD dogs at the same levels as in the cVitD dogs due to increased turnover of 25(OH)D₃ into 24,25-dihydroxycholecalciferol [24,25(OH)2D₃] and 1,25-dihydroxycholecalciferol [1,25(OH)2D₃]. In hVitD dogs, the greater plasma 24,25(OH)2D₃ concentration and the enhanced metabolic clearance rate (MCR) of 1,25(OH)2D₃ indicated upregulated 24-hydroxylase activity. The increased MCR of 1,25(OH)2D₃ decreased plasma 1,25(OH)2D₃ concentrations. In hVitD dogs, the greater production rate of 1,25(OH)2D₃ was consistent with the 12.9-fold greater renal 1{alpha}-hydroxylase gene expression compared with cVitD dogs and compensated to a certain extent for the accelerated MCR of 1,25(OH)2D₃. The moderately decreased plasma 1,25(OH)2D₃ concentration can only partially explain the decreased Ca absorption in the hVitD dogs. Intestinal vitamin D receptor concentrations did not differ between groups and did not account for the decreased Ca absorption. We suggest that 24,25(OH)2D₃ may downregulate Ca absorption.

J. Pharmacol. Exp. Ther. (17)

Garnier, M., P. F. Zaratin, et al. (2003). "Up-Regulation of Regulator of G Protein Signaling 4 Expression in a Model of Neuropathic Pain and Insensitivity to Morphine." *J. Pharmacol. Exp. Ther.* **304**(3): 1299-1306.

<http://jpet.aspetjournals.org/cgi/content/abstract/304/3/1299>

We hypothesized that the up-regulated expression of one or more members of the regulator of G protein signaling (RGS) family can cause an attenuation of signaling via Gi/Go-coupled opioid receptors, and thereby play a role in the development of hyperalgesia and accompanying insensitivity to morphine observed in animal models of neuropathic pain. Accordingly, we examined the mRNA expression of several RGS genes in a rat model of chronic neuropathic pain induced by partial ligation of the sciatic nerve. During the development of hyperalgesia, RGS4 was the only isoform examined whose mRNA levels increased significantly (up to 230%) in the lumbar spinal cord. In situ hybridization studies confirmed that RGS4 is present in the dorsal horn of the spinal cord where μ -opioid receptors (MORs) are also expressed. Overexpression of RGS4 in human embryonic kidney 293 cells stably expressing μ -opioid receptors predictably attenuated opioid agonist-induced inhibition of adenylyl cyclase. This inhibitory effect was overcome partially at high agonist concentrations, supporting the view that morphine insensitivity is promoted by RGS4 overexpression. These studies provide evidence that the up-regulation of RGS4 expression may contribute to changes in pain signal processing that lead to the development of hyperalgesia, and further affect its modulation by morphine.

Gautam, D., S.-J. Han, et al. (2005). "Cholinergic Stimulation of Amylase Secretion from Pancreatic Acinar Cells Studied with Muscarinic Acetylcholine Receptor Mutant Mice." J. Pharmacol. Exp. Ther.: jpet.105.084855.

<http://jpet.aspetjournals.org/cgi/content/abstract/jpet.105.084855v1>

Muscarinic acetylcholine receptors (mAChRs) expressed by pancreatic acinar cells play an important role in mediating acetylcholine-dependent stimulation of digestive enzyme secretion. To examine the potential roles of M1 and M3 mAChRs in this activity, we used M1 and M3 receptor single knockout (KO) and M1/M3 receptor double KO mice as novel experimental tools. Specifically, we examined the ability of the muscarinic agonist, carbachol, to stimulate amylase secretion in vitro, using dispersed pancreatic acini prepared from wild-type and mAChR mutant mice. Quantitative RT-PCR studies using RNA prepared from mouse pancreatic acini showed that deletion of the M1 or M3 mAChR genes did not lead to significantly altered mRNA levels of the remaining mAChR subtypes. Moreover, immunoprecipitation studies with M1 and M3 mAChR-selective antisera demonstrated that both mAChR subtypes are expressed by mouse pancreatic acini. Strikingly, carbachol-induced stimulation of amylase secretion was significantly impaired in acinar preparations from both M1 and M3 receptor single KO mice and completely abolished in acinar preparations from M1/M3 receptor double KO mice. However, another pancreatic secretagogue, bombesin, retained its ability to fully stimulate amylase secretion in acinar preparations from M1/M3 receptor double KO mice. Taken together these studies support the concept that cholinergic stimulation of pancreatic amylase secretion is mediated by a mixture of M1 and M3 mAChRs and that other mAChR subtypes do not make a significant contribution to this activity. These findings clarify the long-standing question regarding the molecular nature of the mAChR subtypes mediating the secretion of digestive enzymes from the exocrine pancreas.

Han, X., G. Lee, et al. (2005). "HEME-REVERSIBLE IMPAIRMENT OF CYP2B1/2 INDUCTION IN HEME DEPLETED RAT HEPATOCYTES IN PRIMARY CULTURE: TRANSLATIONAL CONTROL BY A HEPATIC eIF2 α KINASE?" J. Pharmacol. Exp. Ther.: jpet.105.084699.

<http://jpet.aspetjournals.org/cgi/content/abstract/jpet.105.084699v1>

The role of heme in the phenobarbital-mediated induction of CYP2B1/2 was reexamined in rat

hepatocytes in monolayer culture, acutely depleted of heme by treatment with either DDEP (3,5-dicarbethoxy-2,6-dimethyl-4-ethyl-1,4-dihydropyridine) or NMPP (N-methylprotoporphyrins). The findings revealed that such acute hepatic heme depletion markedly impaired CYP2B1/2 protein induction, an effect that was reversible by heme resupplementation. However, TaqMan analyses of hepatic mRNA isolated from these heme depleted cells revealed that this impairment was not due to faulty transcriptional activation of either CYP2B1 or CYP2B2 gene expression as previously proposed, thereby confirming literature reports that heme is not a transcriptional regulator of the CYP2B1/2 gene. In contrast, the rate of de novo CYP2B1/2 protein synthesis was found to be dramatically inhibited in both DDEP- and NMPP-treated hepatocytes. Concurrently, a marked (>80%) suppression of de novo hepatocellular protein synthesis was also observed, along with a significantly enhanced phosphorylation of the α -subunit of the eukaryotic initiation factor eIF2 (eIF2 α), as monitored by the phosphorylated eIF2 α /total eIF2 α ratio in these heme-depleted cells. Indeed, the parallel reversal of all these three effects by heme supplementation suggests that this impaired CYP2B1 induction most likely stems from blocked translational initiation resulting from the activation of a heme-sensitive eIF2 α kinase. Such global suppression of hepatic protein synthesis may disrupt a myriad of vital cellular functions thereby contributing to the clinical symptoms of acute hepatic heme deficient states such as the hepatic porphyrias.

Hsu, S., T. Yamamoto, et al. (2005). "Green Tea Polyphenol-Induced Epidermal Keratinocyte Differentiation Is Associated with Coordinated Expression of p57/KIP2 and Caspase 14." J. Pharmacol. Exp. Ther. **312**(3): 884-890.

<http://jpet.aspetjournals.org/cgi/content/abstract/312/3/884>

Epigallocatechin-3-gallate (EGCG), the most abundant polyphenol in green tea, exerts chemopreventive effects by selectively inducing apoptosis in tumor cells. In contrast, EGCG accelerates terminal differentiation in normal human epidermal keratinocytes (NHEK) mediated partially by up-regulation of p57/KIP2, a cyclin-dependent kinase inhibitor that confers growth arrest and differentiation. However, it is unclear if EGCG modulates caspase 14, a unique regulator of epithelial cell terminal differentiation associated with cornification. Here, we examined the effect of EGCG on caspase 14 expression in NHEK and correlated the protein and mRNA expression of p57/KIP2 with those of caspase 14 in either normal keratinocytes or p57/KIP2-expressing tumor cells (OSC2, an oral squamous cell carcinoma cell line). Additionally, paraffin-embedded normal and untreated psoriatic (aberrant keratinization) skin sections from humans were assessed for caspase 14 by immunohistochemistry. In NHEK, EGCG induced the expression of caspase 14 mRNA and protein levels within a 24-h period. The expression of p57/KIP2 in OSC2 cells was adequate to induce caspase 14 in the absence of EGCG; this induction of caspase 14 was down-regulated by transforming growth factor- β 1. In human psoriatic skin samples, caspase 14 staining in the upper epidermis was reduced, especially in nuclear areas. These results suggest that, in addition to p57/KIP2, EGCG-induced terminal differentiation of epidermal keratinocytes involves up-regulation of caspase 14. Further understanding of how EGCG modulates cellular differentiation may be useful in developing green tea preparations for selected clinical applications.

Kam, K. W. L., J. S. Qi, et al. (2004). "Estrogen Reduces Cardiac Injury and Expression of β 1-Adrenoceptor upon Ischemic Insult in the Rat Heart." J. Pharmacol. Exp. Ther. **309**(1): 8-15.

<http://jpet.aspetjournals.org/cgi/content/abstract/309/1/8>

To test the hypothesis that estrogen confers cardioprotection by suppressing the expression of β -adrenoceptor (β -AR), we first correlated the infarct size in response to ischemic insult

and β -AR stimulation with the expression of β 1-AR in sham, ovariectomized (Ovx) and estrogen replaced (Ovx + E2) rats. When β -AR is being activated during ischemia, the infarct size was significantly greater in Oxv than in the sham and Oxv + E2 rats. There is a negative correlation between the infarct size and the expression level of β 1-AR as revealed by Western blotting and supported by binding analysis. Incubation of ventricular myocytes from Oxv rats with estrogen at 10^{-9} M for 24 and 48 h, but not 12 h, significantly reduced lactate dehydrogenase release when the myocytes are subjected to simulated ischemia. The cardioprotective effect of 24 h estrogen incubation was accompanied by a reduction in the protein expression level of β 1-AR, which is estrogen receptor-dependent, whereas the lack of protection of 12-h estrogen incubation was not accompanied by any alterations in the expression level of β 1-AR. Together, the result from present study suggested that it is most likely that the cardioprotective effect of long-term estrogen replacement is due to suppressing the enhanced expression of cardiac β 1-AR in the Oxv rats, which in turn reduces cardiac injury when β -AR is activated by sympathetic hyperactivity during ischemia. Therefore, suppression of the enhanced expression of cardiac β 1-AR in Oxv rats represents a novel cardioprotective mechanism of estrogen replacement therapy.

Lee, S.-J., D. A. Bell, et al. (2005). "Recombinant CYP3A4*17 Is Defective in Metabolizing the Hypertensive Drug Nifedipine, and the CYP3A4*17 Allele May Occur on the Same Chromosome as CYP3A5*3, Representing a New Putative Defective CYP3A Haplotype." *J. Pharmacol. Exp. Ther.* **313**(1): 302-309.

<http://jpet.aspetjournals.org/cgi/content/abstract/313/1/302>

Genetic variation in CYP3A activity may influence the rate of the metabolism and elimination of CYP3A substrates in humans. We previously reported four new CYP3A4 coding variants in three different racial groups. In the present study, we examined metabolism of nifedipine by the recombinant forms of these allelic variants. Metabolism of nifedipine by the L293P (CYP3A4*18), M445T (CYP3A4*3), and P467S (CYP3A4*19) allelic variants was not significantly different from wild-type CYP3A4*1. However, F189S (CYP3A4*17) exhibited a >99% decrease in both V_{max} and CL_{max} of nifedipine compared with CYP3A4*1. Of 72 racially diverse individuals, CYP3A4*17 was identified in 1 of 24 Caucasian samples [1:5 Eastern European (Adygei ethnic group)]. Genotyping of an extended set of 276 genomic DNAs of Caucasians (100 from the Coriell Repository and an additional 176 from the United States) for CYP3A4*17 detected no additional individuals containing the CYP3A4*17 allele. However, additional genotyping of four more Adygei samples available from Coriell detected an additional individual carrying the CYP3A4*17 allele. New specific polymerase chain reaction-restriction fragment length polymorphism genotyping procedures were developed for the major splice variant of CYP3A5 (CYP3A5*3) and CYP3A4*17. Genotyping revealed that the two individuals carrying CYP3A4*17 were either homozygous or heterozygous for the more frequent CYP3A5*3 allele, suggesting that the two alleles may exist on the same chromosome as a new putative CYP3A poor metabolizer haplotype. We predict that individuals who are homozygous for defective alleles of both of these genes would metabolize CYP3A substrates poorly. The new genetic tests will be useful in future clinical studies to investigate genotype/phenotype associations.

Serrano, M. A., R. I. R. Macias, et al. (2003). "Effect of Ursodeoxycholic Acid on the Impairment Induced by Maternal Cholestasis in the Rat Placenta-Maternal Liver Tandem Excretory Pathway." *J. Pharmacol. Exp. Ther.* **305**(2): 515-524.

<http://jpet.aspetjournals.org/cgi/content/abstract/305/2/515>

We investigated the effects of ursodeoxycholic acid (UDCA; 60 μ g/day/100 g b.wt.) on the

impairment induced by maternal obstructive cholestasis during pregnancy (OCP) in the rat placenta-maternal liver tandem excretory pathway. A blunted catheter was implanted in the common bile duct on day 14 of pregnancy, and the tip was cut on day 21. [¹⁴C]Glycocholate (GC) was then administered through the umbilical artery of "in situ" perfused placenta (placental transfer test) or through the maternal jugular vein (biliary secretion test), and GC bile output was measured. OCP impaired both GC placental transfer and maternal biliary secretion. UDCA moderately improved the latter but had a more marked beneficial effect on GC placental transfer. Histological examination revealed trophoblast atrophy and structural alterations, e.g., loss of apical membrane microvilli in OCP placentas. Gene expression level was investigated by real-time quantitative reverse transcription-polymerase chain reaction and Western blot analysis. OCP reduced both placental lactogen II (a trophoblast-specific gene) mRNA and the functional amount of epithelial tissue, determined by transplacental diffusion of antipyrin. Using a rapid filtration technique, impairment in the ATP-dependent GC transport across trophoblast apical plasma membranes obtained from OCP placentas was found. UDCA partially prevented all these changes. The expression level of organic anion transporters Oatp1, Oatp2, and Oatp4, and multidrug resistance-associated proteins Mrp1, Mrp2, and Mrp3 in whole placenta were not affected or were moderately affected by OCP but greatly enhanced by UDCA. In summary, UDCA partially prevents deleterious effects of OCP on the rat placenta-maternal liver tandem excretory pathway, mainly by preserving trophoblast structure and function.

Summers, M. A., M. S. O'Dorisio, et al. (2003). "A Lymphocyte-Generated Fragment of Vasoactive Intestinal Peptide with VPAC1 Agonist Activity and VPAC2 Antagonist Effects." J. Pharmacol. Exp. Ther. **306**(2): 638-645.

<http://jpet.aspetjournals.org/cgi/content/abstract/306/2/638>

Vasoactive intestinal peptide receptors 1 (VPAC1) and 2 (VPAC2) have been identified in humans. Cell lines expressing only VPAC1 (HT-29) or VPAC2 (Molt-4b) were identified using real-time reverse transcriptase polymerase chain reaction. Vasoactive intestinal peptide (VIP) and related peptides, VIP-6-28, VIP4-28, and VIP10-28, previously isolated from cultures of human leukocytes, were evaluated for their ability to bind to VPAC1 and VPAC2 and to increase the levels of cAMP in HT-29 and Molt-4b cells. VIP bound to membranes of HT-29 colon carcinoma cells and Molt-4b lymphoblasts with high affinity ($K_D = 1.6 \{+/-\} 0.2$ and $1.7 \{+/-\} 0.9$ nM, respectively). VIP4-28 also demonstrated high-affinity binding ($K_D = 1.7 \{+/-\} 0.2$ and $1.7 \{+/-\} 0.7$ nM in HT-29 and Molt-4b, respectively). VIP and VIP4-28 are potent VPAC1 agonists, inducing maximal 200- and 400-fold increases in cAMP, respectively. VIP demonstrated weak VPAC2 agonist activity, inducing a maximal 14-fold increase in cAMP. VIP4-28 had no VPAC2 agonist activity but demonstrated potent VPAC2 antagonist activity. VIP4-28 inhibited VPAC2-mediated increases in cAMP in Molt-4b cells up to 95%, but had no antagonistic effect on VPAC1. Lymphoblasts did not hydrolyze VIP4-28 to a form with VPAC1 antagonist activity. VIP4-28 thus is a lymphocyte-generated VIP fragment with potent agonist activity for VPAC1 and potent antagonist activity for VPAC2.

Takane, H., D. Kobayashi, et al. (2004). "Haplotype-Oriented Genetic Analysis and Functional Assessment of Promoter Variants in the MDR1 (ABCB1) Gene." J. Pharmacol. Exp. Ther. **311**(3): 1179-1187.

<http://jpet.aspetjournals.org/cgi/content/abstract/311/3/1179>

Recently, a number of nucleotide variants have been described in the multidrug resistance 1 (MDR1/ABCB1) gene; however, most studies have focused on the coding region. In the present study, we identified promoter variants of the MDR1 gene and evaluated their phenotypic

consequences using a reporter gene assay and the real-time polymerase chain reaction method. Ten allelic variants were detected in the promoter region (approximately 2 kilobases), seven of which were newly identified. Certain mutations occurred simultaneously, and a total of 10 haplotypes were observed. These promoter polymorphisms were found more frequently in Japanese than Caucasians. Some haplotypes were associated with changes in luciferase activity and placental and hepatic mRNA levels. We also determined DNA methylation status in the proximal promoter region of the MDR1 gene. The promoter region around potential binding sites for transcription factors was found to be hypomethylated and thus likely to be independent of the gene expression. Nucleotide and/or haplotype variants not only in the coding region but also in the promoter region of the MDR1 gene may be important for interindividual differences of P-glycoprotein expression.

Tertyshnikova, S., R. J. Knox, et al. (2005). "BL-1249 [(5,6,7,8-Tetrahydro-naphthalen-1-yl)-[2-(1H-tetrazol-5-yl)-phenyl]-amine]: A Putative Potassium Channel Opener with Bladder-Relaxant Properties." *J. Pharmacol. Exp. Ther.* **313**(1): 250-259.

<http://jpet.aspetjournals.org/cgi/content/abstract/313/1/250>

BL-1249 [(5,6,7,8-tetrahydro-naphthalen-1-yl)-[2-(1H-tetrazol-5-yl)-phenyl]-amine] produced a concentration-dependent membrane hyperpolarization of cultured human bladder myocytes, assessed as either a reduction in fluorescence of the voltage-sensitive dye bis-(1,2-dibutylbarbituric acid)trimethine oxonol ($EC_{50} = 1.26 \pm 0.6 \mu M$) or by direct electrophysiological measurement ($EC_{50} = 1.49 \pm 0.08 \mu M$). BL-1249 also produced a membrane hyperpolarization of acutely dissociated rat bladder myocytes. Voltage-clamp studies in human bladder cells revealed that BL-1249 activated an instantaneous, noninactivating current that reversed near EK. The BL-1249-evoked outward K^+ current was insensitive to blockade by glyburide, tetraethylammonium, iberiotoxin, 4-aminopyridine, apamin, or Mg^{2+} . However, the current was inhibited by extracellular Ba^{2+} (10 mM). In in vitro organ bath experiments, BL-1249 produced a concentration-dependent relaxation of 30 mM KCl-induced contractions in rat bladder strips ($EC_{50} = 1.12 \pm 0.37 \mu M$), yet had no effect on aortic strips up to the highest concentration tested (10 μM). The bladder relaxation produced by BL-1249 was partially blocked by Ba^{2+} (1 and 10 mM) but not by apamin, iberiotoxin, 4-aminopyridine, glyburide, or tetraethylammonium. In an anesthetized rat model, BL-1249 (1 mg/kg i.v.) decreased the number of isovolumic contractions, without significantly affecting blood pressure. Thus, BL-1249 behaves as a potassium channel activator that exhibits bladder versus vascular selectivity both in vitro and in vivo. A survey of potassium channels exhibiting sensitivity to extracellular Ba^{2+} at millimolar concentration revealed that the expression of the K2P2.1 (TREK-1) channel was relatively high in human bladder cells versus human aortic cells, suggesting this channel as a possible candidate target for BL-1249.

Valenzano, K. J., W. Miller, et al. (2004). "DiPOA ([8-(3,3-Diphenyl-propyl)-4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]dec-3-yl]-acetic Acid), a Novel, Systemically Available, and Peripherally Restricted Mu Opioid Agonist with Antihyperalgesic Activity: I. In Vitro Pharmacological Characterization and Pharmacokinetic Properties." *J. Pharmacol. Exp. Ther.* **310**(2): 783-792.

<http://jpet.aspetjournals.org/cgi/content/abstract/310/2/783>

Mu opioid receptors are present throughout the central and peripheral nervous systems. Peripheral inflammation causes an increase in mu receptor levels on peripheral terminals of primary afferent neurons. Recent studies indicate that activation of peripheral mu receptors produces antihyperalgesic effects in animals and humans. Here, we describe the in vitro pharmacological and in vivo pharmacokinetic properties of a novel, highly potent, and peripherally

restricted mu opioid agonist, [8-(3,3-diphenyl-propyl)-4-oxo-1-phenyl-1,3,8-triaza-spiro[4.5]dec-3-yl]-acetic acid (DiPOA). In a radioligand binding assay, DiPOA inhibited [3H]-diprenorphine binding to recombinant human mu receptors with a K_i value of ~ 0.8 nM. The rank order of affinity for DiPOA binding to recombinant human opioid receptors was $\mu > \kappa \approx \text{ORL-1} \gg \delta$. DiPOA showed potent agonist effects in a human mu receptor guanosine 5'-O-(3-[35S]thio)triphosphate functional assay, with an EC_{50} value of ~ 33 nM and efficacy of $\sim 85\%$ {normalized to the mu agonist, [D-Ala2,MePhe4,Gly(ol)5]enkephalin}. Low potency agonist activity was also seen at ORL-1 and kappa receptors. DiPOA bound competitively to the opioid binding site of human mu receptors as demonstrated by a parallel rightward shift in its concentration-response curve in the presence of increasing concentrations of naltrexone. High and sustained (≥ 5 h) plasma levels for DiPOA were achieved following intraperitoneal administration at 3 and 10 mg/kg; central nervous system penetration, however, was $\leq 4\%$ of the plasma concentration, even at levels exceeding 1500 ng/ml. As such, DiPOA represents a systemically available, peripherally restricted small molecule mu opioid agonist that will aid in understanding the role played by mu opioid receptors in the periphery.

Xu, J., L. Lecanu, et al. (2003). "Inhibition of Adrenal Cortical Steroid Formation by Procaine Is Mediated by Reduction of the cAMP-Induced 3-Hydroxy-3-methylglutaryl-coenzyme A Reductase Messenger Ribonucleic Acid Levels." *J. Pharmacol. Exp. Ther.* **307**(3): 1148-1157.

<http://jpet.aspetjournals.org/cgi/content/abstract/307/3/1148>

Elevated glucocorticoid levels are associated with many diseases, including age-related depression, hypertension, Alzheimer's disease, and acquired immunodeficiency syndrome. Cortisol-lowering agents could provide useful complementary therapy for these disorders. We examined the effect of procaine and procaine in a pharmaceutical formulation on adrenal cortical steroid formation. Procaine inhibited dibutyryl cyclic AMP (dbcAMP)-induced corticosteroid synthesis by murine Y1 and human H295R adrenal cells in a dose-dependent manner without affecting basal steroid formation. Treatment of rats with the procaine-based formulation reduced circulating corticosterone levels. This steroidogenesis-inhibiting activity of procaine was not observed in Leydig cells, suggesting that the effect was specific to adrenocortical cells. In search of the mechanism underlying this inhibitory effect on cAMP-induced corticosteroidogenesis, procaine was found to affect neither the cAMP-dependent protein kinase activity nor key proteins involved in cholesterol transport into mitochondria, cytochrome P450 side chain cleavage enzyme expression, and enzymatic activities associated with cholesterol metabolism to final steroid products. However, procaine reduced in a dose-dependent manner the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA) activity and the dbcAMP-induced HMG-CoA reductase mRNA levels by affecting mRNA stability. These data suggest that the inhibitory effect of procaine on cAMP-induced corticosteroid formation is due to the reduced synthesis of cholesterol. This modulatory effect of procaine on HMG-CoA reductase mRNA expression was also seen in dbcAMP-stimulated Hepa1-6 mouse liver hepatoma cells. Taken together, these results suggest that procaine may provide a pharmacological means for the control of hormone-induced HMG-CoA reductase mRNA expression and hypercortisolemia.

Yip, L. and Y. N. Kwok (2004). "Role of Adenosine A2A Receptor in the Regulation of Gastric Somatostatin Release." *J. Pharmacol. Exp. Ther.* **309**(2): 804-815.

<http://jpet.aspetjournals.org/cgi/content/abstract/309/2/804>

Adenosine has been demonstrated to inhibit gastric acid secretion. In the rat stomach, this inhibitory effect may be mediated indirectly by increasing the release of somatostatin-like immunoreactivity (SLI). Results show that adenosine analogs augmented SLI release in the

isolated vascularly perfused rat stomach. The rank order of potency of the analogs in stimulating SLI release was 2-p-(2-carboxyethyl)phenethylamino-5'-N-ethylcarboxamidoadenosine (CGS 21680) {approx} 5'-N-ethylcarboxamidoadenosine > 2-chloroadenosine > R-(-)-N6-(2-phenylisopropyl)adenosine > 1-deoxy-1-[6-[[[(3-iodophenyl)methyl]amino]-9H-purin-9-yl]-N-methyl- β -D-ribofuranuronamide > N6-cyclopentyladenosine {approx} N6-cyclohexyladenosine > S-(+)-N6-(2-phenylisopropyl) adenosine, suggesting the involvement of the A2A receptor. In agreement, 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a] [1,3,5]triazin-5-ylamino]ethyl)phenol (ZM 241385), an A2A receptor antagonist, was shown to abolish the adenosine- and CGS 21680-stimulated SLI release. Immunohistochemical studies reveal the presence of A2A receptor immunoreactivity on the gastric plexi and mucosal D-cells, but not on parietal cells and G-cells, suggesting that adenosine may act directly on D-cells or indirectly on the gastric plexi to augment SLI release. The present study also demonstrates that the structure of the mucosal A2A receptor is identical to that in the rat brain, and that alternative splicing of this gene does not occur. A real-time reverse transcription-polymerase chain reaction assay has also been established to quantify the levels of A2A receptor mRNA. Results show that gastric tissues contained significantly lower levels of A2A receptor mRNA compared with the striatum. The lowest level was detected in the mucosa. In conclusion, adenosine may act on A2A receptors to augment SLI release and consequently control gastric acid secretion.

Yip, L., H. C. H. Leung, et al. (2004). "Effect of Omeprazole on Gastric Adenosine A1 and A2A Receptor Gene Expression and Function." *J. Pharmacol. Exp. Ther.* **311**(1): 180-189.

<http://jpet.aspetjournals.org/cgi/content/abstract/311/1/180>

Adenosine has been shown to inhibit immunoreactive gastrin (IRG) release and to stimulate somatostatin-like immunoreactivity (SLI) release by activating adenosine A1 and A2A receptors, respectively. Since the synthesis and release of gastrin and somatostatin are regulated by the acid secretory state of the stomach, the effect of achlorhydria on A1 and A2A receptor gene expression and function was examined. Omeprazole-induced achlorhydria was shown to suppress A1 and A2A receptor gene expression in the antrum and corporeal mucosa, but not in the corporeal muscle. Omeprazole treatment produced reciprocal changes in A1 receptor and gastrin gene expression, and parallel changes in A2A receptor and somatostatin gene expression. The localization of A1 and A2A receptors on gastrin-secreting G-cells and somatostatin-secreting D-cells, respectively, suggests that changes in adenosine receptor expression may modulate the synthesis and release of gastrin and somatostatin. Thus, the effect of omeprazole on adenosine receptor-mediated changes in IRG and SLI release was also examined in the vascularly perfused rat stomach. After omeprazole treatment, the A1 receptor-mediated inhibition of IRG and SLI release induced by N6-cyclopentyladenosine (A1 receptor-selective agonist) was not altered, but the A2A receptor-mediated augmentation of SLI release induced by 2-p-(2-carboxyethyl)-phenethylamino-5'-N-ethylcarboxamidoadenosine (A2A-selective agonist) was significantly attenuated. These findings agree well with the corresponding omeprazole-induced decrease in antral A2A receptor mRNA expression. Overall, the present study suggests that adenosine receptor gene expression and function may be altered by omeprazole treatment. Acid-dependent changes in adenosine receptor expression may represent a novel purinergic regulatory feedback mechanism in controlling gastric acid secretion.

Yip, L., H. C. H. Leung, et al. (2004). "Role of Adenosine A1 Receptor in the Regulation of Gastrin Release." *J. Pharmacol. Exp. Ther.* **310**(2): 477-487.

<http://jpet.aspetjournals.org/cgi/content/abstract/310/2/477>

Adenosine has been demonstrated to inhibit gastric acid secretion. In the rat stomach, this

inhibitory effect may be mediated indirectly by the inhibition of gastrin release. Results show that the A1 receptor agonist N6-cyclopentyladenosine (CPA) suppressed immunoreactive gastrin (IRG) release in a concentration-dependent manner. CPA significantly inhibited IRG release at 0.001 μ M and maximally inhibited IRG release at 1 μ M. At concentrations of 0.001 to 0.1 μ M, the A2A receptor-selective agonist 2-p-(2-carboxyethyl)phenethylamino-5'-N-ethylcarboxamidoadenosine and A3 receptor-selective agonist 1-deoxy-1-[6-[[[3-iodophenyl)methyl]amino]-9H-purin-9-yl]-N-methyl- β -D-ribofuranuronamide, had no effect on IRG release, suggesting the involvement of A1 receptors. In agreement, the A1 receptor-selective antagonist 8-cyclopentyl-1,3-dipropylxanthine abolished adenosine-induced inhibition of IRG release. Results of immunohistochemistry experiments reveal the presence of A1 receptor immunoreactivity on mucosal G-cells and D-cells, and the gastric plexi, but not parietal cells, suggesting that adenosine may act directly on G-cells or indirectly on the gastric plexi to modulate IRG release. The structure of the mucosal A1 receptor was found to be identical to that in the rat brain. Alternative splicing within the coding region of this receptor did not occur. A real-time reverse transcription-polymerase chain reaction assay was developed to measure gastric A1 receptor gene expression. The highest level of gastric A1 receptor mRNA was found in the corporeal muscle. However, this level was significantly lower in comparison with the striatum. In conclusion, this study shows that adenosine may suppress IRG release, at least in part, by activating A1 receptors localized on G-cells and may consequently result in an inhibition of gastric acid secretion.

Yu, X., X. Mao, et al. (2003). "Morphine and Endomorphins Differentially Regulate μ -Opioid Receptor mRNA in SHSY-5Y Human Neuroblastoma Cells." *J. Pharmacol. Exp. Ther.* **306**(2): 447-454.

<http://jpet.aspetjournals.org/cgi/content/abstract/306/2/447>

A sensitive quantitative-competitive reverse transcriptase-polymerase chain reaction method was developed to measure μ -opioid receptor (MOR) mRNA expression in SHSY-5Y neuroblastoma cells. Differentiation of SHSY-5Y cells with either retinoic acid (RA) or 12-o-tetradecanoyl-phorbol-13-acetate (TPA) significantly increased MOR mRNA levels. Morphine treatment (10 μ M) for 24 h decreased MOR mRNA levels in control, as well as RA- and TPA-differentiated cells. In contrast, chronic exposure to the opioid peptides endomorphin-1 or endomorphin-2 significantly increased MOR mRNA levels in undifferentiated and RA-differentiated cells. An opioid antagonist, naloxone, reversed the morphine and endomorphin-1 and -2 effects on MOR mRNA levels in undifferentiated SHSY-5Y cells, but naloxone had differential reversing effects on the agonists' regulation of MOR mRNA in RA- or TPA-differentiated cells. To investigate whether the changes in MOR mRNA expression paralleled changes in MOR receptor function, intracellular cAMP accumulation in SHSY-5Y cells was measured. After chronic treatment with morphine, forskolin-induced cAMP levels in SHSY-5Y cells were significantly higher than those of untreated control cells. In contrast, forskolin-induced cAMP accumulation levels were lower in cells treated with endomorphin-1 or -2 than in untreated control cells. Together, our studies indicate that the opioid alkaloid morphine and the opioid peptides endomorphin-1 and -2 differentially regulate MOR mRNA expression and MOR function in SHSY-5Y cells.

Zhang, E. Y., D.-J. Fu, et al. (2004). "Genetic Polymorphisms in Human Proton-Dependent Dipeptide Transporter PEPT1: Implications for the Functional Role of Pro586." *J. Pharmacol. Exp. Ther.* **310**(2): 437-445.

<http://jpet.aspetjournals.org/cgi/content/abstract/310/2/437>

The human proton-dependent dipeptide transporter (PEPT1, gene SLC15A1) is important for intestinal absorption of di- and tripeptides and a variety of peptidomimetic compounds. Using a DNA polymorphism discovery panel of 44 ethnically diverse individuals, nine nonsynonymous and four synonymous coding-region single-nucleotide polymorphisms (SNPs) were identified in PEPT1. HeLa cells were transiently transfected with plasmids constructed by site-directed mutagenesis for each of the nine nonsynonymous variants. Quantitative polymerase chain reaction showed that the mRNA transcription level of all of the mutants was comparable with the mRNA transcription level of the reference sequence in transfected HeLa cells. Functional analysis in transiently transfected HeLa cells revealed that all nonsynonymous variants retained similar pH-dependent activity and K_t values for [glycyl-1,2-¹⁴C]glycylsarcosine (Gly-Sar) uptake as the reference PEPT1. In addition, a group of seven peptide-like drugs showed inhibitory effect on Gly-Sar uptake by these variants comparable with the reference, suggesting conserved drug recognition. Of the nine nonsynonymous SNPs, a single SNP (P586L) demonstrated significantly reduced transport capacity as evidenced by a much lower V_{max} value. This was consistent with lower immunoreactive protein level (Western analysis) and lower plasma membrane expression (immunocytochemical analysis). Therefore, Pro586 may have profound effect on PEPT1 translation, degradation, and/or membrane insertion.

J. Physiol. (16)

Akerstrom, T., A. Steensberg, et al. (2005). "Exercise induces interleukin-8 expression in human skeletal muscle." *J. Physiol.* **563**(2): 507-516.

<http://jp.physoc.org/cgi/content/abstract/563/2/507>

Skeletal muscle has been recognized as an endocrine organ, and muscle cell cultures express several cytokines with potential hormonal effects. Interleukin-8 (IL-8), a chemokine, which induces angiogenesis, is expressed in working muscles; however, the cell source of origin has not been identified. We aimed to elucidate if IL-8 protein is: (1) expressed in contracting muscle fibres and (2) whether there is a release of IL-8 from exercising muscle. Seventeen healthy male volunteers were included in two independent protocols: 3 h of ergometer bicycle exercise at 60% of [IMG]/medium/tjp_731_mu1.gif" ALT="{tjp_731_mu1}"> (n = 6) or rest (n = 5), and 3 h of two-legged knee-extensor exercise at 60% of maximal workload (n = 6). Repetitive muscle biopsy samples were obtained from the vastus lateralis in all experiments. A marked increase in IL-8 mRNA was found in muscle biopsy samples obtained after exercise. A marked IL-8 protein expression was demonstrated within the cytoplasm of muscle fibres in biopsy samples obtained in the recovery phase following 3 h of bicycle exercise, and the peak occurred 3-6 h postexercise. A small transient net release of IL-8 from working muscle was found at 1.5 h of knee-extensor exercise. However, the small release of IL-8 from muscle did not result in an increase in the systemic plasma concentration of IL-8, suggesting that muscle-derived IL-8 may play a local role, e.g. in angiogenesis.

Amberg, G. C., S. A. Baker, et al. (2002). "Characterization of the A-type potassium current in murine gastric antrum." *J. Physiol.* **544**(2): 417-428.

<http://jp.physoc.org/cgi/content/abstract/544/2/417>

Amberg, G. C., S. D. Koh, et al. (2002). "Contribution of Kv4 channels toward the A-type potassium current in murine colonic myocytes." J. Physiol. **544**(2): 403-415.

<http://jp.physoc.org/cgi/content/abstract/544/2/403>

Beauvois, M. C., A. Arredouani, et al. (2004). "Atypical Ca²⁺-induced Ca²⁺ release from a sarco-endoplasmic reticulum Ca²⁺-ATPase 3-dependent Ca²⁺ pool in mouse pancreatic {beta}-cells." J. Physiol. **559**(1): 141-156.

<http://jp.physoc.org/cgi/content/abstract/559/1/141>

The contribution of Ca²⁺ release from intracellular stores to the rise in the free cytosolic Ca²⁺ concentration ([Ca²⁺]_c) triggered by Ca²⁺ influx was investigated in mouse pancreatic {beta}-cells. Depolarization of {beta}-cells by 45 mM K⁺ (in the presence of 15 mM glucose and 0.1 mM diazoxide) evoked two types of [Ca²⁺]_c responses: a monotonic and sustained elevation; or a sustained elevation superimposed by a transient [Ca²⁺]_c peak (TCP) (40-120 s after the onset of depolarization). Simultaneous measurements of [Ca²⁺]_c and voltage-dependent Ca²⁺ current established that the TCP did not result from a larger Ca²⁺ current. Abolition of the TCP by thapsigargin and its absence in sarco-endoplasmic reticulum Ca²⁺-ATPase 3 (SERCA3) knockout mice show that it is caused by Ca²⁺ mobilization from the endoplasmic reticulum. A TCP could not be evoked by the sole depolarization of {beta}-cells but required a rise in [Ca²⁺]_c pointing to a Ca²⁺-induced Ca²⁺ release (CICR). This CICR did not involve inositol 1,4,5-trisphosphate (IP3) receptors (IP3Rs) because it was resistant to heparin. Nor did it involve ryanodine receptors (RyRs) because it persisted after blockade of RyRs with ryanodine, and was not mimicked by caffeine, a RyR agonist. Moreover, RyR1 and RyR2 mRNA were not found and RyR3 mRNA was only slightly expressed in purified {beta}-cells. A CICR could also be detected in a limited number of cells in response to glucose. Our data demonstrate, for the first time in living cells, the existence of an atypical CICR that is independent from the IP3R and the RyR. This CICR is prominent in response to a supraphysiological stimulation with high K⁺, but plays little role in response to glucose in non-obese mouse pancreatic {beta}-cells.

Blessing, E. M., S. G. Solomon, et al. (2004). "Chromatic and spatial properties of parvocellular cells in the lateral geniculate nucleus of the marmoset (*Callithrix jacchus*)." J. Physiol. **557**(1): 229-245.

<http://jp.physoc.org/cgi/content/abstract/557/1/229>

The parvocellular (PC) division of the afferent visual pathway is considered to carry neuronal signals which underlie the red-green dimension of colour vision as well as high-resolution spatial vision. In order to understand the origin of these signals, and the way in which they are combined, the responses of PC cells in dichromatic (red-green colour-blind) and trichromatic marmosets were compared. Visual stimuli included coloured and achromatic gratings, and spatially uniform red and green lights presented at varying temporal phases and frequencies. The sensitivity of PC cells to red-green chromatic modulation was found to depend primarily on the spectral separation between the medium- and long-wavelength-sensitive cone pigments (20 or 7 nm) in the two trichromatic marmoset phenotypes studied. The temporal frequency dependence of chromatic sensitivity was consistent with centre-surround interactions. Some evidence for chromatic selectivity was seen in peripheral PC cells. The receptive field dimensions of parvocellular cells were similar in dichromatic and trichromatic animals, but the achromatic contrast sensitivity of cells was slightly higher (by about 30%) in dichromats than in trichromats. These data support the

hypothesis that the primary role of the PC is to transmit high-acuity spatial signals, with red-green opponent signals appearing as an additional response dimension in trichromatic animals.

Britton, F. C., S. Ohya, et al. (2002). "Comparison of the properties of CLCA1 generated currents and ICl(Ca) in murine portal vein smooth muscle cells." J. Physiol. **539**(1): 107-117.

<http://jp.physoc.org/cgi/content/abstract/539/1/107>

Chen, C.-Y., E.-h. Ling, et al. (2002). "Synaptic transmission in nucleus tractus solitarius is depressed by Group II and III but not Group I presynaptic metabotropic glutamate receptors in rats." J. Physiol. **538**(3): 773-786.

<http://jp.physoc.org/cgi/content/abstract/538/3/773>

Cho, S. Y., E. A. Beckett, et al. (2005). "A pH-sensitive potassium conductance (TASK) and its function in the murine gastrointestinal tract." J. Physiol.: jphysiol.2005.084574.

<http://jp.physoc.org/cgi/content/abstract/jphysiol.2005.084574v1>

The excitability of smooth muscles is regulated, in part, by background K⁺ conductances that determine resting membrane potential. However, the K⁺ conductances so far described in gastrointestinal (GI) muscles are not sufficient to explain the negative resting potentials of these cells. Here we describe expression of two-pore K⁺ channels of the TASK family in murine small and large intestinal muscles. TASK-2, cloned from murine intestinal muscles, resulted in a pH sensitive, time-dependent, non-inactivating K⁺ conductance with slow activation kinetics. A similar conductance was found in native intestinal myocytes using whole-cell patch clamp conditions. The pH- sensitive current was blocked by local anesthetics. Lidocaine, bupivacaine, and acidic pH, depolarized circular muscle cells in intact muscles and decreased amplitude and frequency of slow waves. The effects of lidocaine were not blocked by tetraethylammonium chloride, 4-aminopyridine, glibenclamide, apamin, or MK- 499. However, depolarization by acidic pH was abolished by pre-treatment with lidocaine, suggesting that lidocaine-sensitive K⁺ channels were responsible for pH-sensitive changes in membrane potential. The kinetics of activation, sensitivity to pH, and pharmacology of the conductance in intestinal myocytes and the expression of TASK-1 and TASK-2 in these cells suggest that the pH-sensitive background conductance is encoded by TASK genes. This conductance appears to contribute significantly to resting potential and may regulate excitability of GI muscles.

Fekete, A., A. Vannay, et al. (2004). "Sex differences in the alterations of Na⁺,K⁺-ATPase following ischaemia-reperfusion injury in the rat kidney." J. Physiol. **555**(2): 471-480.

<http://jp.physoc.org/cgi/content/abstract/555/2/471>

Postischaemic acute renal failure (ARF) is influenced by sex. Na⁺,K⁺-ATPase (NKA) plays a crucial role in the pathogenesis of postischaemic ARF. We tested the impact of sex on mRNA, protein expression, cellular distribution and enzyme activity of NKA following renal ischaemia-reperfusion (I-R) injury. The left renal pedicle of uninephrectomized female (F) and male (M) Wistar rats was clamped for 55 min followed by 2 h (T2) and 16 h (T16) of reperfusion.

Uninephrectomized, sham-operated F and M rats served as controls (n= 6 per group). Blood urea nitrogen, serum creatinine and renal histology were evaluated to detect the severity of postischaemic ARF. mRNA expression of NKA $\{\alpha\}$ 1 and $\{\beta\}$ 1 subunits were detected by RT-PCR. The effect of I-R on cellular distribution was compared by Triton X-100 extraction. Cellular proteins were divided into Triton-insoluble and Triton-soluble fractions and assessed by Western blot. NKA enzyme activity was also determined. After the ischaemic insult blood urea nitrogen and serum creatinine were higher and renal histology showed more rapid progression in M versus F ($P < 0.05$). mRNA expression of the NKA $\{\alpha\}$ 1 subunit decreased in I-R groups versus controls, but was higher in F versus M both in control and I-R groups ($P < 0.05$). However, protein levels of the NKA $\{\alpha\}$ 1 subunit in total tissue homogenate did not differ in controls, but were higher in F versus M in I-R groups ($P < 0.05$). Triton X-100 extractability was lower in F versus M at T16 ($P < 0.05$). NKA enzyme activity was the same in controls, but was higher in F versus M in I-R groups (T2: 14.9 $\{\pm\}$ 2.3 versus 9.15 $\{\pm\}$ 2.21 U) (T16: 11.7 $\{\pm\}$ 4.1 versus 5.65 $\{\pm\}$ 2.3 U; $P < 0.05$). mRNA and protein expression of the NKA $\{\beta\}$ 1 subunit did not differ between F and M in any of the protocol. We concluded that NKA is more protected from the detrimental effects of postischaemic injury in females. Higher mRNA and protein expression of the NKA $\{\alpha\}$ 1 subunit and higher enzyme activity might be additional contributing factors to the improved postischaemic renal function of female rats.

Goto, K., N. M. Rummery, et al. (2004). "Attenuation of conducted vasodilatation in rat mesenteric arteries during hypertension: role of inwardly rectifying potassium channels." *J. Physiol.* **561**(1): 215-231.

<http://jp.physoc.org/cgi/content/abstract/561/1/215>

The present study was designed to elucidate whether the conduction of vasomotor responses mediated by endothelium-derived hyperpolarizing factor (EDHF) in rat mesenteric arteries is altered during hypertension. Iontophoresed acetylcholine (ACh; 500 ms) caused EDHF-mediated hyperpolarization and vasodilatation at the local site and these responses spread through the endothelium to remote sites in 12-week-old Wistar-Kyoto rats (WKY). Conducted responses were significantly attenuated in age-matched spontaneously hypertensive rats (SHR) although the rate of decay with distance did not change. Inhibition of inwardly rectifying potassium (Kir) channels (30 $\{\mu\}$ M barium) eliminated the difference between WKY and SHR by attenuating conducted responses in WKY but not SHR. At the local site, barium (30 $\{\mu\}$ M) significantly reduced the duration but not the amplitude of ACh-induced hyperpolarization in WKY only. Barium had no effect when the iontophoretic stimulus was reduced to 350 ms. After blockade of EDHF in SHR, ACh elicited a depolarization which our indirect data suggest spreads along the vessel in the endothelium. Messenger RNA expression of Kir2.0 genes did not differ between the strains nor did the amplitude of K⁺-induced hyperpolarization, which was abolished by disruption of the endothelium. Immunohistochemistry revealed a decrease in connexin (Cx)37 but not Cx40 or Cx43 protein in endothelial cells of SHR compared to WKY. Results suggest that conduction of EDHF-mediated responses in WKY, but not in SHR, is facilitated by activation of Kir channels at the site of ACh application and not by differences in endothelial connexin expression. Lack of Kir channel involvement in hypertension may result from reduction in the duration of the hyperpolarization due to the development of ACh-mediated depolarization, rather than to any difference in Kir subunit expression or function.

Nakamura, T., M. Matsui, et al. (2004). "M3 muscarinic acetylcholine receptor plays a critical role in parasympathetic control of salivation in mice." *J. Physiol.* **558**(2): 561-575.

<http://jp.physoc.org/cgi/content/abstract/558/2/561>

The M1 and M3 subtypes are the major muscarinic acetylcholine receptors in the salivary gland

and M3 is reported to be more abundant. However, despite initial reports of salivation abnormalities in M3-knockout (M3KO) mice, it is still unclear which subtype is functionally relevant in physiological salivation. In the present study, salivary secretory function was examined using mice lacking specific subtype(s) of muscarinic receptor. The carbachol-induced $[Ca^{2+}]_i$ increase was markedly impaired in submandibular gland cells from M3KO mice and completely absent in those from M1/M3KO mice. This demonstrates that M3 and M1 play major and minor roles, respectively, in the cholinergically induced $[Ca^{2+}]_i$ increase. Two-dimensional Ca^{2+} -imaging analysis revealed the patchy distribution of M1 in submandibular gland acini, in contrast to the ubiquitous distribution of M3. In vivo administration of a high dose of pilocarpine (10 mg kg⁻¹, S.C.) to M3KO mice caused salivation comparable to that in wild-type mice, while no salivation was induced in M1/M3KO mice, indicating that salivation in M3KO mice is caused by an M1-mediated $[Ca^{2+}]_i$ increase. In contrast, a lower dose of pilocarpine (1 mg kg⁻¹, S.C.) failed to induce salivation in M3KO mice, but induced abundant salivation in wild-type mice, indicating that M3-mediated salivation has a lower threshold than M1-mediated salivation. In addition, M3KO mice, but not M1KO mice, had difficulty in eating dry food, as shown by frequent drinking during feeding, suggesting that salivation during eating is mediated by M3 and that M1 plays no practical role in it. These results show that the M3 subtype is essential for parasympathetic control of salivation and a reasonable target for the drug treatment and gene therapy of xerostomia, including Sjogren's syndrome.

Pilegaard, H., B. Saltin, et al. (2003). "Exercise induces transient transcriptional activation of the PGC-1 α gene in human skeletal muscle." J. Physiol. **546**(3): 851-858.

<http://jp.physoc.org/cgi/content/abstract/546/3/851>

Sergeeva, O. A., A. N. Chepkova, et al. (2003). "Taurine-induced long-lasting enhancement of synaptic transmission in mice: role of transporters." J. Physiol. **550**(3): 911-919.

<http://jp.physoc.org/cgi/content/abstract/550/3/911>

Sotty, F., M. Danik, et al. (2003). "Distinct electrophysiological properties of glutamatergic, cholinergic and GABAergic rat septohippocampal neurons: novel implications for hippocampal rhythmicity." J. Physiol. **551**(3): 927-943.

<http://jp.physoc.org/cgi/content/abstract/551/3/927>

Strutz-Seebohm, N., G. Seebohm, et al. (2005). "Regulation of GluR1 abundance in hippocampal neurons by serum- and glucocorticoid-inducible kinase 3." J. Physiol.: jphysiol.2004.079582.

<http://jp.physoc.org/cgi/content/abstract/jphysiol.2004.079582v1>

Phosphatidylinositol 3-Kinase (PI3-kinase) is activated during and required for hippocampal glutamate receptor-dependent long-term potentiation. It mediates the delivery of AMPA receptors to the neuronal surface. Among the downstream targets of PI3-kinase are three members of the serum- and glucocorticoid-inducible kinase family, SGK1, SGK2 and SGK3. We show here that, in *Xenopus* oocytes expressing the AMPA subunit GluR1, SGK3 and to a lesser extent SGK2 but not SGK1 increases glutamate-induced currents by increasing the abundance of GluR1 protein in

the cell membrane. We further show Sgk3 mRNA expression in the hippocampus by RT-PCR and in situ hybridisation. According to Western blotting the hippocampal abundance of GluR1 is significantly lower in gene-targeted mice lacking SGK3 (Sgk3^{-/-}) than in their wild type littermates (Sgk3^{+/+}). The present observations disclose a novel mechanism in the regulation of GluR1.

Wanaverbecq, N., S. J. Marsh, et al. (2003). "The plasma membrane calcium-ATPase as a major mechanism for intracellular calcium regulation in neurones from the rat superior cervical ganglion." *J. Physiol.* **550**(1): 83-101.

<http://jp.physoc.org/cgi/content/abstract/550/1/83>

J. Thorac. Cardiovasc. Surg. (3)

Absi, T. S., T. M. Sundt, III, et al. (2003). "Altered patterns of gene expression distinguishing ascending aortic aneurysms from abdominal aortic aneurysms: complementary DNA expression profiling in the molecular characterization of aortic disease." *J. Thorac. Cardiovasc. Surg.* **126**(2): 344-357.

<http://jtcs.ctsnetjournals.org/cgi/content/abstract/126/2/344>

OBJECTIVES: The purpose of this study was to profile altered patterns of gene expression that characterize degenerative ascending thoracic aortic aneurysms and to compare these patterns with those observed for infrarenal abdominal aortic aneurysms. **METHODS:** Full-thickness aortic wall tissues were obtained during surgical repair of degenerative thoracic aortic aneurysms and infrarenal abdominal aortic aneurysms (n = 4 each), with normal thoracic and abdominal aortas from organ transplant donors used as control preparations. Radiolabeled complementary DNA was prepared for each specimen and hybridized to complementary DNA microarrays, and differential levels of gene expression between aneurysmal and normal aortic tissues at each site were assessed by parametric statistics. **RESULTS:** Of 1185 genes examined, 112 (9.5%) were differentially expressed (P < .05) between thoracic aortic aneurysms and normal thoracic aorta, with 105 increased and 7 decreased. There were 104 genes (8.8%) differentially expressed between infrarenal abdominal aortic aneurysms and normal abdominal aorta (65 increased and 39 decreased). Quantitative increases in expression for 97 genes were unique to thoracic aortic aneurysms, whereas increases for 61 genes were unique to infrarenal abdominal aortic aneurysms. Although 8 gene products were significantly altered in both thoracic and infrarenal abdominal aortic aneurysms, these changes were directionally concordant for only 4 (matrix metalloproteinase 9/gelatinase B, v-src-1 oncogene, mitogen-activated protein kinase 9, and intercellular adhesion molecule 1/CD54). Results for 9 genes were independently confirmed by quantitative reverse transcriptase-polymerase chain reaction. **CONCLUSIONS:** Thoracic aortic aneurysms and infrarenal abdominal aortic aneurysms exhibit distinct patterns of gene expression relative to normal aorta from the same sites, with most alterations being unique to each disease. Degenerative aneurysms arising in different locations are thus characterized by a high degree of molecular heterogeneity, reflecting different pathophysiologic mechanisms.

D'Cunha, J., A. L. Corfits, et al. (2002). "Molecular staging of lung cancer: Real-time polymerase chain reaction estimation of lymph node micrometastatic tumor cell burden in stage I non-small cell lung

cancer--preliminary results of cancer and leukemia group B trial 9761." J. Thorac. Cardiovasc. Surg. **123**(3): 484-491.

<http://jtcs.ctsnetjournals.org/cgi/content/abstract/123/3/484>

Objective: The 5-year survival for patients with surgically resected stage I non-small cell lung cancer is only 60% to 70%, probably because of undetected systemic occult micrometastases. Detection of occult micrometastases in lymph nodes by reverse-transcriptase polymerase chain reaction for carcinoembryonic antigen messenger RNA in non-small cell lung cancer has not been reported. Detection of occult micrometastases by standard reverse-transcriptase polymerase chain reaction provides only yes or no answers about their presence, whereas quantitative real-time reverse-transcriptase polymerase chain reaction permits reproducible quantitation of target molecules. This study evaluated the ability of quantitative reverse-transcriptase polymerase chain reaction to quantitate lymph node occult metastases with carcinoembryonic antigen messenger RNA as a tumor marker. **Methods:** Standard reverse-transcriptase polymerase chain reaction and quantitative reverse-transcriptase polymerase chain reaction for carcinoembryonic antigen messenger RNA were performed on 232 lymph nodes from 53 patients with stage I disease (node negative according to histologic examination). Quantitative reverse-transcriptase polymerase chain reaction determined carcinoembryonic antigen messenger RNA quantity by detecting fluorescence increase at a threshold polymerase chain reaction cycle. Threshold polymerase chain reaction cycle values were correlated with standard curves created from serially diluted carcinoembryonic antigen-positive HTB-174 tumor cells to estimate the number of micrometastatic tumor cells in a lymph node. **Results:** Detection rates of occult metastases were similar for standard reverse-transcriptase polymerase chain reaction and quantitative reverse-transcriptase polymerase chain reaction at 38 of 232 (16.4 %) and 59 of 232 (25.4 %), respectively. Upstaging rates among 53 cases of stage I non-small cell lung cancer were also similar for standard reverse-transcriptase polymerase chain reaction and quantitative reverse-transcriptase polymerase chain reaction at 23 of 53 (43.4 %) and 30 of 53 (56.6%), respectively. Comparison of positive lymph node stations according to quantitative reverse-transcriptase polymerase chain reaction (threshold polymerase chain reaction cycle <45) with HTB-174 tumor cell standard curves yielded estimates of metastatic tumor cell burden of 1.07×10^3 to 3.24×10^5 cells per lymph node station (median 7190 tumor cells per lymph node station). **Conclusions:** Standard and quantitative real-time reverse-transcriptase polymerase chain reaction for carcinoembryonic antigen detected occult metastases in patients with stage I non-small cell lung cancer at similar rates; both upstaged about 50% of cases. Quantitative reverse-transcriptase polymerase chain reaction allows estimation of the number of metastatic cells per lymph node, however, which potentially allows greater precision in predicting recurrence risk.

Lord, R. V. N., J. M. Park, et al. (2003). "Vascular endothelial growth factor and basic fibroblast growth factor expression in esophageal adenocarcinoma and Barrett esophagus." J. Thorac. Cardiovasc. Surg. **125**(2): 246-253.

<http://jtcs.ctsnetjournals.org/cgi/content/abstract/125/2/246>

Objective: This study was undertaken to investigate the role of the angiogenic factors vascular endothelial growth factor and basic fibroblast growth factor in the development and progression of Barrett esophagus and adenocarcinomas of the esophagus and gastroesophageal junction. **Methods:** Vascular endothelial growth factor and basic fibroblast growth factor messenger RNA expression levels, relative to the control gene encoding {beta}-actin, were measured by using a quantitative reverse transcription-polymerase chain reaction method (ABI 7700 Sequence Detector system) in specimens of Barrett intestinal metaplasia (n = 16), dysplasia (n = 11), adenocarcinoma (n = 15), and matching normal squamous esophageal tissues (n = 35). Vascular endothelial growth factor and basic fibroblast growth factor protein expression and CD31+ microvessel density were assessed by means of immunohistochemistry in 25 tissue sections that

included representative areas for each of these Barrett stages. Results: Expression levels were significantly increased in adenocarcinoma compared with in either normal squamous mucosa ($P < .0001$ for both genes) or intestinal metaplasia (vascular endothelial growth factor, $P = .002$; basic fibroblast growth factor, $P < .0001$). Vascular endothelial growth factor levels were also significantly higher in cancer tissues compared with dysplasia tissues ($P = .024$, Mann-Whitney U test). Basic fibroblast growth factor expression was also significantly increased in Barrett dysplastic mucosa compared with in intestinal metaplasia or normal esophageal mucosa. Microvessel density was generally higher in adenocarcinoma compared with in preneoplastic Barrett tissues. The pattern of vascular endothelial growth factor and basic fibroblast growth factor protein expression was similar to the messenger RNA expression pattern, with the exception that mucin-containing goblet cells stained intensely for vascular endothelial growth factor and only weak vascular endothelial growth factor staining was present in some adenocarcinomas. Conclusions: Vascular endothelial growth factor and basic fibroblast growth factor messenger RNA expression levels are significantly upregulated in esophageal and gastroesophageal junction adenocarcinomas, suggesting a role for these angiogenic factors in the development of these cancers. Vascular endothelial growth factor and basic fibroblast growth factor messenger RNA expression levels are also increased in some Barrett esophagus tissues, with this increase occurring at an earlier stage for basic fibroblast growth factor than for vascular endothelial growth factor. Basic fibroblast growth factor protein expression pattern is similar to the messenger RNA expression pattern, but unlike the messenger RNA findings, vascular endothelial growth factor protein expression is strongest in goblet cells.

J. Virol. (128)

Albu, D. I., A. Jones-Trower, et al. (2003). "Intranasal Vaccination Using Interleukin-12 and Cholera Toxin Subunit B as Adjuvants To Enhance Mucosal and Systemic Immunity to Human Immunodeficiency Virus Type 1 Glycoproteins." *J. Virol.* **77**(10): 5589-5597.

<http://jvi.asm.org/cgi/content/abstract/77/10/5589>

We have investigated the induction of protective mucosal immunity to human immunodeficiency virus type 1 (HIV-1) isolate 89.6 by intranasal (i.n.) immunization of mice with gp120 and gp140 together with interleukin-12 (IL-12) and cholera toxin subunit B (CTB) as adjuvants. It was found that both IL-12 and CTB were required to elicit mucosal antibody responses and that i.n. immunization resulted in increased total, immunoglobulin G1 (IgG1), and IgG2a anti-HIV-1 antibody levels in serum; increased total, IgG1, IgG2a, and IgA antibody expression in bronchoalveolar lavage fluids; and increased IgA antibody levels in vaginal washes. Levels of anti-HIV-1 antibodies in both sera and secretions were higher in groups immunized with gp140 than in those immunized with gp120. However, only gp120-specific mucosal antibodies demonstrated neutralizing activity against HIV-1 89.6. Taken together, the results show that IL-12 and CTB act synergistically to enhance both systemic and local mucosal antibody responses to HIV-1 glycoproteins and that even though gp140 induces higher antibody titers than gp120, only gp120-specific mucosal antibodies interfere with virus infectivity.

Ambrose, Z., V. Boltz, et al. (2004). "In Vitro Characterization of a Simian Immunodeficiency Virus-Human Immunodeficiency Virus (HIV) Chimera Expressing HIV Type 1 Reverse Transcriptase To Study Antiviral Resistance in Pigtail Macaques." *J. Virol.* **78**(24): 13553-13561.

<http://jvi.asm.org/cgi/content/abstract/78/24/13553>

Antiviral resistance is a significant obstacle in the treatment of human immunodeficiency virus type 1 (HIV-1)-infected individuals. Because nonnucleoside reverse transcriptase inhibitors (NNRTIs) specifically target HIV-1 reverse transcriptase (RT) and do not effectively inhibit simian immunodeficiency virus (SIV) RT, the development of animal models to study the evolution of antiviral resistance has been problematic. To facilitate in vivo studies of NNRTI resistance, we examined whether a SIV that causes immunopathogenesis in pigtail macaques could be made sensitive to NNRTIs. Two simian-human immunodeficiency viruses (SHIVs) were derived from the genetic background of SIV_{mne}: SIV-RT-YY contains RT substitutions intended to confer NNRTI susceptibility (V181Y and L188Y), and RT-SHIV_{mne} contains the entire HIV-1 RT coding region. Both mutant viruses grew to high titers in vitro but had reduced fitness relative to wild-type SIV_{mne}. Although the HIV-1 RT was properly processed into p66 and p51 subunits in RT-SHIV_{mne} particles, the RT-SHIV_{mne} virions had lower levels of RT per viral genomic RNA than HIV-1. Correspondingly, there was decreased RT activity in RT-SHIV_{mne} and SIV-RT-YY particles. HIV-1 and RT-SHIV_{mne} were similarly susceptible to the NNRTIs efavirenz, nevirapine, and UC781. However, SIV-RT-YY was less sensitive to NNRTIs than HIV-1 or RT-SHIV_{mne}. Classical NNRTI resistance mutations were selected in RT-SHIV_{mne} after in vitro drug treatment and were monitored in a sensitive allele-specific real-time RT-PCR assay. Collectively, these results indicate that RT-SHIV_{mne} may be a useful model in macaques for the preclinical evaluation of NNRTIs and for studies of the development of drug resistance in vivo.

Apetrei, C., B. Gormus, et al. (2004). "Direct Inoculation of Simian Immunodeficiency Virus from Sooty Mangabeys in Black Mangabeys (*Lophocebus aterrimus*): First Evidence of AIDS in a Heterologous African Species and Different Pathologic Outcomes of Experimental Infection." J. Virol. **78**(21): 11506-11518.

<http://jvi.asm.org/cgi/content/abstract/78/21/11506>

A unique opportunity for the study of the role of serial passage and cross-species transmission was offered by a series of experiments carried out at the Tulane National Primate Research Center in 1990. To develop an animal model for leprosy, three black mangabeys (BkMs) (*Lophocebus aterrimus*) were inoculated with lepromatous tissue that had been serially passaged in four sooty mangabeys (SMs) (*Cercocebus atys*). All three BkMs became infected with simian immunodeficiency virus from SMs (SIV_{sm}) by day 30 postinoculation (p.i.) with lepromatous tissue. One (BkMG140) died 2 years p.i. from causes unrelated to SIV, one (BkMG139) survived for 10 years, whereas the third (BkMG138) was euthanized with AIDS after 5 years. Histopathology revealed a high number of giant cells in tissues from BkMG138, but no SIV-related lesions were found in the remaining two BkMs. Four-color immunofluorescence revealed high levels of SIV_{sm} associated with both giant cells and T lymphocytes in BkMG138 and no detectable SIV in the remaining two. Serum viral load (VL) showed a significant increase (>1 log) during the late stage of the disease in BkMG138, as opposed to a continuous decline in VL in the remaining two BkMs. With the progression to AIDS, neopterin levels increased in BkMG138. This study took on new significance when phylogenetic analysis unexpectedly showed that all four serially inoculated SMs were infected with different SIV_{sm} lineages prior to the beginning of the experiment. Furthermore, the strain infecting the BkMs originated from the last SM in the series. Therefore, the virus infecting BkMs has not been serially passaged. In conclusion, we present the first compelling evidence that direct cross-species transmission of SIV may induce AIDS in heterologous African nonhuman primate (NHP) species. The results showed that cross-species-transmitted SIV_{sm} was well controlled in two of three BkMs for 2 and 10 years, respectively. Finally, this case of AIDS in an African monkey suggests that the dogma of SIV nonpathogenicity in African NHP hosts should be reconsidered.

Apetrei, C., M. J. Metzger, et al. (2005). "Detection and Partial Characterization of Simian Immunodeficiency Virus SIVsm Strains from Bush Meat Samples from Rural Sierra Leone." J. Virol. **79**(4): 2631-2636.

<http://jvi.asm.org/cgi/content/abstract/79/4/2631>

Human immunodeficiency virus type 2 (HIV-2) originated from simian immunodeficiency viruses (SIVs) that naturally infect sooty mangabeys (SMs; *Cercocebus atys*). In order to further investigate the relationship between HIV-2 and SIVsm, the SIV specific to the SM, we characterized seven new SIVsm strains from SMs sold in Sierra Leone markets as bush meat. The gag, pol, and env sequences showed that, while the viruses of all seven SMs belonged to the SIVsm-HIV-2 lineage, they were highly divergent viruses, in spite of the fact that most of the samples originated from the same geographical region. They clustered in three lineages, two of which have been previously reported. Two of the new SIVsm strains clustered differently in gag and env phylogenetic trees, suggesting SIVsm recombination that had occurred in the past. In spite of the fact that our study doubles the number of known SIVsm strains from wild SMs, none of the simian strains were close to the groups in which HIV-2 was epidemic (groups A and B).

Auwerx, J., M. Stevens, et al. (2004). "The Phenylmethylthiazolylthiourea Nonnucleoside Reverse Transcriptase (RT) Inhibitor MSK-076 Selects for a Resistance Mutation in the Active Site of Human Immunodeficiency Virus Type 2 RT." J. Virol. **78**(14): 7427-7437.

<http://jvi.asm.org/cgi/content/abstract/78/14/7427>

The phenylmethylthiazolylthiourea (PETT) derivative MSK-076 shows, besides high potency against human immunodeficiency virus type 1 (HIV-1), marked activity against HIV-2 (50% effective concentration, 0.63 {micro}M) in cell culture. Time-of-addition experiments pointed to HIV-2 reverse transcriptase (RT) as the target of action of MSK-076. Recombinant HIV-2 RT was inhibited by MSK-076 at 23 {micro}M. As was also found for HIV-1 RT, MSK-076 inhibited HIV-2 RT in a noncompetitive manner with respect to dGTP and poly(rC){middle dot}oligo(dG) as the substrate and template-primer, respectively. MSK-076 selected for A101P and G112E mutations in HIV-2 RT and for K101E, Y181C, and G190R mutations in HIV-1 RT. The selected mutated strains of HIV-2 were fully resistant to MSK-076, and the mutant HIV-2 RT enzymes into which the A101P and/or G112E mutation was introduced by site-directed mutagenesis showed more than 50-fold resistance to MSK-076. Mapping of the resistance mutations to the HIV-2 RT structure ascertained that A101P is located at a position equivalent to the nonnucleoside RT inhibitor (NNRTI)-binding site of HIV-1 RT. G112E, however, is distal to the putative NNRTI-binding site in HIV-2 RT but close to the active site, implying a novel molecular mode of action and mechanism of resistance. Our findings have important implications for the development of new NNRTIs with pronounced activity against a wider range of lentiviruses.

Avery, P. R. and E. A. Hoover (2004). "Gamma Interferon/Interleukin 10 Balance in Tissue Lymphocytes Correlates with Down Modulation of Mucosal Feline Immunodeficiency Virus Infection." J. Virol. **78**(8): 4011-4019.

<http://jvi.asm.org/cgi/content/abstract/78/8/4011>

Understanding the early cytokine response to lentiviral infections may be critical to the design of prevention and treatment strategies. By using the feline immunodeficiency virus (FIV) model, we have documented an interleukin 10 (IL10)-dominated response in lymphoid tissue CD4+ and CD8+ T lymphocytes within the first 4 weeks after mucosal FIV infection. This profile coincided

with the period of high tissue viral replication. By 10 weeks postinfection, tissue viral levels decreased significantly, and gamma interferon (IFN γ) production in CD8 $^{+}$ T cells had increased to restore the IL10/IFN γ ratio to control levels. Concurrently, increased production of IL6 and viral RNA was detected in macrophages. These temporal associations of viral replication with cytokine balance in tissues suggest roles for IL10 in the permissive stage of infection and IFN γ in the subsequent down modulation of lentiviral infection.

Babik, J. M. and M. Holodniy (2003). "Impact of Highly Active Antiretroviral Therapy and Immunologic Status on Hepatitis C Virus Quasispecies Diversity in Human Immunodeficiency Virus/Hepatitis C Virus-Coinfected Patients." *J. Virol.* **77**(3): 1940-1950.

<http://jvi.asm.org/cgi/content/abstract/77/3/1940>

This study analyzes the effect of highly active antiretroviral therapy (HAART), and thus immunologic status, on hepatitis C virus (HCV) load and quasispecies diversity in patients coinfecting with the human immunodeficiency virus (HIV) and HCV. Three cohorts of coinfecting patients were analyzed retrospectively over a period of 7 to 10 months: group A was antiretroviral drug naive at baseline and then on HAART for the remainder of the study, group B did not receive antiretroviral therapy at any point, and group C was on HAART for the entire study. HCV quasispecies diversity was analyzed by sequencing hypervariable region 1. In a longitudinal analysis, there was no significant change from baseline in any immunologic, virologic, or quasispecies parameter in any of the three groups. However, in comparison to groups A and B, group C had significantly higher CD4 $^{+}$ - and CD8 $^{+}$ -cell counts, a trend toward a higher HCV load, and significantly increased number of HCV clones, entropy, genetic distance, and ratio of nonsynonymous substitutions per nonsynonymous site to synonymous substitutions per synonymous site (Ka/Ks). In addition, CD4 $^{+}$ -cell count was positively correlated with HCV load, genetic distance, and Ka. Interestingly, patients infected with HCV genotype 2 or 3 had a significantly higher CD4 $^{+}$ -cell count, HCV load, genetic distance, and Ka/Ks than those infected with genotype 1. These results suggest that there is no immediate effect of HAART on HCV but that, with prolonged HAART, immune restoration results in an increase in HCV load and quasispecies diversity.

Badal, V., L. S. H. Chuang, et al. (2003). "CpG Methylation of Human Papillomavirus Type 16 DNA in Cervical Cancer Cell Lines and in Clinical Specimens: Genomic Hypomethylation Correlates with Carcinogenic Progression." *J. Virol.* **77**(11): 6227-6234.

<http://jvi.asm.org/cgi/content/abstract/77/11/6227>

Infection with genital human papillomaviruses (HPVs) is the primary cause of cervical cancer. The infection is widespread, and little is known about the secondary factors associated with progression from subclinical infection to invasive carcinoma. Here we report that HPV genomes are efficiently targeted in vivo by CpG methylation, a well-known mechanism of transcriptional repression. Indeed, it has been shown previously that in vitro-methylated HPV type 16 (HPV-16) DNA is transcriptionally repressed after transfection into cell cultures. By using a scan with the restriction enzyme M crBC , we observed a conserved profile of CpG hyper- and hypomethylation throughout the HPV-16 genomes of the tumor-derived cell lines SiHa and CaSki. Methylation is particularly high in genomic segments overlying the late genes, while the long control region (LCR) and the oncogenes are unmethylated in the single HPV-16 copy in SiHa cells. In 81 patients from two different cohorts, the LCR and the E6 gene of HPV-16 DNA were found to be hypermethylated in 52% of asymptomatic smears, 21.7% of precursor lesions, and 6.1% of invasive carcinomas. This suggests that neoplastic transformation may be suppressed by CpG methylation, while demethylation occurs as the cause of or concomitant with neoplastic

progression. These prevalences of hyper- and hypomethylation also indicate that CpG methylation plays an important role in the papillomavirus life cycle, which takes place in asymptomatic infections and precursor lesions but not in carcinomas. Bisulfite modification revealed that in most of the HPV-16 genomes of CaSki cells and of asymptomatic patients, all 11 CpG dinucleotides that overlap with the enhancer and the promoter were methylated, while in SiHa cells and cervical lesions, the same 11 or a subset of CpGs remained unmethylated. Our report introduces papillomaviruses as models to study the mechanism of CpG methylation, opens research on the importance of this mechanism during the viral life cycle, and provides a marker relevant for the etiology and diagnosis of cervical cancer.

Bartee, E., M. Mansouri, et al. (2004). "Downregulation of Major Histocompatibility Complex Class I by Human Ubiquitin Ligases Related to Viral Immune Evasion Proteins." *J. Virol.* **78**(3): 1109-1120.

<http://jvi.asm.org/cgi/content/abstract/78/3/1109>

Poxviruses and gamma-2 herpesviruses share the K3 family of viral immune evasion proteins that inhibit the surface expression of glycoproteins such as major histocompatibility complex class I (MHC-I), B7.2, ICAM-1, and CD95(Fas). K3 family proteins contain an amino-terminal PHD/LAP or RING-CH domain followed by two transmembrane domains. To examine whether human homologues are functionally related to the viral immunoevasins, we studied seven membrane-associated RING-CH (MARCH) proteins. All MARCH proteins located to subcellular membranes, and several MARCH proteins reduced surface levels of known substrates of the viral K3 family. Two closely related proteins, MARCH-IV and MARCH-IX, reduced surface expression of MHC-I molecules. In the presence of MARCH-IV or MARCH-IX, MHC-I was ubiquitinated and rapidly internalized by endocytosis, whereas MHC-I molecules lacking lysines in their cytoplasmic tail were resistant to downregulation. The amino-terminal regions containing the RING-CH domain of several MARCH proteins examined catalyzed multiubiquitin formation in vitro, suggesting that MARCH proteins are ubiquitin ligases. The functional similarity of the MARCH family and the K3 family suggests that the viral immune evasion proteins were derived from MARCH proteins, a novel family of transmembrane ubiquitin ligases that seems to target glycoproteins for lysosomal destruction via ubiquitination of the cytoplasmic tail.

Bosch, I., K. Khaja, et al. (2002). "Increased Production of Interleukin-8 in Primary Human Monocytes and in Human Epithelial and Endothelial Cell Lines after Dengue Virus Challenge." *J. Virol.* **76**(11): 5588-5597.

<http://jvi.asm.org/cgi/content/abstract/76/11/5588>

The more severe form of dengue virus infection, dengue hemorrhagic fever, is characterized by plasma leakage and derangements in hemostasis. As elevated interleukin-8 (IL-8) levels have been observed in sera from patients with more severe disease manifestations, a study was initiated to look at the effect of dengue virus infection in vitro on proinflammatory cytokine secretion and expression. A significant increase in IL-8 levels in the culture supernatant of primary human monocytes infected with dengue 2 virus (D2V) New Guinea C (NGC) was found by enzyme-linked immunosorbent assay. Additionally, by reverse transcriptase PCR, the mRNA was also augmented. Among the proinflammatory cytokines and their mRNAs measured (IL-6, IL-1[β], IL-8, and tumor necrosis factor α), IL-8 showed the greatest change following D2V infection. Similarly, two cell lines, 293T (a human epithelial cell line) and ECV304 (an endothelial cell line), were permissive to D2V NGC and responded to the infection by increasing the synthesis of IL-8. Nuclear factor κ B (NF- κ B) and nuclear factor IL-6 (NFIL-6) are primary mediators of IL-8 expression. We studied the transcriptional regulation of IL-8 in the ECV304 and 293T cell lines and found that the induction of IL-8 gene expression involved the

activation of NF- κ B ($P = 0.001$) and, to a lesser extent, the activation of NFIL-6 in ECV304 cells only. We next observed by the chromatin immunoprecipitation procedure in vivo acetylation of core histones bound to the IL-8 promoter after D2V infection. IL-8 produced by infected monocytes and also IL-8 that may be produced by endothelial or other epithelial cells is associated with the hyperacetylation of histones bound to the IL-8 promoter in addition to the activation of transcription by NF- κ B. We hypothesize that the overall increase in IL-8 synthesis observed in this in vitro study may play a role in the pathogenesis of the plasma leakage seen in dengue hemorrhagic fever and dengue shock syndrome.

Bowles, D. E., J. E. Rabinowitz, et al. (2003). "Marker Rescue of Adeno-Associated Virus (AAV) Capsid Mutants: a Novel Approach for Chimeric AAV Production." *J. Virol.* **77**(1): 423-432.

<http://jvi.asm.org/cgi/content/abstract/77/1/423>

Marker rescue, the restoration of gene function by replacement of a defective gene with a normal one by recombination, has been utilized to produce novel adeno-associated virus (AAV) vectors. AAV serotype 2 (AAV2) clones containing wild-type terminal repeats, an intact rep gene, and a mutated cap gene, served as the template for marker rescue. When transfected alone in 293 cells, these AAV2 mutant plasmids produced noninfectious AAV virions that could not bind heparin sulfate after infection with adenovirus dl309 helper virus. However, the mutation in the cap gene was corrected after cotransfection with AAV serotype 3 (AAV3) capsid DNA fragments, resulting in the production of AAV2/AAV3 chimeric viruses. The cap genes from several independent marker rescue experiments were PCR amplified, cloned, and then sequenced. Sequencing results confirmed not only that homologous recombination occurred but, more importantly, that a mixed population of AAV chimeras carrying 16 to 2,200 bp throughout different regions of the type 3 cap gene were generated in a single marker rescue experiment. A 100% correlation was observed between infectivity and the ability of the chimeric virus to bind heparin sulfate. In addition, many of the AAV2/AAV3 chimeras examined exhibited differences at both the nucleotide and amino acid levels, suggesting that these chimeras may also exhibit unique infectious properties. Furthermore, AAV helper plasmids containing these chimeric cap genes were able to function in the triple transfection method to generate recombinant AAV. Together, the results suggest that DNA from other AAV serotypes can rescue AAV capsid mutants and that marker rescue may be a powerful, yet simple, technique to map, as well as develop, chimeric AAV capsids that display different serotype-specific properties.

Brilot, F., V. Geenen, et al. (2004). "Coxsackievirus B4 Infection of Human Fetal Thymus Cells." *J. Virol.* **78**(18): 9854-9861.

<http://jvi.asm.org/cgi/content/abstract/78/18/9854>

The infection of human fetal thymus organ cultures (FTOC) with coxsackievirus B4 E2 (CVB4 E2) was investigated. Both positive- and negative-strand viral RNA were detected by real-time quantitative reverse transcription-PCR (RT-PCR) in CVB4 E2-infected FTOC, which supported high yields of virus production ($\sim 10^6$ 50% tissue culture infective doses/ml), and in flow-sorted thymocyte populations for 7 days after inoculation. Cortical CD4⁺ CD8⁺ thymocytes were found to be the principal targets of infection. Inoculation of human FTOC with CVB4 E2 led to a marked and progressive depletion of immature thymocytes (CD4⁺ CD8⁺ cells) with no enhancement of Annexin V-positive cells. CVB4 E2 replication caused significant major histocompatibility complex (MHC) class I upregulation on these cells. MHC class I upregulation was correlated with positive- and negative-strand RNA quantitative detection and the release of infectious particles. In addition, chloroquine treatment of FTOC and single-thymocyte suspensions suggested that MHC class I upregulation on thymocytes was the result of direct infection rather than caused by production of

soluble factors such as alpha interferon. Thus, CVB4 E2 can infect human fetal thymocytes, which subsequently results in quantitative and qualitative abnormalities of these cells.

Buckman, J. S., W. J. Bosche, et al. (2003). "Human Immunodeficiency Virus Type 1 Nucleocapsid Zn²⁺ Fingers Are Required for Efficient Reverse Transcription, Initial Integration Processes, and Protection of Newly Synthesized Viral DNA." *J. Virol.* **77**(2): 1469-1480.

<http://jvi.asm.org/cgi/content/abstract/77/2/1469>

Human immunodeficiency virus type 1 (HIV-1) containing mutations in the nucleocapsid (NC) Zn²⁺ finger domains have greatly reduced infectivity, even though genome packaging is largely unaffected in certain cases. To examine replication defects, viral DNA (vDNA) was isolated from cells infected with viruses containing His-to-Cys changes in their Zn²⁺ fingers (NCH23C and NCH44C), an integrase mutant (IND116N), a double mutant (NCH23C/IND116N), or wild-type HIV-1. In vitro assays have established potential roles for NC in reverse transcription and integration. In vivo results for these processes were obtained by quantitative PCR, cloning of PCR products, and comparison of the quantity and composition of vDNA generated at discrete points during reverse transcription. Quantitative analysis of the reverse transcription intermediates for these species strongly suggests decreased stability of the DNA produced. Both Zn²⁺ finger mutants appear to be defective in DNA synthesis, with the minus- and plus-strand transfer processes being affected while interior portions of the vDNA remain more intact. Sequences obtained from PCR amplification and cloning of 2-LTR circle junction fragments revealed that the NC mutants had a phenotype similar to the IN mutant; removal of the terminal CA dinucleotides necessary for integration of the vDNA is disabled by the NC mutations. Thus, the loss of infectivity in these NC mutants in vivo appears to result from defective reverse transcription and integration processes stemming from decreased protection of the full-length vDNA. Finally, these results indicate that the chaperone activity of NC extends from the management of viral RNA through to the full-length vDNA.

Candotti, D., N. Etiz, et al. (2004). "Identification and Characterization of Persistent Human Erythrovirus Infection in Blood Donor Samples." *J. Virol.* **78**(22): 12169-12178.

<http://jvi.asm.org/cgi/content/abstract/78/22/12169>

The presence of human erythrovirus DNA in 2,440 blood donations from the United Kingdom and sub-Saharan Africa (Ghana, Malawi, and South Africa) was screened. Sensitive qualitative and real-time quantitative PCR assays revealed a higher prevalence of persistent infection with the simultaneous presence of immunoglobulin G (IgG) and viral DNA (0.55 to 1.3%) than previously reported. This condition was characterized by a low viral load (median, 558 IU/ml; range, 42 to 135,000 IU/ml), antibody-complexed virus, free specific IgG, and potentially infectious free virus. Human erythrovirus genotype 1 (formerly parvovirus B19) was prevalent in the United Kingdom, Malawi, and South Africa. In contrast, only human erythrovirus genotype 3 (erythrovirus variant V9) was prevalent in Ghana. Genotype 3 had considerable genetic diversity, clustering in two probable subtypes. Genotype 1-based antibody assays failed to detect 38.5% of Ghanaian samples containing antibodies to genotype 3 virus but did not fail to detect cases of persistent infection. This study indicates a potential African origin of genotype 3 human erythrovirus and considerable shortcomings in the tools currently used to diagnose erythrovirus infection.

Candotti, D., J. Temple, et al. (2003). "Frequent Recovery and Broad Genotype 2 Diversity Characterize

Hepatitis C Virus Infection in Ghana, West Africa." *J. Virol.* **77**(14): 7914-7923.

<http://jvi.asm.org/cgi/content/abstract/77/14/7914>

Hepatitis C virus (HCV) infection is thought to mostly become chronic and rarely resolves. HCV infection was serologically screened in 4,984 samples from Ghanaian blood donors, and 1.3% prevalence was found. At least 53% of confirmed anti-HCV carriers had no detectable viral RNA and were considered to have cleared the virus and recovered from the infection. Confirmation was authenticated by the presence of antibodies specific to at least two viral antigens, mostly NS3 and E2. Reactivity to HCV core antigens was lower in Ghanaian than United Kingdom blood donors. The minority of chronically infected donors carried a viral load significantly lower than an unselected comparative group of United Kingdom blood donors (2.5×10^5 versus 2.9×10^6 IU/ml; $P = 0.004$). HCV genotype 2 was largely predominant (87%). Sequence clustering was similarly broad in the E1/E2 and NS5 regions. The phylogenetic diversity and the incapacity to distinguish subtypes within genotype 2 in our and others' West African strains suggested that West Africa may be the origin of HCV genotype 2. The genetic diversity extended to the identification of strains clearly separated from known subtypes of genotype 2 and genotype 1. One strain appears to be part of a new HCV genotype. HCV infection in Ghana is characterized by a high rate of recovery and the predominance of broadly divergent genotype 2 strains.

Cheng, C.-H., S.-M. Liu, et al. (2002). "Analysis of the Complete Genome Sequence of the Hz-1 Virus Suggests that It Is Related to Members of the Baculoviridae." *J. Virol.* **76**(18): 9024-9034.

<http://jvi.asm.org/cgi/content/abstract/76/18/9024>

We report the complete sequence of a large rod-shaped DNA virus, called the Hz-1 virus. This virus persistently infects the *Heliothis zea* cell lines. The Hz-1 virus has a double-stranded circular DNA genome of 228,089 bp encoding 154 open reading frames (ORFs) and also expresses a persistence-associated transcript 1, PAT1. The G+C content of the Hz-1 virus genome is 41.8%, with a gene density of one gene per 1.47 kb. Sequence analysis revealed that a 9.6-kb region at 43.6 to 47.8 map units harbors five cellular genes encoding proteins with homology to dUTP pyrophosphatase, matrix metalloproteinase, deoxynucleoside kinase, glycine hydroxymethyltransferase, and ribonucleotide reductase large subunit. Other cellular homologs were also detected dispersed in the viral genome. Several baculovirus homologs were detected in the Hz-1 virus genome. These include PxOrf-70, PxOrf-29, AcOrf-81, AcOrf-96, AcOrf-22, VLF-1, RNA polymerase LEF-8 (orf50), and two structural proteins, p74 and p91. The Hz-1 virus p74 homolog shows high structural conservation with a double transmembrane domain at its C terminus. Phylogenetic analysis of the p74 revealed that the Hz-1 virus is evolutionarily distant from the baculoviruses. Another distinctive feature of the Hz-1 virus genome is a gene that is involved in insect development. However, the remainder of the ORFs (81%) encoded proteins that bear no homology to any known proteins. In conclusion, the sequence differences between the Hz-1 virus and the baculoviruses outnumber the similarities and suggest that the Hz-1 virus may form a new family of viruses distantly related to the Baculoviridae.

Cologna, R., P. M. Armstrong, et al. (2005). "Selection for Virulent Dengue Viruses Occurs in Humans and Mosquitoes." *J. Virol.* **79**(2): 853-859.

<http://jvi.asm.org/cgi/content/abstract/79/2/853>

Dengue is the most common mosquito-borne viral disease in humans. The spread of both mosquito vectors and viruses has led to the resurgence of epidemic dengue fever (a self-limited

flu-like syndrome) and the emergence of dengue hemorrhagic fever (severe dengue with bleeding abnormalities) in urban centers of the tropics. There are no animal or laboratory models of dengue disease; indirect evidence suggests that dengue viruses differ in virulence, including their pathogenicities for humans and epidemic potential. We developed two assay systems (using human dendritic cells and *Aedes aegypti* mosquitoes) for measuring differences in virus replication that correlate with the potential to cause hemorrhagic dengue and increased virus transmission. Infection and growth experiments showed that dengue serotype 2 viruses causing dengue hemorrhagic fever epidemics (Southeast Asian genotype) can outcompete viruses that cause dengue fever only (American genotype). This fact implies that Southeast Asian genotype viruses will continue to displace other viruses, causing more hemorrhagic dengue epidemics.

Courgnaud, V., B. Abela, et al. (2003). "Identification of a New Simian Immunodeficiency Virus Lineage with a vpu Gene Present among Different Cercopithecus Monkeys (*C. mona*, *C. cephus*, and *C. nictitans*) from Cameroon." *J. Virol.* **77**(23): 12523-12534.

<http://jvi.asm.org/cgi/content/abstract/77/23/12523>

During a large serosurvey of wild-caught primates from Cameroon, we found 2 mona monkeys (*Cercopithecus mona*) out of 8 and 47 mustached monkeys (*Cercopithecus cephus*) out of 302 with human immunodeficiency virus (HIV)-simian immunodeficiency virus (SIV) cross-reactive antibodies. In this report, we describe the full-length genome sequences of two novel SIVs, designated SIVmon-99CMCL1 and SIVmus-01CM1085, isolated from one mona (CML1) and one mustached (1085) monkey, respectively. Interestingly, these viruses displayed the same genetic organization (i.e., presence of a vpu homologue) as members of the SIVcpz-HIV type 1 lineage and SIVgsn isolated from greater spot-nosed monkeys (*Cercopithecus nictitans*). Phylogenetic analyses of SIVmon and SIVmus revealed that these viruses were genetically distinct from other known primate lentiviruses but were more closely related to SIVgsn all across their genomes, thus forming a monophyletic lineage within the primate lentivirus family, which we designated the SIVgsn lineage. Interestingly, mona, mustached, and greater spot-nosed monkeys are phylogenetically related species belonging to three different groups of the genus *Cercopithecus*, the *C. mona*, *C. cephus*, and *Cercopithecus mitis* groups, respectively. The presence of new viruses closely related to SIVgsn in two other species reinforces the hypothesis that a recombination event between ancestral SIVs from the family Cercopithecinae is the origin of the present SIVcpz that is widespread among the chimpanzee population.

Courgnaud, V., P. Formenty, et al. (2003). "Partial Molecular Characterization of Two Simian Immunodeficiency Viruses (SIV) from African Colobids: SIVwrc from Western Red Colobus (*Piliocolobus badius*) and SIVolc from Olive Colobus (*Procolobus verus*)." *J. Virol.* **77**(1): 744-748.

<http://jvi.asm.org/cgi/content/abstract/77/1/744>

In order to study primate lentivirus evolution in the Colobinae subfamily, in which only one simian immunodeficiency virus (SIV) has been described to date, we screened additional species from the three different genera of African colobus monkeys for SIV infection. Blood was obtained from 13 West African colobids, and HIV cross-reactive antibodies were observed in 5 of 10 *Piliocolobus badius*, 1 of 2 *Procolobus verus*, and 0 of 1 *Colobus polykomos* specimens. Phylogenetic analyses of partial pol sequences revealed that the new SIVs were more closely related to each other than to the other SIVs and especially did not cluster with the previously described SIVcol from *Colobus guereza*. This study presents evidence that the three genera of African colobus monkeys are naturally infected with an SIV and indicates also that there was no coevolution between virus and hosts at the level of the Colobinae subfamily.

Courgnaud, V., M. Salemi, et al. (2002). "Characterization of a Novel Simian Immunodeficiency Virus with a vpu Gene from Greater Spot-Nosed Monkeys (*Cercopithecus nictitans*) Provides New Insights into Simian/Human Immunodeficiency Virus Phylogeny." *J. Virol.* **76**(16): 8298-8309.

<http://jvi.asm.org/cgi/content/abstract/76/16/8298>

In the present study, we describe a new simian immunodeficiency virus (SIV), designated SIVgsn, naturally infecting greater spot-nosed monkeys (*Cercopithecus nictitans*) in Cameroon. Together with SIVsyk, SIVgsn represents the second virus isolated from a monkey belonging to the *Cercopithecus mitis* group of the *Cercopithecus* genus. Full-length genome sequence analysis of two SIVgsn strains, SIVgsn-99CM71 and SIVgsn-99CM166, revealed that despite the close phylogenetic relationship of their hosts, SIVgsn was highly divergent from SIVsyk. First of all, they differ in their genomic organization. SIVgsn codes for a vpu homologue, so far a unique feature of the members of the SIVcpz/human immunodeficiency virus type 1 (HIV-1) lineage, and detailed phylogenetic analyses of various regions of the viral genome indicated that SIVgsn might be a mosaic of sequences with different evolutionary histories. SIVgsn was related to SIVsyk in Gag and part of Pol and related to SIVcpz in Env, and the middle part of the genome did not cluster significantly with any of the known SIV lineages. When comparing the two SIVgsn Env sequences with that of SIVcpz, a remarkable conservation was seen in the V3 loop, indicating a possible common origin for the envelopes of these two viruses. The habitats of the two subspecies of chimpanzees infected by SIVcpz overlap the geographic ranges of greater spot-nosed monkeys and other monkey species, allowing cross-species transmission and recombination between coinfecting viruses. The complex genomic structure of SIVgsn, the presence of a vpu gene, and its relatedness to SIVcpz in the envelope suggest a link between SIVgsn and SIVcpz and provide new insights about the origin of SIVcpz in chimpanzees.

Courgnaud, V., S. Van Dooren, et al. (2004). "Simian T-Cell Leukemia Virus (STLV) Infection in Wild Primate Populations in Cameroon: Evidence for Dual STLV Type 1 and Type 3 Infection in Agile Mangabeys (*Cercocebus agilis*)." *J. Virol.* **78**(9): 4700-4709.

<http://jvi.asm.org/cgi/content/abstract/78/9/4700>

Three types of human T-cell leukemia virus (HTLV)-simian T-cell leukemia virus (STLV) (collectively called primate T-cell leukemia viruses [PTLVs]) have been characterized, with evidence for zoonotic origin from primates for HTLV type 1 (HTLV-1) and HTLV-2 in Africa. To assess human exposure to STLVs in western Central Africa, we screened for STLV infection in primates hunted in the rain forests of Cameroon. Blood was obtained from 524 animals representing 18 different species. All the animals were wild caught between 1999 and 2002; 328 animals were sampled as bush meat and 196 were pets. Overall, 59 (11.2%) of the primates had antibodies cross-reacting with HTLV-1 and/or HTLV-2 antigens; HTLV-1 infection was confirmed in 37 animals, HTLV-2 infection was confirmed in 9, dual HTLV-1 and HTLV-2 infection was confirmed in 10, and results for 3 animals were indeterminate. Prevalences of infection were significantly lower in pets than in bush meat, 1.5 versus 17.0%, respectively. Discriminatory PCRs identified STLV-1, STLV-3, and STLV-1 and STLV-3 in HTLV-1-, HTLV-2-, and HTLV-1- and HTLV-2-cross-reactive samples, respectively. We identified for the first time STLV-1 sequences in mustached monkeys (*Cercopithecus cephus*), talapoints (*Miopithecus ogouensis*), and gorillas (*Gorilla gorilla*) and confirmed STLV-1 infection in mandrills, African green monkeys, agile mangabeys, and crested mona and greater spot-nosed monkeys. STLV-1 long terminal repeat (LTR) and env sequences revealed that the strains belonged to different PTLV-1 subtypes. A high prevalence of PTLV infection was observed among agile mangabeys (*Cercocebus agilis*); 89% of bush meat was infected with STLV. Cocirculation of STLV-1 and STLV-3 and STLV-1-STLV-3 coinfections were identified among the agile mangabeys. Phylogenetic analyses of partial LTR

sequences indicated that the agile mangabey STLV-3 strains were more related to the STLV-3 CTO604 strain isolated from a red-capped mangabey (*Cercocebus torquatus*) from Cameroon than to the STLV-3 PH969 strain from an Eritrean baboon or the PPA-F3 strain from a baboon in Senegal. Our study documents for the first time that (i) a substantial proportion of wild-living monkeys in Cameroon is STLV infected, (ii) STLV-1 and STLV-3 cocirculate in the same primate species, (iii) coinfection with STLV-1 and STLV-3 occurs in agile mangabeys, and (iv) humans are exposed to different STLV-1 and STLV-3 subtypes through handling primates as bush meat.

Dang, X., M. K. Axthelm, et al. (2005). "Rearrangement of Simian Virus 40 Regulatory Region Is Not Required for Induction of Progressive Multifocal Leukoencephalopathy in Immunosuppressed Rhesus Monkeys." *J. Virol.* **79**(3): 1361-1366.

<http://jvi.asm.org/cgi/content/abstract/79/3/1361>

Rearrangements of the JC virus (JCV) regulatory region (RR) are consistently found in the brains of patients with progressive multifocal leukoencephalopathy (PML), whereas the archetype RR is present in their kidneys. In addition, the C terminus of the large T antigen (T-Ag) shows greater variability in PML than does the rest of the coding region. To determine whether similar changes in simian virus 40 (SV40) are necessary for disease induction in monkeys, we sequenced the SV40 RR and the C terminus of the T-Ag from the brain of simian/human immunodeficiency virus (SHIV)-infected monkey 18429, which presented spontaneously with an SV40-associated PML-like disease, as well as from the peripheral blood mononuclear cells (PBMC), kidneys, and brains of SV40-seronegative, SHIV-infected monkeys 21289 and 21306, which were inoculated with the 18429 brain SV40 isolate. These animals developed both SV40-associated PML and meningoencephalitis. Thirteen types of SV40 RR were characterized. Compared to the SV40 archetype, we identified RRs with variable deletions in either the origin of replication, the 21-bp repeat elements, or the late promoter, as well as deletions or duplications of the 72-bp enhancer. The archetype was the most prominent RR in the brain of monkey 18429. Shortly after inoculation, a wide range of RRs could be found in the PBMC of monkeys 21289 and 21306. However, the archetype RR became the predominant type in their blood, kidneys, and brains at the time of sacrifice. On the contrary, the T-Ag C termini remained identical in all compartments of the three animals. These results indicate that unlike JCV in humans, rearrangements of SV40 RR are not required for brain disease induction in immunosuppressed monkeys.

De Tomassi, A., M. Pizzuti, et al. (2002). "Cell Clones Selected from the Huh7 Human Hepatoma Cell Line Support Efficient Replication of a Subgenomic GB Virus B Replicon." *J. Virol.* **76**(15): 7736-7746.

<http://jvi.asm.org/cgi/content/abstract/76/15/7736>

Tamarins (*Saguinus* species) infected by GB virus B (GBV-B) have recently been proposed as an acceptable surrogate model for hepatitis C virus (HCV) infection. The availability of infectious genomic molecular clones of both viruses will permit chimeric constructs to be tested for viability in animals. Studies in cells with parental and chimeric constructs would also be very useful for both basic research and drug discovery. For this purpose, a convenient host cell type supporting replication of in vitro-transcribed GBV-B RNA should be identified. We constructed a GBV-B subgenomic selectable replicon based on the sequence of a genomic molecular clone proved to sustain infection in tamarins. The corresponding in vitro-transcribed RNA was used to transfect the Huh7 human hepatoma cell line, and intracellular replication of transfected RNA was shown to occur, even though in a small percentage of transfected cells, giving rise to antibiotic-resistant clones. Sequence analysis of GBV-B RNA from some of those clones showed no adaptive mutations with respect to the input sequence, whereas the host cells sustained higher GBV-B

RNA replication than the original Huh7 cells. The enhancement of replication depending on host cell was shown to be a feature common to the majority of clones selected. The replication of GBV-B subgenomic RNA was susceptible to inhibition by known inhibitors of HCV to a level similar to that of HCV subgenomic RNA.

Dvorin, J. D., P. Bell, et al. (2002). "Reassessment of the Roles of Integrase and the Central DNA Flap in Human Immunodeficiency Virus Type 1 Nuclear Import." *J. Virol.* **76**(23): 12087-12096.

<http://jvi.asm.org/cgi/content/abstract/76/23/12087>

Human immunodeficiency virus type 1 (HIV-1) can infect nondividing cells productively because the nuclear import of viral nucleic acids occurs in the absence of cell division. A number of viral factors that are present in HIV-1 preintegration complexes (PICs) have been assigned functions in nuclear import, including an essential valine at position 165 in integrase (IN-V165) and the central polypurine tract (cPPT). In this article, we report a comparison of the replication and infection characteristics of viruses with disruptions in the cPPT and IN-V165. We found that viruses with cPPT mutations still replicated productively in both dividing and nondividing cells, while viruses with a mutation at IN-V165 did not. Direct observation of the subcellular localization of HIV-1 cDNAs by fluorescence in situ hybridization revealed that cDNAs synthesized by both mutant viruses were readily detected in the nucleus. Thus, neither the cPPT nor the valine residue at position 165 of integrase is essential for the nuclear import of HIV-1 PICs.

Edwards, R. H., D. Sitki-Green, et al. (2004). "Potential Selection of LMP1 Variants in Nasopharyngeal Carcinoma." *J. Virol.* **78**(2): 868-881.

<http://jvi.asm.org/cgi/content/abstract/78/2/868>

Seven distinct sequence variants of the Epstein-Barr virus latent membrane protein 1 (LMP1) have been identified by distinguishing amino acid changes in the carboxy-terminal domain. In this study the transmembrane domains are shown to segregate identically with the distinct carboxy-terminal amino acid sequences. Since strains of LMP1 have been shown to differ in abundance between blood and throat washes, nasopharyngeal carcinomas (NPCs) from areas of endemicity and nonendemicity with matching blood were analyzed by using a heteroduplex tracking assay to distinguish LMP1 variants. Striking differences were found between the compartments with the Ch1 strain prevalent in the NPCs from areas of endemicity and nonendemicity and the B958 strain prevalent in the blood of the endemic samples, whereas multiple strains of LMP1 were prevalent in the blood of the nonendemic samples. The possible selection against the B958 strain appearing in the tumor was highly significant ($P < 0.0001$). Sequence analysis of the full-length LMP1 variants revealed changes in many of the known and computer-predicted HLA-restricted epitopes with changes in key positions in multiple, potential epitopes for the specific HLA of the patients. These amino acid substitutions at key positions in the LMP1 epitopes may result in a reduced cytotoxic-T-lymphocyte response. These data indicate that strains with specific variants of LMP1 are more likely to be found in NPC. The predominance of specific LMP1 variants in NPC could reflect differences in the biologic or molecular properties of the distinct forms of LMP1 or possible immune selection.

Fenaux, M., P. G. Halbur, et al. (2002). "Cloned Genomic DNA of Type 2 Porcine Circovirus Is Infectious When Injected Directly into the Liver and Lymph Nodes of Pigs: Characterization of Clinical Disease, Virus Distribution, and Pathologic Lesions." *J. Virol.* **76**(2): 541-551.

<http://jvi.asm.org/cgi/content/abstract/76/2/541>

Infection of animals with a molecular viral clone is critical to study the genetic determinants of viral replication and virulence in the host. Type 2 porcine circovirus (PCV2) has been incriminated as the cause of postweaning multisystemic wasting syndrome (PMWS), an emerging disease in pigs. We report here for the first time the construction and use of an infectious molecular DNA clone of PCV2 to characterize the disease and pathologic lesions associated with PCV2 infection by direct in vivo transfection of pigs with the molecular clone. The PCV2 molecular clone was generated by ligating two copies of the complete PCV2 genome in tandem into the pBluescript SK (pSK) vector and was shown to be infectious in vitro when transfected into PK-15 cells. Forty specific-pathogen-free pigs at 4 weeks of age were randomly assigned to four groups of 10 each. Group 1 pigs served as uninoculated controls. Pigs in group 2 were each inoculated intranasally with about 1.9×10^5 50% tissue culture infective doses of a homogeneous PCV2 live virus stock derived from the molecular clone. Pigs in group 3 were each injected intrahepatically with 200 {micro}g of the cloned PCV2 plasmid DNA, and pigs in group 4 were each injected into the superficial iliac lymph nodes with 200 {micro}g of the cloned PCV2 plasmid DNA. Animals injected with the cloned PCV2 plasmid DNA developed infection resembling that induced by intranasal inoculation with PCV2 live virus stock. Seroconversion to PCV2-specific antibody was detected in the majority of pigs from the three inoculated groups at 35 days postinoculation (DPI). Viremia, beginning at 14 DPI and lasting 2 to 4 weeks, was detected in the majority of the pigs from all three inoculated groups. There were no remarkable clinical signs of PMWS in control or any of the inoculated pigs. Gross lesions in pigs of the three inoculated groups were similar and were characterized by systemically enlarged, tan lymph nodes and lungs that failed to collapse. Histopathological lesions and PCV2-specific antigen were detected in numerous tissues and organs, including brain, lung, heart, kidney, tonsil, lymph nodes, spleen, ileum, and liver of infected pigs. This study more definitively characterizes the clinical course and pathologic lesions exclusively attributable to PCV2 infection. The data from this study indicate that the cloned PCV2 genomic DNA may replace infectious virus for future PCV2 pathogenesis and immunization studies. The data also suggest that PCV2, although essential for development of PMWS, may require other factors or agents to induce the full spectrum of clinical signs and lesions associated with advanced cases of PMWS.

Fenaux, M., T. Opriessnig, et al. (2003). "Immunogenicity and Pathogenicity of Chimeric Infectious DNA Clones of Pathogenic Porcine Circovirus Type 2 (PCV2) and Nonpathogenic PCV1 in Weanling Pigs." *J. Virol.* **77**(20): 11232-11243.

<http://jvi.asm.org/cgi/content/abstract/77/20/11232>

Porcine circovirus type 2 (PCV2) is the primary causative agent of postweaning multisystemic wasting syndrome (PMWS), whereas the ubiquitous porcine circovirus type 1 (PCV1) is nonpathogenic for pigs. We report here the construction and characterization of two chimeric infectious DNA clones of PCV1 and PCV2. The chimeric PCV1-2 clone contains the PCV2 capsid gene cloned in the backbone of the nonpathogenic PCV1 genome. A reciprocal chimeric PCV2-1 DNA clone was also constructed by replacing the PCV2 capsid gene with that of PCV1 in the backbone of the PCV2 genome. The PCV1, PCV2, and chimeric PCV1-2 and PCV2-1 DNA clones were all shown to be infectious in PK-15 cells, and their growth characteristics in vitro were determined and compared. To evaluate the immunogenicity and pathogenicity of the chimeric infectious DNA clones, 40 specific-pathogen-free (SPF) pigs were randomly assigned into five groups of eight pigs each. Group 1 pigs received phosphate-buffered saline as the negative control. Group 2 pigs were each injected in the superficial inguinal lymph nodes with 200 {micro}g of the PCV1 infectious DNA clone. Group 3 pigs were each similarly injected with 200 {micro}g of the PCV2 infectious DNA clone, group 4 pigs were each injected with 200 {micro}g of the chimeric PCV1-2 infectious DNA clone, and group 5 pigs were each injected with 200 {micro}g of the reciprocal chimeric PCV2-1 infectious DNA clone. As expected, seroconversion to

antibodies to the PCV2 capsid antigen was detected in group 3 and group 4 pigs. Group 2 and 5 pigs all seroconverted to PCV1 antibody. Gross and microscopic lesions in various tissues of animals inoculated with the PCV2 infectious DNA clone were significantly more severe than those found in pigs inoculated with PCV1, chimeric PCV1-2, and reciprocal chimeric PCV2-1 infectious DNA clones. These data indicated that the chimeric PCV1-2 virus with the immunogenic ORF2 capsid gene of pathogenic PCV2 cloned into the nonpathogenic PCV1 genomic backbone induces a specific antibody response to the pathogenic PCV2 capsid antigen but is attenuated in pigs. Future studies are warranted to evaluate the usefulness of the chimeric PCV1-2 infectious DNA clone as a genetically engineered live-attenuated vaccine against PCV2 infection and PMWS.

Francois, F. and M. E. Klotman (2003). "Phosphatidylinositol 3-Kinase Regulates Human Immunodeficiency Virus Type 1 Replication following Viral Entry in Primary CD4+ T Lymphocytes and Macrophages." *J. Virol.* **77**(4): 2539-2549.

<http://jvi.asm.org/cgi/content/abstract/77/4/2539>

Human immunodeficiency virus (HIV) gp120 induces multiple cellular signaling pathways, including the phosphatidylinositol 3-kinase (PI3-kinase) pathway. The role of the PI3-kinase pathway in HIV-1 replication is not understood. Here we examined whether HIV-1 gp120 upregulates the PI3-kinase pathway and whether PI3-kinase activity plays a role in virus replication in primary human CD4+ T cells and macrophages. Soluble and virion-associated HIV-1 gp120 induced calcium mobilization and phosphorylation of the PI3-kinase downstream effectors PKB/Akt and p70 S6 kinase. gp120-induced PI3-kinase activity and calcium mobilization were inhibited by pertussis toxin and blocking antibodies directed against CCR5 and CXCR4, suggesting that the signaling is mediated through the chemokine receptor. The PI3-kinase inhibitor LY294002 inhibited infection of CD4+ T cells and macrophages with X4 and R5 HIV-1-pseudotyped viruses at concentrations that did not induce cell toxicity or downregulate HIV-1 coreceptor expression. When gp120-induced signaling was bypassed with the vesicular stomatitis virus G envelope protein, infection was still sensitive to PI3-kinase inhibition, suggesting that basal PI3-kinase activity is required for infection. LY294002 inhibited HIV-1 infection when added after viral entry and did not affect formation of the HIV-1 reverse transcriptase products R/U5 and long terminal repeat/Gag in the presence of the inhibitor. However, when the inhibitor was added after viral integration had occurred, no inhibition of HIV infection was observed. Our studies show that inhibition of the PI3-kinase signaling pathway suppresses virus infection post-viral entry and post-reverse transcription but prior to HIV gene expression. This type of host-virus interaction has implications for anti-HIV therapeutics that target cellular signaling machinery.

Friebe, A., A. Siegling, et al. (2004). "Immunomodulatory Effects of Inactivated Parapoxvirus Ovis (Orf Virus) on Human Peripheral Immune Cells: Induction of Cytokine Secretion in Monocytes and Th1-Like Cells." *J. Virol.* **78**(17): 9400-9411.

<http://jvi.asm.org/cgi/content/abstract/78/17/9400>

Inactivated parapoxvirus ovis (Orf virus; PPVO) recently displayed strong immunostimulating and modulating capacities in several animal models for acute and chronic virus infections through the induction of gamma interferon (IFN- γ) as a key mediator of antiviral activity. The data presented in this work demonstrate that inactivated PPVO has strong effects on cytokine secretion by human immune cells, including the upregulation of inflammatory and Th1-related cytokines (IFN- γ , tumor necrosis factor alpha [TNF- α], interleukin 6 [IL-6], IL-8, IL-12, and IL-18) as well as anti-inflammatory and Th2-related cytokines (IL-4, IL-10, and IL-1 receptor antagonist [IL-1ra]). Studies on the mechanism of action revealed virus particles to be

the effective components of the preparation. The virus particles activate monocytes or other antigen-presenting cells (APC), e.g., plasmacytoid dendritic cells, through signaling over CD14 and a Toll-like receptor and the intracellular presence of certain PPVO-specific components. The activation of monocytes or APC is followed by the release of early proinflammatory cytokines (TNF- α , IL-6, and IL-8) as well as the Th1-related cytokines IL-12 and IL-18. Both IL-18 and IL-12 are involved in PPVO-mediated IFN- γ release by T cells and/or NK cells. The proinflammatory response is accompanied by the induction of anti-inflammatory and Th2-related cytokines (IL-4, IL-10, and IL-1ra), which exert a limiting effect on the inflammatory response induced by PPVO. We conclude that the induction of a natural immune response with physiologically significant amounts of different cytokines and with antiviral potential might provide advantages over existing antiviral immunotherapies.

Fuller, D. H., P. A. Rajakumar, et al. (2002). "Induction of Mucosal Protection against Primary, Heterologous Simian Immunodeficiency Virus by a DNA Vaccine." *J. Virol.* **76**(7): 3309-3317.

<http://jvi.asm.org/cgi/content/abstract/76/7/3309>

An effective vaccine against human immunodeficiency virus (HIV) should protect against mucosal transmission of genetically divergent isolates. As a safe alternative to live attenuated vaccines, the immunogenicity and protective efficacy of a DNA vaccine containing simian immunodeficiency virus (SIV) strain 17E-Fr (SIV/17E-Fr) gag-pol-env was analyzed in rhesus macaques. Significant levels of cytotoxic T lymphocytes (CTL), but low to undetectable serum antibody responses, were observed following multiple immunizations. SIV-specific mucosal antibodies and CTL were also detected in rectal washes and gut-associated lymphoid tissues, respectively. Vaccinated and naive control monkeys were challenged intrarectally with SIV strain DeltaB670 (SIV/DeltaB670), a primary isolate whose env is 15% dissimilar to that of the vaccine strain. Four of seven vaccinees were protected from infection as determined by the inability to identify viral RNA or DNA sequences in the peripheral blood and the absence of anamnestic antibody responses postchallenge. This is the first report of mucosal protection against a primary pathogenic, heterologous isolate of SIV by using a commercially viable vaccine approach. These results support further development of a DNA vaccine for protection against HIV.

Gaddis, N. C., E. Chertova, et al. (2003). "Comprehensive Investigation of the Molecular Defect in vif-Deficient Human Immunodeficiency Virus Type 1 Virions." *J. Virol.* **77**(10): 5810-5820.

<http://jvi.asm.org/cgi/content/abstract/77/10/5810>

Replication of human immunodeficiency virus type 1 (HIV-1) in primary blood lymphocytes, certain T-cell lines (nonpermissive cells), and most likely in vivo is highly dependent on the virally encoded Vif protein. Evidence suggests that Vif acts late in the viral life cycle during assembly, budding, and/or maturation to counteract the antiviral activity of the CEM15 protein and possibly other antiviral factors. Because HIV-1 virions produced in the absence of Vif are severely restricted at a postentry, preintegration step of infection, it is presumed that such virions differ from wild-type virions in some way. In the present study, we established a protocol for producing large quantities of vif-deficient HIV-1 (HIV-1/ Δ vif) from an acute infection of nonpermissive T cells and performed a thorough examination of the defect in these virions. Aside from the expected lack of Vif, we observed no apparent abnormalities in the packaging, modification, processing, or function of proteins in Δ vif virions. In addition, we found no consistent defect in the ability of Δ vif virions to perform intravirion reverse transcription under a variety of assay conditions, suggesting that the reverse transcription complexes in these particles can behave normally under cell-free conditions. Consistent with this finding, neither the placement of the primer tRNA^{Lys} nor its ability to promote reverse transcription in an in vitro assay was

affected by a lack of Vif. Based on the inability of this comprehensive analysis to uncover molecular defects in Δ vif virions, we speculate that such defects are likely to be subtle and/or rare.

Garner-Hamrick, P. A., J. M. Fostel, et al. (2004). "Global Effects of Human Papillomavirus Type 18 E6/E7 in an Organotypic Keratinocyte Culture System." *J. Virol.* **78**(17): 9041-9050.

<http://jvi.asm.org/cgi/content/abstract/78/17/9041>

The effects of human papillomavirus type 18 (HPV-18) E6 and E7 proteins on global patterns of host gene expression in primary human keratinocytes grown in organotypic raft culture system were assessed. Primary human keratinocytes were infected with retroviruses that express the wild-type HPV-18 E6 and E7 genes from the native differentiation-dependent HPV enhancer-promoter. Total RNA was isolated from raft cultures and used to generate probes for querying Affymetrix U95A microarrays, which contain >12,500 human gene sequences. Quadruplicate arrays of each E6/E7-transduced and empty vector-transduced samples were analyzed by 16 pairwise comparisons. Transcripts altered in ≥ 12 comparisons were selected for further analysis. With this approach, HPV-18 E6/E7 expression significantly altered the expression of 1,381 genes. A large increase in transcripts associated with DNA and RNA metabolism was observed, with major increases noted for transcription factors, splicing factors, and DNA replication elements, among others. Multiple genes associated with protein translation were downregulated. In addition, major alterations were found in transcripts associated with the cell cycle and cell differentiation. Our study provides a systematic description of transcript changes brought about by HPV-18 E6/E7 in a physiologically relevant model and should furnish a solid source of information to guide future studies.

Geels, M. J., M. Cornelissen, et al. (2003). "Identification of Sequential Viral Escape Mutants Associated with Altered T-Cell Responses in a Human Immunodeficiency Virus Type 1-Infected Individual." *J. Virol.* **77**(23): 12430-12440.

<http://jvi.asm.org/cgi/content/abstract/77/23/12430>

Control of viremia in natural human immunodeficiency virus type 1 (HIV-1) infection in humans is associated with a virus-specific T-cell response. However, still much is unknown with regard to the extent of CD8⁺ cytotoxic T-lymphocyte (CTL) responses required to successfully control HIV-1 infection and to what extent CTL epitope escape can account for rises in viral load and ultimate progression to disease. In this study, we chose to monitor through full-length genome sequence of replication-competent biological clones the modifications that occurred within predicted CTL epitopes and to identify whether the alterations resulted in epitope escape from CTL recognition. From an extensive analysis of 59 biological HIV-1 clones generated over a period of 4 years from a single individual in whom the viral load was observed to rise, we identified the locations in the genome of five CD8⁺ CTL epitopes. Fixed mutations were identified within the p17, gp120, gp41, Nef, and reverse transcriptase genes. Using a gamma interferon ELISPOT assay, we identified for four of the five epitopes with fixed mutations a complete loss of T-cell reactivity against the wild-type epitope and a partial loss of reactivity against the mutant epitope. These results demonstrate the sequential accumulation of CTL escape in a patient during disease progression, indicating that multiple combinations of T-cell epitopes are required to control viremia.

Goebel, S. J., B. Hsue, et al. (2004). "Characterization of the RNA Components of a Putative Molecular

Switch in the 3' Untranslated Region of the Murine Coronavirus Genome." *J. Virol.* **78**(2): 669-682.

<http://jvi.asm.org/cgi/content/abstract/78/2/669>

RNA virus genomes contain cis-acting sequence and structural elements that participate in viral replication. We previously identified a bulged stem-loop secondary structure at the upstream end of the 3' untranslated region (3' UTR) of the genome of the coronavirus mouse hepatitis virus (MHV). This element, beginning immediately downstream of the nucleocapsid gene stop codon, was shown to be essential for virus replication. Other investigators discovered an adjacent downstream pseudoknot in the 3' UTR of the closely related bovine coronavirus (BCoV). This pseudoknot was also shown to be essential for replication, and it has a conserved counterpart in every group 1 and group 2 coronavirus. In MHV and BCoV, the bulged stem-loop and pseudoknot are, in part, mutually exclusive, because of the overlap of the last segment of the stem-loop and stem 1 of the pseudoknot. This led us to hypothesize that they form a molecular switch, possibly regulating a transition occurring during viral RNA synthesis. We have now performed an extensive genetic analysis of the two components of this proposed switch. Our results define essential and nonessential components of these structures and establish the limits to which essential parts of each element can be destabilized prior to loss of function. Most notably, we have confirmed the interrelationship of the two putative switch elements. Additionally, we have identified a pseudoknot loop insertion mutation that appears to point to a genetic interaction between the pseudoknot and a distant region of the genome.

Goncalvez, A. P., R. Men, et al. (2004). "Chimpanzee Fab Fragments and a Derived Humanized Immunoglobulin G1 Antibody That Efficiently Cross-Neutralize Dengue Type 1 and Type 2 Viruses." *J. Virol.* **78**(23): 12910-12918.

<http://jvi.asm.org/cgi/content/abstract/78/23/12910>

Passive immunization with monoclonal antibodies from humans or nonhuman primates represents an attractive alternative to vaccines for prevention of illness caused by dengue viruses (DENV) and other flaviviruses, including the West Nile virus. In a previous study, repertoire cloning to recover Fab fragments from bone marrow mRNA of chimpanzees infected with all four DENV serotypes (dengue virus serotype 1 [DENV-1] to DENV-4) was described. In that study, a humanized immunoglobulin G1 (IgG1) antibody that efficiently neutralized DENV-4 was recovered and characterized. In this study, the phage library constructed from the chimpanzees was used to recover Fab antibodies against the other three DENV serotypes. Serotype-specific neutralizing Fabs were not identified. Instead, we recovered DENV-neutralizing Fabs that specifically precipitated the envelope protein and were cross-reactive with all four DENV serotypes. Three of the Fabs competed with each other for binding to DENV-1 and DENV-2, although each of these Fabs contained a distinct complementarity determining region 3 (CDR3)-H sequence. Fabs that shared an identical or nearly identical CDR3-H sequences cross-neutralized DENV-1 and DENV-2 at a similar high 50% plaque reduction neutralization test (PRNT50) titer, ranging from 0.26 to 1.33 {micro}g/ml, and neutralized DENV-3 and DENV-4 but at a titer 10- to 20-fold lower. One of these Fabs, 1A5, also neutralized the West Nile virus most efficiently among other flaviviruses tested. Fab 1A5 was converted to a full-length antibody in combination with human sequences for production in mammalian CHO cells. Humanized IgG1 1A5 proved to be as efficient as Fab 1A5 for cross-neutralization of DENV-1 and DENV-2 at a titer of 0.48 and 0.95 {micro}g/ml, respectively. IgG1 1A5 also neutralized DENV-3, DENV-4, and the West Nile virus at a PRNT50 titer of approximately 3.2 to 4.2 {micro}g/ml. This humanized antibody represents an attractive candidate for further development of immunoprophylaxis against DENV and perhaps other flavivirus-associated diseases.

Goncalvez, A. P., R. H. Purcell, et al. (2004). "Epitope Determinants of a Chimpanzee Fab Antibody That Efficiently Cross-Neutralizes Dengue Type 1 and Type 2 Viruses Map to Inside and in Close Proximity to Fusion Loop of the Dengue Type 2 Virus Envelope Glycoprotein." *J. Virol.* **78**(23): 12919-12928.

<http://jvi.asm.org/cgi/content/abstract/78/23/12919>

The epitope determinants of chimpanzee Fab antibody 1A5, which have been shown to be broadly reactive to flaviviruses and efficient for cross-neutralization of dengue virus type 1 and type 2 (DENV-1 and DENV-2), were studied by analysis of DENV-2 antigenic variants. Sequence analysis showed that one antigenic variant contained a Gly-to-Val substitution at position 106 within the flavivirus-conserved fusion peptide loop of the envelope protein (E), and another variant contained a His-to-Gln substitution at position 317 in E. Substitution of Gly106Val in DENV-2 E reduced the binding affinity of Fab 1A5 by approximately 80-fold, whereas substitution of His317Gln had little or no effect on antibody binding compared to the parental virus. Treatment of DENV-2 with {beta}-mercaptoethanol abolished binding of Fab 1A5, indicating that disulfide bridges were required for the structural integrity of the Fab 1A5 epitope. Binding of Fab 1A5 to DENV-2 was competed by an oligopeptide containing the fusion peptide sequence as shown by competition enzyme-linked immunosorbent assay. Both DENV-2 antigenic variants were shown to be attenuated, or at least similar to the parental virus, when evaluated for growth in cultured cells or for neurovirulence in mice. Fab 1A5 inhibited low pH-induced membrane fusion of mosquito C6/36 cells infected with DENV-1 or DENV-2, as detected by reduced syncytium formation. Both substitutions in DENV-2 E lowered the pH threshold for membrane fusion, as measured in a fusion-from-within assay. In the three-dimensional structure of E, Gly106 in domain II and His317 in domain III of the opposite E monomer were spatially close. From the locations of these amino acids, Fab 1A5 appears to recognize a novel epitope that has not been mapped before with a flavivirus monoclonal antibody.

Gordon, M., T. De Oliveira, et al. (2003). "Molecular Characteristics of Human Immunodeficiency Virus Type 1 Subtype C Viruses from KwaZulu-Natal, South Africa: Implications for Vaccine and Antiretroviral Control Strategies." *J. Virol.* **77**(4): 2587-2599.

<http://jvi.asm.org/cgi/content/abstract/77/4/2587>

The KwaZulu-Natal region of South Africa is experiencing an explosive outbreak of human immunodeficiency virus type 1 (HIV-1) subtype C infections. Understanding the genetic diversity of C viruses and the biological consequences of this diversity is important for the design of effective control strategies. We analyzed the protease gene, the first 935 nucleotides of reverse transcriptase, and the C2V5 envelope region of a representative set of 72 treatment-naïve patients from KwaZulu-Natal and correlated the results with amino acid signature and resistance patterns. Phylogenetic analysis revealed multiple clusters or "lineages" of HIV-1 subtype C that segregated with other C viruses from southern Africa. The same pattern was observed for both black and Indian subgroups and for retrospective specimens collected prior to 1990, indicating that multiple sublineages of HIV-1 C have been present in KwaZulu-Natal since the early stages of the epidemic. With the exception of three nonnucleoside reverse transcriptase inhibitor mutations, no primary resistance mutations were identified. Numerous accessory polymorphisms were present in the protease, but none were located at drug-binding or active sites of the enzyme. One frequent polymorphism, I93L, was located near the protease/reverse transcriptase cleavage site. In the envelope, disruption of the glycosylation motif at the beginning of V3 was associated with the presence of an extra protein kinase C phosphorylation site at codon 11. Many polymorphisms were embedded within cytotoxic T lymphocyte or overlapping cytotoxic T-lymphocyte/T-helper epitopes, as defined for subtype B. This work forms a baseline for future studies aimed at understanding the impact of genetic diversity on vaccine efficacy and on natural susceptibility to antiretroviral drugs.

Goto, Y., Y. Nishimura, et al. (2002). "Association of Plasma Viral RNA Load with Prognosis in Cats Naturally Infected with Feline Immunodeficiency Virus." J. Virol. **76**(19): 10079-10083.

<http://jvi.asm.org/cgi/content/abstract/76/19/10079>

We measured the quantity of plasma feline immunodeficiency virus (FIV) RNA using a real-time sequence detecting system. Plasma viral RNA load was shown to correlate with the clinical stage, survival time, and disease progression in naturally FIV-infected cats. The present study indicates that the plasma viral RNA load can be used as a clinical marker representing the impairment of the immune system and predicting the clinical outcome in FIV-infected cats.

Grivel, J.-C., F. Santoro, et al. (2003). "Pathogenic Effects of Human Herpesvirus 6 in Human Lymphoid Tissue Ex Vivo." J. Virol. **77**(15): 8280-8289.

<http://jvi.asm.org/cgi/content/abstract/77/15/8280>

Human herpesvirus 6 (HHV-6) is a potentially immunosuppressive agent that has been suggested to act as a cofactor in the progression of human immunodeficiency virus disease. However, the lack of suitable experimental models has hampered the elucidation of the mechanisms of HHV-6-mediated immune suppression. Here, we used ex vivo lymphoid tissue to investigate the cellular tropism and pathogenic mechanisms of HHV-6. Viral strains belonging to both HHV-6 subgroups (A and B) were able to productively infect human tonsil tissue fragments in the absence of exogenous stimulation. The majority of viral antigen-expressing cells were CD4+ T lymphocytes expressing a nonnaive phenotype, while CD8+ T cells were efficiently infected only with HHV-6A. Accordingly, HHV-6A infection resulted in the depletion of both CD4+ and CD8+ T cells, whereas in HHV-6B-infected tissue CD4+ T cells were predominantly depleted. The expression of different cellular antigens was dramatically altered in HHV-6-infected tissues: whereas CD4 was upregulated, both CD46, which serves as a cellular receptor for HHV-6, and CD3 were downmodulated. However, CD3 downmodulation was restricted to infected cells, while the loss of CD46 expression was generalized. Moreover, HHV-6 infection markedly enhanced the production of the CC chemokine RANTES, whereas other cytokines and chemokines were only marginally affected. These results provide the first evidence, in a physiologically relevant study model, that HHV-6 can severely affect the physiology of secondary lymphoid organs through direct infection of T lymphocytes and modulation of key membrane receptors and chemokines.

Guidoboni, M., M. Ponzoni, et al. (2005). "Latent Membrane Protein 1 Deletion Mutants Accumulate in Reed-Sternberg Cells of Human Immunodeficiency Virus-Related Hodgkin's Lymphoma." J. Virol. **79**(4): 2643-2649.

<http://jvi.asm.org/cgi/content/abstract/79/4/2643>

The origin and biological significance of deletions at the 3' end of the Epstein-Barr virus (EBV)-encoded latent membrane protein 1 (LMP-1) gene are still controversial. We herein demonstrate that LMP-1 deletion mutants are highly associated with human immunodeficiency virus-related Hodgkin's lymphoma (HIV-HL) of Italian patients (29 of 31 cases; 93.5%), a phenomenon that is not due to a peculiar distribution of EBV strains in this area. In fact, although HIV-HL patients are infected by multiple EBV variants, we demonstrate that LMP-1 deletion mutants preferentially accumulate within neoplastic tissues. Subcloning and sequencing of the 3' LMP-1 ends of two HIV-HL genes in which both variants were present showed the presence of molecular signatures

suggestive of a likely derivation of the LMP-1 deletion mutant from a nondeletion ancestor. This phenomenon likely occurs within tumor cells in vivo, as shown by the detection of both LMP-1 variants in single microdissected Reed-Sternberg cells, and may at least in part explain the high prevalence of LMP-1 deletions associated with HIV-HL.

Gurney, K. B., J. Elliott, et al. (2005). "Binding and Transfer of Human Immunodeficiency Virus by DC-SIGN+ Cells in Human Rectal Mucosa." J. Virol. **79**(9): 5762-5773.

<http://jvi.asm.org/cgi/content/abstract/79/9/5762>

The role of DC-SIGN on human rectal mucosal dendritic cells is unknown. Using highly purified human rectal mucosal DC-SIGN+ cells and an ultrasensitive real-time reverse transcription-PCR assay to quantify virus binding, we found that HLA-DR+/DC-SIGN+ cells can bind and transfer more virus than the HLA-DR+/DC-SIGN- cells. Greater than 90% of the virus bound to total mucosal mononuclear cells (MMCs) was accounted for by the DC-SIGN+ cells, which comprise only 1 to 5% of total MMCs. Significantly, anti-DC-SIGN antibodies blocked 90% of the virus binding when more-physiologic amounts of virus inoculum were used. DC-SIGN expression in the rectal mucosa was significantly correlated with the interleukin-10 (IL-10)/IL-12 ratio ($r = 0.58$, $P < 0.002$; $n = 26$) among human immunodeficiency virus (HIV)-positive patients. Ex vivo and in vitro data implicate the role of IL-10 in upregulating DC-SIGN expression and downregulating expression of the costimulatory molecules CD80/CD86. Dendritic cells derived from monocytes (MDDCs) in the presence of IL-10 render the MDDCs less responsive to maturation stimuli, such as lipopolysaccharide and tumor necrosis factor alpha, and migration to the CCR7 ligand macrophage inflammatory protein 3 β . Thus, an increased IL-10 environment could render DC-SIGN+ cells less immunostimulatory and migratory, thereby dampening an effective immune response. DC-SIGN and the IL-10/IL-12 axis may play significant roles in the mucosal transmission and pathogenesis of HIV type 1.

Haijema, B. J., H. Volders, et al. (2004). "Live, Attenuated Coronavirus Vaccines through the Directed Deletion of Group-Specific Genes Provide Protection against Feline Infectious Peritonitis." J. Virol. **78**(8): 3863-3871.

<http://jvi.asm.org/cgi/content/abstract/78/8/3863>

Feline infectious peritonitis (FIP) is a fatal immunity-mediated disease caused by mutants of a ubiquitous coronavirus. Since previous attempts to protect cats under laboratory and field conditions have been largely unsuccessful, we used our recently developed system of reverse genetics (B. J. Haijema, H. Volders, and P. J. M. Rottier, *J. Virol.* 77:4528-4538, 2003) for the development of a modified live FIP vaccine. With this objective, we deleted the group-specific gene cluster open reading frame 3abc or 7ab and obtained deletion mutant viruses that not only multiplied well in cell culture but also showed an attenuated phenotype in the cat. At doses at which the wild-type virus would be fatal, the mutants with gene deletions did not cause any clinical symptoms. They still induced an immune response, however, as judged from the high levels of virus-neutralizing antibodies. The FIP virus (FIPV) mutant lacking the 3abc cluster and, to a lesser extent, the mutant missing the 7ab cluster, protected cats against a lethal homologous challenge; no protection was obtained with the mutant devoid of both gene clusters. Our studies show that the deletion of group-specific genes from the coronavirus genome results in live attenuated candidate vaccines against FIPV. More generally, our approach may allow the development of vaccines against infections with other pathogenic coronaviruses, including that causing severe acute respiratory syndrome in humans.

Haijema, B. J., H. Volders, et al. (2003). "Switching Species Tropism: an Effective Way To Manipulate the Feline Coronavirus Genome." J. Virol. **77**(8): 4528-4538.

<http://jvi.asm.org/cgi/content/abstract/77/8/4528>

Feline infectious peritonitis virus (FIPV), a coronavirus, is the causative agent of an invariably lethal infection in cats. Like other coronaviruses, FIPV contains an extremely large positive-strand RNA genome of ca. 30 kb. We describe here the development and use of a reverse genetics strategy for FIPV based on targeted RNA recombination that is analogous to what has been described for the mouse hepatitis virus (MHV) (L. Kuo et al., J. Virol. 74:1393-1406, 2000). In this two-step process, we first constructed by targeted recombination a mutant of FIPV, designated mFIPV, in which the ectodomain of the spike glycoprotein was replaced by that of MHV. This switch allowed for the selection of the recombinant virus in murine cells: mFIPV grows to high titers in these cells but has lost the ability to grow in feline cells. In a second, reverse process, mFIPV was used as the recipient, and the reintroduction of the FIPV spike now allowed for selection of candidate recombinants by their regained ability to grow in feline cells. In this fashion, we reconstructed a wild-type recombinant virus (r-wtFIPV) and generated a directed mutant FIPV in which the initiation codon of the nonstructural gene 7b had been disrupted (FIPV{Delta}7b). The r-wtFIPV was indistinguishable from its parental virus FIPV 79-1146 not only for its growth characteristics in tissue culture but also in cats, exhibiting a highly lethal phenotype. FIPV{Delta}7b had lost the expression of its 7b gene but grew unimpaired in cell culture, confirming that the 7b glycoprotein is not required *in vitro*. We establish the second targeted RNA recombination system for coronaviruses and provide a powerful tool for the genetic engineering of the FIPV genome.

Hauck, B. and W. Xiao (2003). "Characterization of Tissue Tropism Determinants of Adeno-Associated Virus Type 1." J. Virol. **77**(4): 2768-2774.

<http://jvi.asm.org/cgi/content/abstract/77/4/2768>

Muscle is an attractive target for gene delivery because of its mass and because vectors can be delivered in a noninvasive fashion. Adeno-associated virus (AAV) has been shown to be effective for muscle-targeted gene transfer. Recent progress in characterization of AAV serotype 1 (AAV1) and AAV6 demonstrated that these two AAV serotypes are far more efficient in transducing muscle than is the traditionally used AAV2. Since all cis elements are identical in these vectors, the potential determinants for their differences in transducing muscle appear to be located within the AAV capsid proteins. In the present study, a series of AAV capsid mutants were generated to identify the major regions affecting AAV transduction efficiency in muscle. Replacement of amino acids 350 to 736 of AAV2 VP1 with the corresponding amino acids from VP1 of AAV1 resulted in a hybrid vector that behaved very similarly to AAV1 *in vitro* and *in vivo* in muscle. Characterization of additional mutants carrying smaller regions of the AAV1 VP1 amino acid sequence in the AAV2 capsid protein suggested that amino acids 350 to 430 of VP1 function as a major tissue tropism determinant. Further analysis showed that the heparin binding domain and the major antigenic determinants in the AAV capsid region were not necessary for the efficiency of AAV1 transduction of muscle.

Havenga, M. J. E., A. A. C. Lemckert, et al. (2002). "Exploiting the Natural Diversity in Adenovirus Tropism for Therapy and Prevention of Disease." J. Virol. **76**(9): 4612-4620.

<http://jvi.asm.org/cgi/content/abstract/76/9/4612>

Since targeting of recombinant adenovirus vectors to defined cell types in vivo is a major challenge in gene therapy and vaccinology, we explored the natural diversity in human adenovirus tissue tropism. Hereto, we constructed a library of Ad5 vectors carrying fibers from other human serotypes. From this library, we identified vectors that efficiently infect human cells that are important for diverse gene therapy approaches and for induction of immunity. For several medical applications (prenatal diagnosis, artificial bone, vaccination, and cardiovascular disease), we demonstrate the applicability of these novel vectors. In addition, screening cell types derived from different species revealed that cellular receptors for human subgroup B adenoviruses are not conserved between rodents and primates. These results provide a rationale for utilizing elements of human adenovirus serotypes to generate chimeric vectors that improve our knowledge concerning adenovirus biology and widen the therapeutic window for vaccination and many different gene transfer applications.

Henderson, W. W., R. Ruhl, et al. (2004). "Human Immunodeficiency Virus (HIV) Type 1 Vpu Induces the Expression of CD40 in Endothelial Cells and Regulates HIV-Induced Adhesion of B-Lymphoma Cells." *J. Virol.* **78**(9): 4408-4420.

<http://jvi.asm.org/cgi/content/abstract/78/9/4408>

AIDS-related B-cell non-Hodgkin's lymphoma (AIDS-NHL) is a significant cause of morbidity and mortality among individuals infected with human immunodeficiency virus type 1 (HIV-1). AIDS-NHL is clinically and histologically heterogeneous, but common features include an aggressive clinical course and frequent extranodal presentation. HIV-1 infection of nonimmune cells that interact with malignant B cells at extranodal sites may influence both the development and the clinical presentation of disease. Our previous studies have shown that coculture of B-lymphoma (BL) cells with HIV-1-infected endothelial cells (EC) leads to contact activation of EC and firm BL-cell adhesion. The key event promoting EC-BL-cell adhesion was HIV-1 upregulation of endothelial CD40, which allowed induction of vascular cell adhesion molecule 1 (VCAM-1) in a CD40-dependent manner. The present study was designed to identify the HIV-1 protein(s) that influence EC-BL-cell adhesion. When HIV-1 proteins were individually expressed in EC by using recombinant adenoviruses, cultured BL cells adhered exclusively to Vpu-transduced EC. As with HIV-infected EC, adhesive properties were linked to the capacity of Vpu to upregulate CD40, which in turn allowed efficient expression of VCAM-1. When EC were infected with an HIV-1 pseudotype lacking the Vpu gene, CD40 upregulation and BL-cell adhesive properties were lost, indicating an essential role for Vpu in EC-BL-cell interactions. Thus, these data reveal a novel function for HIV-1 Vpu and further suggest a role for Vpu in the development of AIDS-NHL at EC-rich extranodal sites.

Holmgren, S. C., N. A. Patterson, et al. (2005). "The Minor Capsid Protein L2 Contributes to Two Steps in the Human Papillomavirus Type 31 Life Cycle." *J. Virol.* **79**(7): 3938-3948.

<http://jvi.asm.org/cgi/content/abstract/79/7/3938>

Prior studies, which have relied upon the use of pseudovirions generated in heterologous cell types, have led to sometimes conflicting conclusions regarding the role of the minor capsid protein of papillomaviruses, L2, in the viral life cycle. In this study we carry out analyses with true virus particles assembled in the natural host cell to assess L2's role in the viral infectious life cycle. For these studies we used the organotypic (raft) culture system to recapitulate the full viral life cycle of the high-risk human papillomavirus HPV31, which was either wild type or mutant for

L2. After transfection, the L2 mutant HPV31 genome was able to establish itself as a nuclear plasmid in proliferating populations of poorly differentiated (basal-like) human keratinocytes and to amplify its genome to high copy number, support late viral gene expression, and cause formation of virus particles in human keratinocytes that had been induced to undergo terminal differentiation. These results indicate that aspects of both the nonproductive and productive phases of the viral life cycle occur normally in the absence of functional L2. However, upon the analysis of the virus particles generated, we found an approximate 10-fold reduction in the amount of viral DNA encapsidated into L2-deficient virions. Furthermore, there was an over-100-fold reduction in the infectivity of L2-deficient virus. Because the latter deficiency cannot be accounted for solely by the 10-fold decrease in encapsidation, we conclude that L2 contributes to at least two steps in the production of infectious virus.

Horscroft, N., D. Bellows, et al. (2005). "Establishment of a Subgenomic Replicon for Bovine Viral Diarrhea Virus in Huh-7 Cells and Modulation of Interferon-Regulated Factor 3-Mediated Antiviral Response." *J. Virol.* **79**(5): 2788-2796.

<http://jvi.asm.org/cgi/content/abstract/79/5/2788>

We describe the development of a selectable, bi-cistronic subgenomic replicon for bovine viral diarrhea virus (BVDV) in Huh-7 cells, similar to that established for hepatitis C virus (HCV). The selection marker and reporter (Luc-Ubi-Neo) in the BVDV replicon was fused with the amino-terminal protease Npro, and expression of the nonstructural proteins (NS3 to NS5B) was driven by an encephalomyocarditis virus internal ribosome entry site. This BVDV replicon allows us to compare RNA replication of these two related viruses in a similar cellular background and to identify antiviral molecules specific for HCV RNA replication. The BVDV replicon showed similar sensitivity as the HCV replicon to interferons (alpha, beta, and gamma) and 2'-{beta}-C-methyl ribonucleoside inhibitors. Known nonnucleoside inhibitor molecules specific for either HCV or BVDV can be easily distinguished by using the parallel replicon systems. The HCV replicon has been shown to block, via the NS3/4A serine protease, Sendai virus-induced activation of interferon regulatory factor 3 (IRF-3), a key antiviral signaling molecule. Similar suppression of IRF-3-mediated responses was also observed with the Huh-7-BVDV replicon but was independent of NS3/4A protease activity. Instead, the amino-terminal cysteine protease Npro of BVDV appears to be, at least partly, responsible for suppressing IRF-3 activation induced by Sendai virus infection. This result suggests that different viruses, including those closely related, may have developed unique mechanisms for evading host antiviral responses. The parallel BVDV and HCV replicon systems provide robust counterscreens to distinguish viral specificity of small-molecule inhibitors of viral replication and to study the interactions of the viral replication machinery with the host cell innate immune system.

Huang, J., H. Chen, et al. (2003). "Lytic Viral Replication as a Contributor to the Detection of Epstein-Barr Virus in Breast Cancer." *J. Virol.* **77**(24): 13267-13274.

<http://jvi.asm.org/cgi/content/abstract/77/24/13267>

Epstein-Barr virus (EBV) has an accepted association with the epithelial malignancy nasopharyngeal carcinoma and has also been reported in other more controversial carcinoma settings. Evaluation of EBV association with epithelial carcinomas such as breast cancer would benefit from a better understanding of the outcome of EBV infection of these cells. Cell-free preparations of a green fluorescent protein-expressing virus, BX1, were used to infect breast cancer cell lines, which were then examined for EBV gene expression and viral genome copy number. Reverse transcription-PCR analyses revealed that the cells supported a mixture of latency II and lytic EBV gene expression. Lytic Zta and BMRF1 protein expression was detected

by immunohistochemistry, and DNA PCR analyses estimated an EBV copy number of 300 to 600 genomes per infected cell. Evidence for lytic EBV expression was also found in breast tissue, where reverse transcription-PCR analyses detected lytic Zta transcripts in 7 of 10 breast carcinoma tissues and 4 of 10 normal tissues from the same patients. Scattered cells immunoreactive for Zta protein were also detectable in breast carcinoma. Quantitative real-time PCR analysis of EBV-positive breast carcinoma tissues suggested that less than 0.1% of the cells contained viral genomes. We suggest that sporadic lytic EBV infection may contribute to PCR-based detection of EBV in traditionally nonvirally associated epithelial malignancies.

Huang, W., A. Gamarnik, et al. (2003). "Amino Acid Substitutions at Position 190 of Human Immunodeficiency Virus Type 1 Reverse Transcriptase Increase Susceptibility to Delavirdine and Impair Virus Replication." J. Virol. **77**(2): 1512-1523.

<http://jvi.asm.org/cgi/content/abstract/77/2/1512>

Suboptimal treatment of human immunodeficiency virus type 1 (HIV-1) infection with nonnucleoside reverse transcriptase inhibitors (NNRTI) often results in the rapid selection of drug-resistant virus. Several amino acid substitutions at position 190 of reverse transcriptase (RT) have been associated with reduced susceptibility to the NNRTI, especially nevirapine (NVP) and efavirenz (EFV). In the present study, the effects of various 190 substitutions observed in viruses obtained from NNRTI-experienced patients were characterized with patient-derived HIV isolates and confirmed with a panel of isogenic viruses. Compared to wild-type HIV, which has a glycine at position 190 (G190), viruses with 190 substitutions (A, C, Q, S, V, E, or T, collectively referred to as G190X substitutions) were markedly less susceptible to NVP and EFV. In contrast, delavirdine (DLV) susceptibility of these G190X viruses increased from 3 to 300-fold (hypersusceptible) or was only slightly decreased. The replication capacity of viruses with certain 190 substitutions (C, Q, V, T, and E) was severely impaired and was correlated with reduced virion-associated RT activity and incomplete protease (PR) processing of the viral p55gag polyprotein. These defects were the result of inadequate p160gagpol incorporation into virions. Compensatory mutations within RT and PR improved replication capacity, p55gag processing, and RT activity, presumably through increased incorporation of p160gagpol into virions. We observe an inverse relationship between the degree of NVP and EFV resistance and the impairment of viral replication in viruses with substitutions at 190 in RT. These observations may have important implications for the future design and development of antiretroviral drugs that restrict the outgrowth of resistant variants with high replication capacity.

Huder, J. B., J. Boni, et al. (2002). "Identification and Characterization of Two Closely Related Unclassifiable Endogenous Retroviruses in Pythons (*Python molurus* and *Python curtus*)."
J. Virol. **76**(15): 7607-7615.

<http://jvi.asm.org/cgi/content/abstract/76/15/7607>

Boid inclusion body disease (BIBD) is a fatal disorder of boid snakes that is suspected to be caused by a retrovirus. In order to identify this agent, leukocyte cultures (established from *Python molurus* specimens with symptoms of BIBD or kept together with such diseased animals) were assessed for reverse transcriptase (RT) activity. Virus from cultures exhibiting high RT activity was banded on sucrose density gradients, and the RT peak fraction was subjected to highly efficient procedures for the identification of unknown particle-associated retroviral RNA. A 7-kb full retroviral sequence was identified, cloned, and sequenced. This virus contained intact open reading frames (ORFs) for gag, pro, pol, and env, as well as another ORF of unknown function within pol. Phylogenetic analysis showed that the virus is distantly related to viruses from both the B and D types and the mammalian C type but cannot be classified. It is present as a highly

expressed endogenous retrovirus in all *P. molurus* individuals; a closely related, but much less expressed virus was found in all tested *Python curtus* individuals. All other boid snakes tested, including *Python regius*, *Python reticulatus*, *Boa constrictor*, *Eunectes notaeus*, and *Morelia spilota*, were virus negative, independent of whether they had BIBD or not. Virus isolated from *P. molurus* could not be transmitted to the peripheral blood mononuclear cells of *B. constrictor* or *P. regius*. Thus, there is no indication that this novel virus, which we propose to name python endogenous retrovirus (PyERV), is causally linked with BIBD.

Hulse, D. J., R. G. Webster, et al. (2004). "Molecular Determinants within the Surface Proteins Involved in the Pathogenicity of H5N1 Influenza Viruses in Chickens." *J. Virol.* **78**(18): 9954-9964.

<http://jvi.asm.org/cgi/content/abstract/78/18/9954>

Although it is established that the cleavage site and glycosylation patterns in the hemagglutinin (HA) play important roles in determining the pathogenicity of H5 avian influenza viruses, some viruses exist that are not highly pathogenic despite possessing the known characteristics of high pathogenicity (i.e., their HA contains multiple basic amino acids at the cleavage site and has glycosylation patterns similar to that of the highly pathogenic H5 viruses). Currently little is known about the H5N1 viruses that fall into this intermediate category of pathogenicity. We have identified strains of H5N1 avian influenza viruses that have markers typical of high pathogenicity but distinctly differ in their ability to cause disease and death in chickens. By analyzing viruses constructed by reverse-genetic methods and containing recombinant HAs, we established that amino acids 97, 108, 126, 138, 212, and 217 of HA, in addition to those within the cleavage site, affect pathogenicity. Further investigation revealed that an additional glycosylation site within the neuraminidase (NA) protein globular head contributed to the high virulence of the H5N1 virus. Our findings are in agreement with previous observations that suggest that the activities of the HA and NA proteins are functionally linked.

Hussain, A. I., J. A. Johnson, et al. (2003). "Identification and Characterization of Avian Retroviruses in Chicken Embryo-Derived Yellow Fever Vaccines: Investigation of Transmission to Vaccine Recipients." *J. Virol.* **77**(2): 1105-1111.

<http://jvi.asm.org/cgi/content/abstract/77/2/1105>

All currently licensed yellow fever (YF) vaccines are propagated in chicken embryos. Recent studies of chick cell-derived measles and mumps vaccines show evidence of two types of retrovirus particles, the endogenous avian retrovirus (EAV) and the endogenous avian leukosis virus (ALV-E), which originate from the chicken embryonic fibroblast substrates. In this study, we investigated substrate-derived avian retrovirus contamination in YF vaccines currently produced by three manufacturers (YF-vax [Connaught Laboratories], Stamaril [Aventis], and YF-FIOCRUZ [FIOCRUZ-Bio-Manguinhos]). Testing for reverse transcriptase (RT) activity was not possible because of assay inhibition. However, Western blot analysis of virus pellets with anti-ALV RT antiserum detected three distinct RT proteins in all vaccines, indicating that more than one source is responsible for the RTs present in the vaccines. PCR analysis of both chicken substrate DNA and particle-associated RNA from the YF vaccines showed no evidence of the long terminal repeat sequences of exogenous ALV subgroups A to D in any of the vaccines. In contrast, both ALV-E and EAV particle-associated RNA were detected at equivalent titers in each vaccine by RT-PCR. Quantitative real-time RT-PCR revealed 61,600, 348,000, and 1,665,000 ALV-E RNA copies per dose of Stamaril, YF-FIOCRUZ, and YF-vax vaccines, respectively. *ev* locus-specific PCR testing of the vaccine-associated chicken substrate DNA was positive both for the nondefective *ev*-12 locus in two vaccines and for the defective *ev*-1 locus in all three vaccines. Both intact and *ev*-1 pol sequences were also identified in the particle-associated RNA. To

investigate the risks of transmission, serum samples from 43 YF vaccine recipients were studied. None of the samples were seropositive by an ALV-E-based Western blot assay or had detectable EAV or ALV-E RNA sequences by RT-PCR. YF vaccines produced by the three manufacturers all have particles containing EAV genomes and various levels of defective or nondefective ALV-E sequences. The absence of evidence of infection with ALV-E or EAV in 43 YF vaccine recipients suggests low risks for transmission of these viruses, further supporting the safety of these vaccines.

Hussain, S., J. a. Pan, et al. (2005). "Identification of Novel Subgenomic RNAs and Noncanonical Transcription Initiation Signals of Severe Acute Respiratory Syndrome Coronavirus." J. Virol. **79**(9): 5288-5295.

<http://jvi.asm.org/cgi/content/abstract/79/9/5288>

The expression of the genomic information of severe acute respiratory syndrome coronavirus (SARS CoV) involves synthesis of a nested set of subgenomic RNAs (sgRNAs) by discontinuous transcription. In SARS CoV-infected cells, 10 sgRNAs, including 2 novel ones, were identified, which were predicted to be functional in the expression of 12 open reading frames located in the 3' one-third of the genome. Surprisingly, one new sgRNA could lead to production of a truncated spike protein. Sequence analysis of the leader-body fusion sites of each sgRNA showed that the junction sequences and the corresponding transcription-regulatory sequence (TRS) are unique for each species of sgRNA and are consistent after virus passages. For the two novel sgRNAs, each used a variant of the TRS that has one nucleotide mismatch in the conserved hexanucleotide core (ACGAAC) in the TRS. Coexistence of both plus and minus strands of SARS CoV sgRNAs and evidence for derivation of the sgRNA core sequence from the body core sequence favor the model of discontinuous transcription during minus-strand synthesis. Moreover, one rare species of sgRNA has the junction sequence AAA, indicating that its transcription could result from a noncanonical transcription signal. Taken together, these results provide more insight into the molecular mechanisms of genome expression and subgenomic transcription of SARS CoV.

Jana, N. K., L. R. Gray, et al. (2005). "Human Immunodeficiency Virus Type 1 Stimulates the Expression and Production of Secretory Leukocyte Protease Inhibitor (SLPI) in Oral Epithelial Cells: a Role for SLPI in Innate Mucosal Immunity." J. Virol. **79**(10): 6432-6440.

<http://jvi.asm.org/cgi/content/abstract/79/10/6432>

The innate immune response is a key barrier against pathogenic microorganisms such as human immunodeficiency virus type 1 (HIV-1). Because HIV-1 is rarely transmitted orally, we hypothesized that oral epithelial cells participate in the innate immune defense against this virus. We further hypothesized that secretory leukocyte protease inhibitor (SLPI), a 12-kDa mucosal antiviral protein, is a component of the host immune response to this virus. Here we demonstrated constitutive expression and production of SLPI in immortalized human oral keratinocytes. Brief exposure of cells to HIV-1 BaL and HXB2 significantly increased SLPI mRNA and protein production compared to that in mock-exposed cells ($P < 0.01$), as evaluated by real-time quantitative reverse transcription-PCR and enzyme-linked immunosorbent assay. HIV-1-mediated stimulation of SLPI occurred at the transcriptional level, was dose and time dependent, was elicited by heat-inactivated and infectious viruses, and did not depend on cellular infection. Experiments with purified retroviral proteins showed that the stimulatory effect was induced specifically by external envelope glycoproteins from HIV-1 and simian immunodeficiency virus. SLPI responsiveness to HIV-1 was also observed in an unrelated oral epithelial cell line and in normal (nonimmortalized) human oral epithelial cells isolated from healthy uninfected gingival

tissues. In this first report of SLPI regulation by HIV-1, we show that the expression and production of the antimicrobial and anti-inflammatory protein can be stimulated in oral epithelial cells by the virus through interactions with gp120 in the absence of direct infection. These findings indicate that SLPI is a component of the oral mucosal response to HIV-1.

Jin, Y. F., T. Ishibashi, et al. (2002). "Isolation and Analysis of Retroviral Integration Targets by Solo Long Terminal Repeat Inverse PCR." *J. Virol.* **76**(11): 5540-5547.

<http://jvi.asm.org/cgi/content/abstract/76/11/5540>

Upon retroviral infection, the genomic RNA is reverse transcribed to make proviral DNA, which is then integrated into the host chromosome. Although the viral elements required for successful integration have been extensively characterized, little is known about the host DNA structure constituting preferred targets for proviral integration. In order to elucidate the mechanism for the target selection, comparison of host DNA sequences at proviral integration sites may be useful. To achieve simultaneous analysis of the upstream and downstream host DNA sequences flanking each proviral integration site, a Moloney murine leukemia virus-based retroviral vector was designed so that its integrated provirus could be removed by Cre-loxP homologous recombination, leaving a solo long terminal repeat (LTR). Taking advantage of the solo LTR, inverse PCR was carried out to amplify both the upstream and downstream cellular flanking DNA. The method called solo LTR inverse PCR, or SLIP, proved useful for simultaneously cloning the upstream and downstream flanking sequences of individual proviral integration sites from the polyclonal population of cells harboring provirus at different chromosomal sites. By the SLIP method, nucleotide sequences corresponding to 38 independent proviral integration targets were determined and, interestingly, atypical virus-host DNA junction structures were found in more than 20% of the cases. Characterization of retroviral integration sites using the SLIP method may provide useful insights into the mechanism for proviral integration and its target selection.

Jung, Y. T. and C. A. Kozak (2003). "Generation of Novel Syncytium-Inducing and Host Range Variants of Ecotropic Moloney Murine Leukemia Virus in *Mus spicilegus*." *J. Virol.* **77**(9): 5065-5072.

<http://jvi.asm.org/cgi/content/abstract/77/9/5065>

Mus spicilegus is an Eastern European wild mouse species that has previously been reported to harbor an unusual infectious ecotropic murine leukemia virus (MLV) and proviral envelope genes of a novel MLV subgroup. In the present study, *M. spicilegus* neonates were inoculated with Moloney ecotropic MLV (MoMLV). All 17 inoculated mice produced infectious ecotropic virus after 8 to 14 weeks, and two unusual phenotypes distinguished the isolates from MoMLV. First, most of the *M. spicilegus* isolates grew to equal titers on *M. dunni* and SC-1 cells, although MoMLV does not efficiently infect *M. dunni* cells. The deduced amino acid sequence of a representative clone differed from MoMLV by insertion of two serine residues within the VRA of SUenv. Modification of a molecular clone of MoMLV by the addition of these serines produced a virus that grows to high titer in *M. dunni* cells, establishing a role for these two serine residues in host range. A second unusual phenotype was found in only one of the *M. spicilegus* isolates, Spl574. Spl574 produces large syncytia of multinucleated giant cells in *M. dunni* cells, but its replication is restricted in other mouse cell lines. Sequencing and mutagenesis demonstrated that syncytium formation could be attributed to a single amino acid substitution within VRA, S82F. Thus, viruses with altered growth properties are selected during growth in *M. spicilegus*. The mutations associated with the host range and syncytium-inducing variants map to a key region of VRA known to govern interactions with the cell surface receptor, suggesting that the associated phenotypes may result from altered interactions with the unusual ecotropic virus mCAT1 receptor carried by *M. dunni*.

Jung, Y. T., T. Wu, et al. (2003). "Characterization of Recombinant Noncotropic Murine Leukemia Viruses from the Wild Mouse Species *Mus spretus*." *J. Virol.* **77**(23): 12773-12781.

<http://jvi.asm.org/cgi/content/abstract/77/23/12773>

The wild mouse species most closely related to the common laboratory strains contain proviral env genes of the xenotropic/polytropic subgroup of mouse leukemia viruses (MLVs). To determine if the polytropic proviruses of *Mus spretus* contain functional genes, we inoculated neonates with Moloney MLV (MoMLV) or amphotropic MLV (A-MLV) and screened for viral recombinants with altered host ranges. Thymus and spleen cells from MoMLV-inoculated mice were plated on *Mus dunni* cells and mink cells, since these cells do not support the replication of MoMLV, and cells from A-MLV-inoculated mice were plated on ferret cells. All MoMLV-inoculated mice produced ecotropic viruses that resembled their MoMLV progenitor, although some isolates, unlike MoMLV, grew to high titers in *M. dunni* cells. All of the MoMLV-inoculated mice also produced noncotropic virus that was infectious for mink cells. Sequencing of three MoMLV- and two A-MLV-derived noncotropic recombinants confirmed that these viruses contained substantial substitutions that included the regions of env encoding the surface (SU) protein and the 5' end of the transmembrane (TM) protein. The 5' recombination breakpoint for one of the A-MLV recombinants was identified in RNase H. The *M. spretus*-derived env substitutions were nearly identical to the corresponding regions in prototypical laboratory mouse polytropic proviruses, but the wild mouse infectious viruses had a more restricted host range. The *M. spretus* proviruses contributing to these recombinants were also sequenced. The seven sequenced proviruses were 99% identical to one another and to the recombinants; only two of the seven had obvious fatal defects. We conclude that the *M. spretus* proviruses are likely to be recent germ line acquisitions and that they contain functional genes that can contribute to the production of replication-competent virus.

Kato, H., E. Orito, et al. (2002). "Characteristics of Hepatitis B Virus Isolates of Genotype G and Their Phylogenetic Differences from the Other Six Genotypes (A through F)." *J. Virol.* **76**(12): 6131-6137.

<http://jvi.asm.org/cgi/content/abstract/76/12/6131>

Eight hepatitis B virus (HBV) isolates of genotype G were recovered from patients and sequenced over the entire genome. Six of them had a genomic length of 3,248 bp and two had genomic lengths of 3,239 bp (USG15) and 3,113 bp (USG18) due to deletions. The 10 HBV/G isolates, including the 8 sequenced isolates as well as the original isolate (AF160501) and another isolate (B1-89), had a close sequence homology of 99.3 to 99.8% among themselves (excluding USG18 with a long deletion) but of <88.7% to any of the 68 HBV isolates of the other six genotypes with the full-length sequence known. The eight HBV/G isolates possessed an insertion of 36 bp in the core gene and two stop codons in the precore region, as did the AF160501 and B1-89 isolates. The 10 HBV/G isolates clustered on a branch separate from those bearing the other six genotypes (A through F [A-F]) in the phylogenetic tree constructed from full-length sequences of 78 HBV isolates as well as in those constructed from the core, polymerase, X, and envelope genes. Despite two stop codons in the precore region that prohibited the translation of the HBV e antigen (HBeAg), all of the eight patients with HBV/G infection possessed the HBeAg in serum. By restriction fragment length polymorphism of the surface gene, all of the eight patients were found to be coinfecting with HBV of genotype A (HBV/A), which would be responsible for the expression of HBeAg in them. It is worthy of examination to determine how coinfection occurs and whether HBV/G needs HBV/A for replication.

Kaverin, N. V., I. A. Rudneva, et al. (2004). "Structural Differences among Hemagglutinins of Influenza A Virus Subtypes Are Reflected in Their Antigenic Architecture: Analysis of H9 Escape Mutants." J. Virol. **78**(1): 240-249.

<http://jvi.asm.org/cgi/content/abstract/78/1/240>

We used a panel of monoclonal antibodies to H9 hemagglutinin to select 18 escape mutants of mouse-adapted influenza A/Swine/Hong Kong/9/98 (H9N2) virus. Cross-reactions of the mutants with the antibodies and the sequencing of hemagglutinin genes revealed two minimally overlapping epitopes. We mapped the amino acid changes to two areas of the recently reported three-dimensional structure of A/Swine/Hong Kong/9/98 hemagglutinin. The grouping of the antigenically relevant amino acid positions in H9 hemagglutinin differs from the pattern observed in H3 and H5 hemagglutinins. Several positions in site B of H3 hemagglutinin are distributed in two sites of H9 hemagglutinin. Unlike any subtype analyzed so far, H9 hemagglutinin does not contain an antigenic site corresponding to site A in H3 hemagglutinin. Positions 145 and 193 (H3 numbering), which in H3 hemagglutinin belong to sites A and B, respectively, are within one site in H9 hemagglutinin. This finding is consistent with the peculiarity of the three-dimensional structure of the H9 molecule, that is, the absence from H9 hemagglutinin of the lateral loop that forms site A in H3 and the equivalent site in H5 hemagglutinins. The escape mutants analyzed displayed phenotypic variations, including decreased virulence for mice and changes in affinity for sialyl substrates. Our results demonstrate a correlation between intersubtype differences in three-dimensional structure and variations among subtypes in the distribution of antigenic areas. Our findings also suggest that covariation and pleiotropic effects of antibody-selected mutations may be important in the evolution of H9 influenza virus, a possible causative agent of a future pandemic.

Kijak, G. H., V. Simon, et al. (2002). "Origin of Human Immunodeficiency Virus Type 1 Quasispecies Emerging after Antiretroviral Treatment Interruption in Patients with Therapeutic Failure." J. Virol. **76**(14): 7000-7009.

<http://jvi.asm.org/cgi/content/abstract/76/14/7000>

The emergence of antiretroviral (ARV) drug-resistant human immunodeficiency virus type 1 (HIV-1) quasispecies is a major cause of treatment failure. These variants are usually replaced by drug-sensitive ones when the selective pressure of the drugs is removed, as the former have reduced fitness in a drug-free environment. This was the rationale for the design of structured ARV treatment interruption (STI) studies for the management of HIV-1 patients with treatment failure. We have studied the origin of drug-sensitive HIV-1 quasispecies emerging after STI in patients with treatment failure due to ARV drug resistance. Plasma and peripheral blood mononuclear cell samples were obtained the day of treatment interruption (day 0) and 30 and 60 days afterwards. HIV-1 pol and env were partially amplified, cloned, and sequenced. At day 60 drug-resistant variants were replaced by completely or partially sensitive quasispecies. Phylogenetic analyses of pol revealed that drug-sensitive variants emerging after STI were not related to their immediate temporal ancestors but formed a separate cluster, demonstrating that STI leads to the recrudescence and reemergence of a sequestered viral population rather than leading to the back mutation of drug-resistant forms. No evidence for concomitant changes in viral tropism was seen, as deduced from env sequences. This study demonstrates the important role that the reemergence of quasispecies plays in HIV-1 population dynamics and points out the difficulties that may be found when recycling ARV therapies with patients with treatment failure.

Kostrikis, L. G., G. Touloumi, et al. (2002). "Quantitation of Human Immunodeficiency Virus Type 1 DNA Forms with the Second Template Switch in Peripheral Blood Cells Predicts Disease Progression

Independently of Plasma RNA Load." *J. Virol.* **76**(20): 10099-10108.

<http://jvi.asm.org/cgi/content/abstract/76/20/10099>

There are several forms of human immunodeficiency virus type 1 (HIV-1) DNA in peripheral blood T cells and lymph nodes in untreated HIV-1-infected individuals and in patients whose plasma HIV-1 RNA levels are suppressed by long-term combination antiretroviral therapy. However, it remains to be established whether the concentration of HIV-1 DNA in cells predicts the clinical outcome of HIV-1 infection. In this report, we measured the concentration of HIV-1 DNA forms which has undergone the second template switch (STS DNA) and 2-long-terminal-repeat DNA circles in peripheral blood mononuclear cell (PBMC) samples. To do this, we used molecular-beacon-based real-time PCR assays and studied 130 patients with hemophilia in the Multicenter Hemophilia Cohort Study. We assessed the influence of baseline HIV-1 STS DNA levels on the progression of HIV-1 disease in the absence of combination antiretroviral therapy by Kaplan-Meier and Cox regression analysis. Among the patients who progressed to AIDS, the median levels (interquartile ranges) of STS HIV-1 DNA in PBMC were significantly higher than those of patients who remained AIDS free during the 16 years of follow-up (1,017 [235 to 6,059] and 286 [31 to 732] copies per 106 PBMC, respectively; $P < 0.0001$). Rates of progression to death and development of AIDS varied significantly (log rank $P < 0.001$) by quartile distribution of HIV-1 STS DNA levels. After adjustment for age at seroconversion, baseline CD4+ T-cell counts, plasma viral load, and T-cell-receptor excision circles, the relative hazards (RH) of death and AIDS were significantly increased with higher HIV-1 STS DNA levels (adjusted RH, 1.84 [95% confidence interval {CI}, 1.30 to 2.59] and 2.62 [95% CI, 1.75 to 3.93] per 10-fold increase per 106 PBMC, respectively). HIV-1 STS DNA levels in each individual remained steady in longitudinal PBMC samples during 16 years of follow-up. Our findings show that the concentration of HIV-1 STS DNA in PBMC complements the HIV-1 RNA load in plasma in predicting the clinical outcome of HIV-1 disease. This parameter may have important implications for understanding the virological response to combination antiretroviral therapy.

Kuo, L. and P. S. Masters (2002). "Genetic Evidence for a Structural Interaction between the Carboxy Termini of the Membrane and Nucleocapsid Proteins of Mouse Hepatitis Virus." *J. Virol.* **76**(10): 4987-4999.

<http://jvi.asm.org/cgi/content/abstract/76/10/4987>

The coronavirus membrane (M) protein is the most abundant virion protein and the key component in viral assembly and morphogenesis. The M protein of mouse hepatitis virus (MHV) is an integral membrane protein with a short ectodomain, three transmembrane segments, and a large carboxy-terminal endodomain facing the interior of the viral envelope. The carboxy terminus of MHV M has previously been shown to be extremely sensitive to mutation, both in a virus-like particle expression system and in the intact virion. We have constructed a mutant, M{Delta}2, containing a two-amino-acid truncation of the M protein that was previously thought to be lethal. This mutant was isolated by means of targeted RNA recombination with a powerful host range-based selection allowed by the interspecies chimeric virus fMHV (MHV containing the ectodomain of the feline infectious peritonitis virus S protein). Analysis of multiple second-site revertants of the M{Delta}2 mutant has revealed changes in regions of both the M protein and the nucleocapsid (N) protein that can compensate for the loss of the last two residues of the M protein. Our data thus provide the first genetic evidence for a structural interaction between the carboxy termini of the M and N proteins of MHV. In addition, this work demonstrates the efficacy of targeted recombination with fMHV for the systematic genetic analysis of coronavirus structural protein interactions.

Kuo, L. and P. S. Masters (2003). "The Small Envelope Protein E Is Not Essential for Murine Coronavirus Replication." *J. Virol.* **77**(8): 4597-4608.

<http://jvi.asm.org/cgi/content/abstract/77/8/4597>

The importance of the small envelope (E) protein in the assembly of coronaviruses has been demonstrated in several studies. While its precise function is not clearly defined, E is a pivotal player in the morphogenesis of the virion envelope. Expression of the E protein alone results in its incorporation into vesicles that are released from cells, and the coexpression of the E protein with the membrane protein M leads to the assembly of coronavirus-like particles. We have previously generated E gene mutants of mouse hepatitis virus (MHV) that had marked defects in viral growth and produced virions that were aberrantly assembled in comparison to wild-type virions. We have now been able to obtain a viable MHV mutant in which the entire E gene, as well as the nonessential upstream genes 4 and 5a, has been deleted. This mutant (Δ E) was obtained by a targeted RNA recombination method that makes use of a powerful host range-based selection system. The Δ E mutant produces tiny plaques with an unusual morphology compared to plaques formed by wild-type MHV. Despite its low growth rate and low infectious titer, the Δ E mutant is genetically stable, showing no detectable phenotypic changes after several passages. The properties of this mutant provide further support for the importance of E protein in MHV replication, but surprisingly, they also show that E protein is not essential.

Lee, E.-g., A. Alidina, et al. (2003). "Importance of Basic Residues in Binding of Rous Sarcoma Virus Nucleocapsid to the RNA Packaging Signal." *J. Virol.* **77**(3): 2010-2020.

<http://jvi.asm.org/cgi/content/abstract/77/3/2010>

In the context of the Rous sarcoma virus Gag polyprotein, only the nucleocapsid (NC) domain is required to mediate the specificity of genomic RNA packaging. We have previously showed that the *Saccharomyces cerevisiae* three-hybrid system provides a rapid genetic assay to analyze the RNA and protein components of the avian retroviral RNA-Gag interactions necessary for specific encapsidation. In this study, using both site-directed mutagenesis and in vivo random screening in the yeast three-hybrid binding assay, we have examined the amino acids in NC required for genomic RNA binding. We found that we could delete either of the two Cys-His boxes without greatly abrogating either RNA binding or packaging, although the two Cys-His boxes are likely to be required for efficient viral assembly and release. In contrast, substitutions for the Zn-coordinating residues within the boxes did prevent RNA binding, suggesting changes in the overall conformation of the protein. In the basic region between the two Cys-His boxes, three positively charged residues, as well as basic residues flanking the two boxes, were necessary for both binding and packaging. Our results suggest that the stretches of positively charged residues within NC that need to be in a proper conformation appear to be responsible for selective recognition and binding to the packaging signal (Ψ)-containing RNAs.

Lee, L., I. B. Kaplan, et al. (2005). "A Surface Loop of the Potato Leafroll Virus Coat Protein Is Involved in Virion Assembly, Systemic Movement, and Aphid Transmission." *J. Virol.* **79**(2): 1207-1214.

<http://jvi.asm.org/cgi/content/abstract/79/2/1207>

Two acidic domains of the Potato leafroll virus (PLRV) coat protein, separated by 55 amino acids and predicted to be adjacent surface features on the virion, were the focus of a mutational analysis. Eleven site-directed mutants were generated from a cloned infectious cDNA of PLRV and delivered to plants by *Agrobacterium*-mediated mechanical inoculation. Alanine substitutions

of any of the three amino acids of the sequence EWH (amino acids 170 to 172) or of D177 disrupted the ability of the coat protein to assemble stable particles and the ability of the viral RNA to move systemically in four host plant species. Alanine substitution of E109, D173, or E176 reduced the accumulation of virus in agrobacterium-infiltrated tissues, the efficiency of systemic infection, and the efficiency of aphid transmission relative to wild-type virus, but the mutations did not affect virion stability. A structural model of the PLRV capsid predicted that the amino acids critical for virion assembly were located within a depression at the center of a coat protein trimer. The other amino acids that affected plant infection and/or aphid transmission were predicted to be located around the perimeter of the depression. PLRV virions play key roles in phloem-limited virus movement in plant hosts as well as in transport and persistence in the aphid vectors. These results identified amino acid residues in a surface-oriented loop of the coat protein that are critical for virus assembly and stability, systemic infection of plants, and movement of virus through aphid vectors.

Loparev, V. N., A. Gonzalez, et al. (2004). "Global Identification of Three Major Genotypes of Varicella-Zoster Virus: Longitudinal Clustering and Strategies for Genotyping." *J. Virol.* **78**(15): 8349-8358.

<http://jvi.asm.org/cgi/content/abstract/78/15/8349>

By analysis of a single, variable, and short DNA sequence of 447 bp located within open reading frame 22 (ORF22), we discriminated three major varicella-zoster virus (VZV) genotypes. VZV isolates from all six inhabited continents that showed nearly complete homology to ORF22 of the European reference strain Dumas were assigned to the European (E) genotype. All Japanese isolates, defined as the Japanese (J) genotype, were identical in the respective genomic region and proved the most divergent from the E strains, carrying four distinct variations. The remaining isolates carried a combination of E- and J-specific variations in the target sequence and thus were collectively termed the mosaic (M) genotype. Three hundred twenty-six isolates collected in 27 countries were genotyped. A distinctive longitudinal distribution of VZV genotypes supports this approach. Among 111 isolates collected from European patients, 96.4% were genotype E. Consistent with this observation, approximately 80% of the VZV strains from the United States were also genotype E. Similarly, genotype E viruses were dominant in the Asian part of Russia and in eastern Australia. M genotype viruses were strongly dominant in tropical regions of Africa, Indochina, and Central America, and they were common in western Australia. However, genotype M viruses were also identified as a minority in several countries worldwide. Two major intertypic variations of genotype M strains were identified, suggesting that the M genotype can be further differentiated into subgenotypes. These data highlight the direction for future VZV genotyping efforts. This approach provides the first simple genotyping method for VZV strains in clinical samples.

Lovato, L., M. Inman, et al. (2003). "Infection of Cattle with a Bovine Herpesvirus 1 Strain That Contains a Mutation in the Latency-Related Gene Leads to Increased Apoptosis in Trigeminal Ganglia during the Transition from Acute Infection to Latency." *J. Virol.* **77**(8): 4848-4857.

<http://jvi.asm.org/cgi/content/abstract/77/8/4848>

Bovine herpesvirus 1 (BHV-1) is an important pathogen of cattle and infection is usually initiated via the ocular or nasal cavity. After acute infection, the primary site for BHV-1 latency is sensory neurons in the trigeminal ganglia (TG). Reactivation from latency occurs sporadically, resulting in virus shedding and transmission to uninfected cattle. The only abundant viral transcript expressed during latency is the latency-related (LR) RNA. An LR mutant was constructed by inserting three stop codons near the beginning of the LR RNA. This mutant grows to wild-type (wt) efficiency in bovine kidney cells and in the nasal cavity of acutely infected calves. However, shedding of

infectious virus from the eye and TG was dramatically reduced in calves infected with the LR mutant. Calves latently infected with the LR mutant do not reactivate after dexamethasone treatment. In contrast, all calves latently infected with wt BHV-1 or the LR rescued mutant reactivate from latency after dexamethasone treatment. In the present study, we compared the frequency of apoptosis in calves infected with the LR mutant to calves infected with wt BHV-1 because LR gene products inhibit apoptosis in transiently transfected cells. A sensitive TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling) assay and an antibody that detects cleaved caspase-3 were used to identify apoptotic cells in TG. Both assays demonstrated that calves infected with the LR mutant for 14 days had higher levels of apoptosis in TG compared to calves infected with wt BHV-1 or to mock-infected calves. Viral gene expression, except for the LR gene, is extinguished by 14 days after infection, and thus this time frame is operationally defined as the establishment of latency. Real-time PCR analysis indicated that lower levels of viral DNA were present in the TG of calves infected with the LR mutant throughout acute infection. Taken together, these results suggest that the antiapoptotic properties of the LR gene play an important role during the establishment of latency.

Lu, J., P. Sista, et al. (2004). "Relative Replicative Fitness of Human Immunodeficiency Virus Type 1 Mutants Resistant to Enfuvirtide (T-20)." *J. Virol.* **78**(9): 4628-4637.

<http://jvi.asm.org/cgi/content/abstract/78/9/4628>

Resistance to enfuvirtide (ENF; T-20), a fusion inhibitor of human immunodeficiency virus type 1 (HIV-1), is conferred by mutations in the first heptad repeat of the gp41 ectodomain. The replicative fitness of recombinant viruses carrying ENF resistance mutations was studied in growth competition assays. ENF resistance mutations, selected in vitro or in vivo, were introduced into the env gene of HIV-1NL4-3 by site-directed mutagenesis and expressed in HIV-1 recombinants carrying sequence tags in nef. The doubling time of ENF-resistant viruses was highly correlated with decreasing ENF susceptibility ($R^2 = 0.859$; $P < 0.001$). Initial fitness experiments focused on mutants identified by in vitro selection in the presence of ENF (L. T. Rimsy, D. C. Shugars, and T. J. Matthews, *J. Virol.* **72**:986-993, 1998). In the absence of drug, these mutants displayed reduced fitness compared to wild-type virus with a relative order of fitness of wild type > I37T > V38 M > D36S/V38 M; this order was reversed in the presence of ENF. Likewise, recombinant viruses carrying ENF resistance mutations selected in vivo displayed reduced fitness in the absence of ENF with a relative order of wild type > N42T > V38A > N42T/N43K {approx} N42T/N43S > V38A/N42D {approx} V38A/N42T. Fitness and ENF susceptibility were inversely correlated ($r = -0.988$; $P < 0.001$). Similar results were obtained with recombinants expressing molecularly cloned full-length env genes obtained from patient-derived HIV-1 isolates before and after ENF treatment. Further studies are needed to determine whether the reduced fitness of ENF-resistant viruses alters their pathogenicity in vivo.

Lundberg, P., P. Welander, et al. (2003). "A Locus on Mouse Chromosome 6 That Determines Resistance to Herpes Simplex Virus Also Influences Reactivation, While an Unlinked Locus Augments Resistance of Female Mice." *J. Virol.* **77**(21): 11661-11673.

<http://jvi.asm.org/cgi/content/abstract/77/21/11661>

During studies to determine a role for tumor necrosis factor (TNF) in herpes simplex virus type 1 (HSV-1) infection using TNF receptor null mutant mice, we discovered a genetic locus, closely linked to the TNF p55 receptor (Tnfrsf1a) gene on mouse chromosome 6 (c6), that determines resistance or susceptibility to HSV-1. We named this locus the herpes resistance locus, Hrl, and showed that it also mediates resistance to HSV-2. Hrl has at least two alleles, Hrlr, expressed by resistant strains like C57BL/6 (B6), and Hrls, expressed by susceptible strains like 129S6 (129)

and BALB/c. Although Hrl is inherited as an autosomal dominant gene, resistance to HSV-1 is strongly sex biased such that female mice are significantly more resistant than male mice. Analysis of backcrosses between resistant B6 and susceptible 129 mice revealed that a second locus, tentatively named the sex modifier locus, Sml, functions to augment resistance of female mice. Besides determining resistance, Hrl is one of several genes involved in the control of HSV-1 replication in the eye and ganglion. Remarkably, Hrl also affects reactivation of HSV-1, possibly by interaction with some unknown gene(s). We showed that Hrl is distinct from Cmv1, the gene that determines resistance to murine cytomegalovirus, which is encoded in the major NK cell complex just distal of p55 on c6. Hrl has been mapped to a roughly 5-centimorgan interval on c6, and current efforts are focused on obtaining a high-resolution map for Hrl.

Markoff, L., X. Pang, et al. (2002). "Derivation and Characterization of a Dengue Type 1 Host Range-Restricted Mutant Virus That Is Attenuated and Highly Immunogenic in Monkeys." *J. Virol.* **76**(7): 3318-3328.

<http://jvi.asm.org/cgi/content/abstract/76/7/3318>

We recently described the derivation of a dengue serotype 2 virus (DEN2mutF) that exhibited a host range-restricted phenotype; it was severely impaired for replication in cultured mosquito cells (C6/36 cells). DEN2mutF virus had selected mutations in genomic sequences predicted to form a 3' stem-loop structure (3'-SL) that is conserved among all flavivirus species. The 3'-SL constitutes the downstream terminal [~]95 nucleotides of the 3' noncoding region in flavivirus RNA. Here we report the introduction of these same mutational changes into the analogous region of an infectious DNA derived from the genome of a human-virulent dengue serotype 1 virus (DEN1), strain Western Pacific (DEN1WP). The resulting DEN1 mutant (DEN1mutF) exhibited a host range-restricted phenotype similar to that of DEN2mutF virus. DEN1mutF virus was attenuated in a monkey model for dengue infection in which viremia is taken as a correlate of human virulence. In spite of the markedly reduced levels of viremia that it induced in monkeys compared to DEN1WP, DEN1mutF was highly immunogenic. In addition, DEN1mutF-immunized monkeys retained high levels of neutralizing antibodies in serum and were protected from challenge with high doses of the DEN1WP parent for as long as 17 months after the single immunizing dose. Phenotypic revertants of DEN1mutF and DEN2mutF were each detected after a total of 24 days in C6/36 cell cultures. Complete nucleotide sequence analysis of DEN1mutF RNA and that of a revertant virus, DEN1mutFrev, revealed that (i) the DEN1mutF genome contained no additional mutations upstream from the 3'-SL compared to the DEN1WP parent genome and (ii) the DEN1mutFrev genome contained de novo mutations, consistent with our previous hypothesis that the defect in DEN2mutF replication in C6/36 cells was at the level of RNA replication. A strategy for the development of a tetravalent dengue vaccine is discussed.

Maunula, L. and C.-H. von Bonsdorff (2002). "Frequent Reassortments May Explain the Genetic Heterogeneity of Rotaviruses: Analysis of Finnish Rotavirus Strains." *J. Virol.* **76**(23): 11793-11800.

<http://jvi.asm.org/cgi/content/abstract/76/23/11793>

The predominant rotavirus electropherotypes (e-types) during 17 epidemic seasons (1980 through 1997) in Finland were established, and representative virus isolates were studied by nucleotide sequencing and phylogenetic analysis. The virus isolates were either P[8]G1 or P[8]G4 types. The G1 and G4 strains formed one G1 lineage (VP7-G1-1) and one G4 lineage, respectively. Otherwise, they belonged to two P[8] lineages (VP4-P[8]-1 and -2) unrelated to their G types. Phylogenetic analysis of partial sequences of all 11 RNA segments obtained from the strains also revealed genetic diversity among gene segments other than those defining P and G

types. With the exception of segments 1, 3, and 10, the sequences of the other segments could be assigned to 2 to 4 different genetic clusters. The results of this study suggest that, in addition to the RNA segments encoding VP4 and VP7, the other RNA segments may segregate independently as well. In total, the 9 predominant e-types represented 7 different RNA segment combinations when the phylogenetic clusters of their 11 genes were determined. The extensive genetic diversity and number of e-types among rotaviruses are best explained by frequent genetic reassortment.

McGrath, C. F., J. S. Buckman, et al. (2003). "Human Cellular Nucleic Acid-Binding Protein Zn²⁺ Fingers Support Replication of Human Immunodeficiency Virus Type 1 When They Are Substituted in the Nucleocapsid Protein." *J. Virol.* **77**(15): 8524-8531.

<http://jvi.asm.org/cgi/content/abstract/77/15/8524>

A family of cellular nucleic acid binding proteins (CNBPs) contains seven Zn²⁺ fingers that have many of the structural characteristics found in retroviral nucleocapsid (NC) Zn²⁺ fingers. The sequence of the NH₂-terminal NC Zn²⁺ finger of the pNL4-3 clone of human immunodeficiency virus type 1 (HIV-1) was replaced individually with sequences from each of the seven fingers from human CNBP. Six of the mutants were normal with respect to protein composition and processing, full-length genomic RNA content, and infectivity. One of the mutants, containing the fifth CNBP Zn²⁺ finger (CNBP-5) packaged reduced levels of genomic RNA and was defective in infectivity. There appear to be defects in reverse transcription in the CNBP-5 infections. Models of Zn²⁺ fingers were constructed by using computational methods based on available structural data, and atom-atom interactions were determined by the hydropathic orthogonal dynamic analysis of the protein method. Defects in the CNBP-5 mutant could possibly be explained, in part, by restrictions of a set of required atom-atom interactions in the CNBP-5 Zn²⁺ finger compared to mutant and wild-type Zn²⁺ fingers in NC that support replication. The present study shows that six of seven of the Zn²⁺ fingers from the CNBP protein can be used as substitutes for the Zn²⁺ finger in the NH₂-terminal position of HIV-1 NC. This has obvious implications in antiviral therapeutics and DNA vaccines employing NC Zn²⁺ finger mutants.

Melder, D. C., V. S. Pankratz, et al. (2003). "Evolutionary Pressure of a Receptor Competitor Selects Different Subgroup A Avian Leukosis Virus Escape Variants with Altered Receptor Interactions." *J. Virol.* **77**(19): 10504-10514.

<http://jvi.asm.org/cgi/content/abstract/77/19/10504>

A complex interaction between the retroviral envelope glycoproteins and a specific cell surface protein initiates viral entry into cells. The avian leukosis-sarcoma virus (ALV) group of retroviruses provides a useful experimental system for studying the retroviral entry process and the evolution of receptor usage. In this study, we demonstrate that evolutionary pressure on subgroup A ALV [ALV(A)] entry exerted by the presence of a competitive inhibitor, a soluble form of the ALV(A) Tva receptor linked to a mouse immunoglobulin G tag (quail sTva-mIgG), can select different populations of escape variants. This escape population contained three abundant ALV(A) variant viruses, all with mutations in the surface glycoprotein hypervariable regions: a previously identified variant containing the Y142N mutation in the hr1 region; a new variant with two mutations, W141G in hr1 and K261E in vr3; and another new variant with two mutations, W145R in hr1 and K261E. The W141G K261E and W145R K261E viruses escape primarily by lowering their binding affinities for the quail Tva receptor competitive inhibitor while retaining wild-type levels of binding affinity for the chicken Tva receptor. A secondary phenotype of the new variants was an alteration in receptor interference patterns from that of wild-type ALV(A), indicating that the mutant glycoproteins are possibly interacting with other cellular proteins. One result of these

altered interactions was that the variants caused a transient period of cytotoxicity. We could also directly demonstrate that the W141G K261E variant glycoproteins bound significant levels of a soluble form of the TvbS3 ALV receptor in a binding assay. Alterations in the normally extreme specificity of the ALV(A) glycoproteins for Tva may represent an evolutionary first step toward expanding viral receptor usage in response to inefficient viral entry.

Men, R., T. Yamashiro, et al. (2004). "Identification of Chimpanzee Fab Fragments by Repertoire Cloning and Production of a Full-Length Humanized Immunoglobulin G1 Antibody That Is Highly Efficient for Neutralization of Dengue Type 4 Virus." *J. Virol.* **78**(9): 4665-4674.

<http://jvi.asm.org/cgi/content/abstract/78/9/4665>

A safe and effective dengue vaccine is still not available. Passive immunization with monoclonal antibodies from humans or nonhuman primates represents an attractive alternative for the prevention of dengue virus infection. Fab monoclonal antibodies to dengue type 4 virus (DENV-4) were recovered by repertoire cloning of bone marrow mRNAs from an immune chimpanzee and analyzed for antigen binding specificity, VH and VL sequences, and neutralizing activity against DENV-4 in vitro. Fabs 5A7, 3C1, 3E4, and 7G4 were isolated from a library constructed from a chimpanzee following intrahepatic transfection with infectious DENV-4 RNA. Fabs 5H2 and 5D9, which had nearly identical VH sequences but varied in their VL sequences, were recovered from a library constructed from the same chimpanzee after superinfection with a mixture of DENV-1, DENV-2, and DENV-3. In radioimmunoprecipitation, Fab 5A7 precipitated only DENV-4 prM, and Fabs 3E4, 7G4, 5D9, and 5H2 precipitated DENV-4 E but little or no prM. Fab 3E4 and Fab 7G4 competed with each other for binding to DENV-4 in an enzyme-linked immunosorbent assay, as did Fab 3C1 and Fab 5A7. Fab 5H2 recognized an epitope on DENV-4 that was separate from the epitope(s) recognized by other Fabs. Both Fab 5H2 and Fab 5D9 neutralized DENV-4 efficiently with a titer of 0.24 to 0.58 {micro}g/ml by plaque reduction neutralization test (PRNT), whereas DENV-4-neutralizing activity of other Fabs was low or not detected. Fab 5H2 was converted to full-length immunoglobulin G1 (IgG1) by combining it with human sequences. The humanized chimpanzee antibody IgG1 5H2 produced in CHO cells neutralized DENV-4 strains from different geographical origins at a similar 50% plaque reduction (PRNT50) titer of 0.03 to 0.05 {micro}g/ml. The DENV-4 binding affinities were 0.42 nM for Fab 5H2 and 0.24 nM for full-length IgG1 5H2. Monoclonal antibody IgG1 5H2 may prove valuable for passive immunoprophylaxis against dengue virus in humans.

Minisini, R., C. Tulone, et al. (2003). "Constitutive Inositol Phosphate Formation in Cytomegalovirus-Infected Human Fibroblasts Is due to Expression of the Chemokine Receptor Homologue pUS28." *J. Virol.* **77**(8): 4489-4501.

<http://jvi.asm.org/cgi/content/abstract/77/8/4489>

An open reading frame (ORF), US28, with homology to mammalian chemokine receptors has been identified in the genome of human cytomegalovirus (HCMV). Its protein product, pUS28, has been shown to bind several human CC chemokines, including RANTES, MCP-1, and MIP-1{alpha}, and the CX3C chemokine fractalkine with high affinity. Addition of CC chemokines to cells expressing pUS28 was reported to cause a pertussis toxin-sensitive increase in the concentration of cytosolic free Ca²⁺. Recently, pUS28 was shown to mediate constitutive, ligand-independent, and pertussis toxin-insensitive activation of phospholipase C via Gq/11-dependent signaling pathways in transiently transfected COS-7 cells. Since these findings are not easily reconciled with the former observations, we analyzed the role of pUS28 in mediating CC chemokine activation of pertussis toxin-sensitive G proteins in cell membranes and phospholipase C in intact cells. The transmembrane signaling functions of pUS28 were studied in

HCMV-infected cells rather than in cDNA-transfected cells. Since DNA sequence analysis of ORF US28 of different laboratory and clinical strains had revealed amino acid sequence differences in the amino-terminal portion of pUS28, we compared two laboratory HCMV strains, AD169 and Toledo, and one clinical strain, TB40/E. The results showed that infection of human fibroblasts with all three HCMV strains led to a vigorous, constitutively enhanced formation of inositol phosphates which was insensitive to pertussis toxin. This effect was critically dependent on the presence of the US28 ORF in the HCMV genome but was independent of the amino acid sequence divergence of the three HCMV strains investigated. The constitutive activity of pUS28 is not explained by expression of pUS28 at high density in HCMV-infected cells. The pUS28 ligands RANTES and MCP-1 failed to stimulate binding of guanosine 5'-O-(3-[³⁵S]thiotriphosphate to membranes of HCMV-infected cells and did not enhance constitutive activation of phospholipase C in intact HCMV-infected cells. These findings raise the possibility that the effects of CC chemokines and pertussis toxin on G protein-mediated transmembrane signaling previously observed in HCMV-infected cells are either independent of or not directly mediated by the protein product of ORF US28.

Moorman, N. J., D. O. Willer, et al. (2003). "The Gammaherpesvirus 68 Latency-Associated Nuclear Antigen Homolog Is Critical for the Establishment of Splenic Latency." *J. Virol.* **77**(19): 10295-10303.

<http://jvi.asm.org/cgi/content/abstract/77/19/10295>

Open reading frame 73 (ORF 73) is conserved among the gamma-2-herpesviruses (rhadinoviruses) and, in Kaposi's sarcoma-associated herpesvirus (KSHV) and herpesvirus saimiri (HVS), has been shown to encode a latency-associated nuclear antigen (LANA). The KSHV and HVS LANAs have also been shown to be required for maintenance of the viral genome as an episome during latency. LANA binds both the viral latency-associated origin of replication and the host cell chromosome, thereby ensuring efficient partitioning of viral genomes to daughter cells during mitosis of a latently infected cell. In gammaherpesvirus 68 ({gamma}HV68), the role of the LANA homolog in viral infection has not been analyzed. Here we report the construction of a {gamma}HV68 mutant containing a translation termination codon in the LANA ORF (73.STOP). The 73.STOP mutant virus replicated normally in vitro, in both proliferating and quiescent murine fibroblasts. In addition, there was no difference between wild-type (WT) and 73.STOP virus in the kinetics of induction of lethality in mice lacking B and T cells (Rag 1^{-/-}) infected with 1,000 PFU of virus. However, compared to WT virus, the 73.STOP mutant exhibited delayed kinetics of replication in the lungs of immunocompetent C57BL/6 mice. In addition, the 73.STOP mutant exhibited a severe defect in the establishment of latency in the spleen of C57BL/6 mice. Increasing the inoculum of 73.STOP virus partially overcame the acute replication defect observed in the lungs at day 4 postinfection but did not ameliorate the severe defect in the establishment of splenic latency. Thus, consistent with its proposed role in replication of the latent viral episome, LANA appears to be a critical determinant in the establishment of {gamma}HV68 latency in the spleen post-intranasal infection.

Muir, W. B., R. Nichols, et al. (2002). "Phylogenetic Analysis of Varicella-Zoster Virus: Evidence of Intercontinental Spread of Genotypes and Recombination." *J. Virol.* **76**(4): 1971-1979.

<http://jvi.asm.org/cgi/content/abstract/76/4/1971>

A heteroduplex mobility assay was used to identify variants of varicella-zoster virus circulating in the United Kingdom and elsewhere. Within the United Kingdom, 58 segregating sites were found out of the 23,266 examined (0.25%), and nucleotide diversity was estimated to be 0.00063. These are an order of magnitude smaller than comparable estimates from herpes simplex virus

type 1. Sixteen substitutions were nonsynonymous, the majority of which were clustered within surface-expressed proteins. Extensive genetic correlation between widely spaced sites indicated that recombination has been rare. Phylogenetic analysis of varicella-zoster viruses from four continents distinguished at least three major genetic clades. Most geographical regions contained only one of these three strains, apart from the United Kingdom and Brazil, where two or more strains were found. There was minimal genetic differentiation (one or fewer substitutions in 1,895 bases surveyed) between the samples collected from Africa (Guinea Bissau, Zambia) and the Indian subcontinent (Bangladesh, South India), suggesting recent rapid spread and/or low mutation rates. The geographic pattern of strain distribution would favor a major influence of the former. The genetic uniformity of most virus populations makes recombination difficult to detect. However, at least one probable recombinant between two of the major strains was found among the samples originating from Brazil, where mixtures of genotypes co-occur.

Narezkina, A., K. D. Taganov, et al. (2004). "Genome-Wide Analyses of Avian Sarcoma Virus Integration Sites." *J. Virol.* **78**(21): 11656-11663.

<http://jvi.asm.org/cgi/content/abstract/78/21/11656>

The chromosomal features that influence retroviral integration site selection are not well understood. Here, we report the mapping of 226 avian sarcoma virus (ASV) integration sites in the human genome. The results show that the sites are distributed over all chromosomes, and no global bias for integration site selection was detected. However, RNA polymerase II transcription units (protein-encoding genes) appear to be favored targets of ASV integration. The integration frequency within genes is similar to that previously described for murine leukemia virus but distinct from the higher frequency observed with human immunodeficiency virus type 1. We found no evidence for preferred ASV integration sites over the length of genes and immediate flanking regions. Microarray analysis of uninfected HeLa cells revealed that the expression levels of ASV target genes were similar to the median level for all genes represented in the array. Although expressed genes were targets for integration, we found no preference for integration into highly expressed genes. Our results provide a more detailed description of the chromosomal features that may influence ASV integration and support the idea that distinct, virus-specific mechanisms mediate integration site selection. Such differences may be relevant to viral pathogenesis and provide utility in retroviral vector design.

Oshima, M., D. Muriaux, et al. (2004). "Effects of Blocking Individual Maturation Cleavages in Murine Leukemia Virus Gag." *J. Virol.* **78**(3): 1411-1420.

<http://jvi.asm.org/cgi/content/abstract/78/3/1411>

A single protein, termed Gag, is responsible for retrovirus particle assembly. After the assembled virion is released from the cell, Gag is cleaved at several sites by the viral protease (PR). The cleavages catalyzed by PR bring about a wide variety of physical changes in the particle, collectively termed maturation, and convert the particle into an infectious virion. In murine leukemia virus (MLV) maturation, Gag is cleaved at three sites, resulting in formation of the matrix (MA), p12, capsid (CA), and nucleocapsid (NC) proteins. We introduced mutations into MLV that inhibited cleavage at individual sites in Gag. All mutants had lost the intensely staining ring characteristic of immature particles; thus, no single cleavage event is required for this feature of maturation. Mutant virions in which MA was not cleaved from p12 were still infectious, with a specific infectivity only [~]10-fold below that of the wild type. Particles in which p12 and CA could not be separated from each other were noninfectious and lacked a well-delineated core despite the presence of dense material in their interiors. In both of these mutants, the dimeric viral RNA had undergone the stabilization normally associated with maturation, suggesting that this change

may depend upon the separation of CA from NC. Alteration of the C-terminal end of CA blocked CA-NC cleavage but also reduced the efficiency of particle formation and, in some cases, severely disrupted the ability of Gag to assemble into regular structures. This observation highlights the critical role of this region of Gag in assembly.

Oster, B. and P. Hollsberg (2002). "Viral Gene Expression Patterns in Human Herpesvirus 6B-Infected T Cells." J. Virol. **76**(15): 7578-7586.

<http://jvi.asm.org/cgi/content/abstract/76/15/7578>

Herpesvirus gene expression is divided into immediate-early (IE) or {alpha} genes, early (E) or {beta} genes, and late (L) or {gamma} genes on the basis of temporal expression and dependency on other gene products. By using real-time PCR, we have investigated the expression of 35 human herpesvirus 6B (HHV-6B) genes in T cells infected by strain PL-1. Kinetic analysis and dependency on de novo protein synthesis and viral DNA polymerase activity suggest that the HHV-6B genes segregate into six separate kinetic groups. The genes expressed early (groups I and II) and late (groups V and VI) corresponded well with IE and L genes, whereas the intermediate groups III and IV contained E and L genes. Although HHV-6B has characteristics similar to those of other roseoloviruses in its overall gene regulation, we detected three B-variant-specific IE genes. Moreover, genes that were independent of de novo protein synthesis clustered in an area of the viral genome that has the lowest identity to the HHV-6A variant. The organization of IE genes in an area of the genome that differs from that of HHV-6A underscores the distinct differences between HHV-6B and HHV-6A and may provide a basis for further molecular and immunological analyses to elucidate their different biological behaviors.

Ozaki, H., E. A. Govorkova, et al. (2004). "Generation of High-Yielding Influenza A Viruses in African Green Monkey Kidney (Vero) Cells by Reverse Genetics." J. Virol. **78**(4): 1851-1857.

<http://jvi.asm.org/cgi/content/abstract/78/4/1851>

Influenza A viruses are the cause of annual epidemics of human disease with occasional outbreaks of pandemic proportions. The zoonotic nature of the disease and the vast viral reservoirs in the aquatic birds of the world mean that influenza will not easily be eradicated and that vaccines will continue to be needed. Recent technological advances in reverse genetics methods and limitations of the conventional production of vaccines by using eggs have led to a push to develop cell-based strategies to produce influenza vaccine. Although cell-based systems are being developed, barriers remain that need to be overcome if the potential of these systems is to be fully realized. These barriers include, but are not limited to, potentially poor reproducibility of viral rescue with reverse genetics systems and poor growth kinetics and yields. In this study we present a modified A/Puerto Rico/8/34 (PR8) influenza virus master strain that has improved viral rescue and growth properties in the African green monkey kidney cell line, Vero. The improved properties were mediated by the substitution of the PR8 NS gene for that of a Vero-adapted reassortant virus. The Vero growth kinetics of viruses with H1N1, H3N2, H6N1, and H9N2 hemagglutinin and neuraminidase combinations rescued on the new master strain were significantly enhanced in comparison to those of viruses with the same combinations rescued on the standard PR8 master strain. These improvements pave the way for the reproducible generation of high-yielding human and animal influenza vaccines by reverse genetics methods. Such a means of production has particular relevance to epidemic and pandemic use.

Ozbun, M. A. (2002). "Human Papillomavirus Type 31b Infection of Human Keratinocytes and the Onset of Early Transcription." J. Virol. **76**(22): 11291-11300.

<http://jvi.asm.org/cgi/content/abstract/76/22/11291>

Human papillomaviruses (HPVs) cause a number of human tumors and malignancies, including cervical cancers. Epithelial differentiation is required for the complete HPV life cycle and can be achieved using the organotypic (raft) culture system. The CIN-612 9E cell line maintains episomal copies of HPV type 31b (HPV31b), an HPV type associated with cervical cancers. When grown in the raft system, CIN-612 9E cells form a differentiated epithelium such that infectious virions can be synthesized. Many aspects of the later stages of the HPV31b life cycle have been investigated in CIN-612 9E raft tissues. We used a biologically contained homogenization system for efficient virion extraction from raft epithelial tissues. Purified HPV31b virions were used to infect low-passage-number human foreskin keratinocytes and a variety of epithelial cell lines. Newly synthesized, spliced HPV31b transcripts were detected by reverse transcription and PCR (RT-PCR) following HPV31b infection. HPV31b infection was most efficient and reproducible in HaCaT cells. The onset of viral transcription following infection was also investigated using RT-PCR techniques. Spliced E1*1, E2 RNAs were present as early as 4 h postinfection (p.i.), whereas the other major viral transcripts were detected by 8 to 10 h p.i. Furthermore, we characterized the structures and temporal expression of seven novel spliced early transcripts expressed following infection.

Payne, S. L., X.-f. Pei, et al. (2004). "Influence of Long Terminal Repeat and Env on the Virulence Phenotype of Equine Infectious Anemia Virus." J. Virol. **78**(5): 2478-2485.

<http://jvi.asm.org/cgi/content/abstract/78/5/2478>

The molecular clones pSPeIav19 and p19/wenv17 of equine infectious anemia virus (EIAV) differ in env and long terminal repeats (LTRs) and produce viruses (EIAV19 and EIAV17, respectively) of dramatically different virulence phenotypes. These constructs were used to generate a series of chimeric clones to test the individual contributions of LTR, surface (SU), and transmembrane (TM)/Rev regions to the disease potential of the highly virulent EIAV17. The LTRs of EIAV19 and EIAV17 differ by 16 nucleotides in the transcriptional enhancer region. The two viruses differ by 30 amino acids in SU, by 17 amino acids in TM, and by 8 amino acids in Rev. Results from in vivo infections with chimeric clones indicate that both LTR and env of EIAV17 are required for the development of severe acute disease. In the context of the EIAV17 LTR, SU appears to have a greater impact on virulence than does TM. EIAV17SU, containing only the TM/Rev region from the avirulent parent, induced acute disease in two animals, while a similar infectious dose of EIAV17TM (which derives SU from the avirulent parent) did not. Neither EIAV17SU nor EIAV17TM produced lethal disease when administered at infectious doses that were 6- to 30-fold higher than a lethal dose of the parental EIAV17. All chimeric clones replicated in primary equine monocyte-derived macrophages, and there was no apparent correlation between macrophage tropism and virulence phenotype.

Perez, D. R., W. Lim, et al. (2003). "Role of Quail in the Interspecies Transmission of H9 Influenza A Viruses: Molecular Changes on HA That Correspond to Adaptation from Ducks to Chickens." J. Virol. **77**(5): 3148-3156.

<http://jvi.asm.org/cgi/content/abstract/77/5/3148>

H9 influenza viruses have become endemic in land-based domestic poultry in Asia and have

sporadically crossed to pigs and humans. To understand the molecular determinants of their adaptation to land-based birds, we tested the replication and transmission of several 1970s duck H9 viruses in chickens and quail. Quail were more susceptible than chickens to these viruses, and generation of recombinant H9 viruses by reverse genetics showed that changes in the HA gene are sufficient to initiate efficient replication and transmission in quail. Seven amino acid positions on the HA molecule corresponded to adaptation to land-based birds. In quail H9 viruses, the pattern of amino acids at these seven positions is intermediate between those of duck and chicken viruses; this fact may explain the susceptibility of quail to duck H9 viruses. Our findings suggest that quail provide an environment in which the adaptation of influenza viruses from ducks generates novel variants that can cross the species barrier.

Poon, L. L. M., D. K. W. Chu, et al. (2005). "Identification of a Novel Coronavirus in Bats." J. Virol. **79**(4): 2001-2009.

<http://jvi.asm.org/cgi/content/abstract/79/4/2001>

Exotic wildlife can act as reservoirs of diseases that are endemic in the area or can be the source of new emerging diseases through interspecies transmission. The recent emergence of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) highlights the importance of virus surveillance in wild animals. Here, we report the identification of a novel bat coronavirus through surveillance of coronaviruses in wildlife. Analyses of the RNA sequence from the ORF1b and S-gene regions indicated that the virus is a group 1 coronavirus. The virus was detected in fecal and respiratory samples from three bat species (*Miniopterus* spp.). In particular, 63% (12 of 19) of fecal samples from *Miniopterus pusillus* were positive for the virus. These findings suggest that this virus might be commonly circulating in *M. pusillus* in Hong Kong.

Roca, A. L., W. G. Nash, et al. (2005). "Insertional Polymorphisms of Endogenous Feline Leukemia Viruses." J. Virol. **79**(7): 3979-3986.

<http://jvi.asm.org/cgi/content/abstract/79/7/3979>

The number, chromosomal distribution, and insertional polymorphisms of endogenous feline leukemia viruses (enFeLVs) were determined in four domestic cats (Burmese, Egyptian Mau, Persian, and nonbreed) using fluorescent in situ hybridization and radiation hybrid mapping. Twenty-nine distinct enFeLV loci were detected across 12 of the 18 autosomes. Each cat carried enFeLV at only 9 to 16 of the loci, and many loci were heterozygous for presence of the provirus. Thus, an average of 19 autosomal copies of enFeLV were present per cat diploid genome. Only five of the autosomal enFeLV sites were present in all four cats, and at only one autosomal locus, B4q15, was enFeLV present in both homologues of all four cats. A single enFeLV occurred in the X chromosome of the Burmese cat, while three to five enFeLV proviruses occurred in each Y chromosome. The X chromosome and nine autosomal enFeLV loci were telomeric, suggesting that ectopic recombination between nonhomologous subtelomeres may contribute to enFeLV distribution. Since endogenous FeLVs may affect the infectiousness or pathogenicity of exogenous FeLVs, genomic variation in enFeLVs represents a candidate for genetic influences on FeLV leukemogenesis in cats.

Rohayem, J., J. Munch, et al. (2005). "Evidence of Recombination in the Norovirus Capsid Gene." J. Virol. **79**(8): 4977-4990.

<http://jvi.asm.org/cgi/content/abstract/79/8/4977>

Noroviruses are single-stranded RNA viruses with high genomic variability. They have emerged in the last decade as a major cause of acute gastroenteritis. It remains so far unclear whether norovirus evolution is driven by sequence mutation and/or recombination. In this study, we have assessed the occurrence of recombination in the norovirus capsid gene. For this purpose, 69 complete capsid sequences of norovirus strains accessible in GenBank as well as 25 complete capsid sequences generated from norovirus-positive clinical samples were examined. Unreported recombination was detected in about 8% of norovirus strains belonging to genetic clusters I/1 (n = 1), II/1 (n = 1), II/3 (n = 1), II/4 (n = 3), and II/5 (n = 1). Recombination breakpoints were mainly located at the interface of the putative P1-1 and P2 domains of the capsid protein and/or within the P2 domain. The recombination region displayed features such as length, sequence composition (upstream and downstream GC- and AU-rich sequences, respectively), and predicted RNA secondary structure that are characteristic of homologous recombination activators. Our results suggest that recombination in the norovirus capsid gene may naturally occur, involving capsid domains presumably exposed to immunological pressure.

Roque-Afonso, A.-M., D. Ducoulombier, et al. (2005). "Compartmentalization of Hepatitis C Virus Genotypes between Plasma and Peripheral Blood Mononuclear Cells." *J. Virol.* **79**(10): 6349-6357.

<http://jvi.asm.org/cgi/content/abstract/79/10/6349>

Differences in hepatitis C virus (HCV) variants of the highly conserved 5' untranslated region (UTR) have been observed between plasma and peripheral blood mononuclear cells (PBMC). The prevalence and the mechanisms of this compartmentalization are unknown. Plasma and PBMC HCV variants were compared by single-strand conformation polymorphism (SSCP) and by cloning or by genotyping with a line probe assay (LiPA) in 116 chronically infected patients, including 44 liver transplant recipients. SSCP patterns differed between compartments in 43/109 analyzable patients (39%). Differences were significantly more frequent in patients with transplants (21/38 [55%] versus 22/71 [31%]; $P < 0.01$) and in those who acquired HCV through multiple transfusions before 1991 (15/20; 75%) or through drug injection (16/31; 52%) than in those infected through an unknown route (7/29; 24%) or through a single transfusion (5/29; 17%; $P < 0.001$). Cloning of the 5' UTR, LiPA analysis, and nonstructural region 5B sequencing revealed different genotypes in the two compartments from 10 patients (9%). In nine patients, the genotype detected in PBMC was not detected in plasma and was weak or undetectable in the liver in three cases. This genotypic compartmentalization persisted for years in three patients and after liver transplantation in two. The present study shows that a significant proportion of HCV-infected subjects harbor in their PBMC highly divergent variants which were likely acquired through superinfections.

Rose, T. M., J. T. Ryan, et al. (2003). "Analysis of 4.3 Kilobases of Divergent Locus B of Macaque Retroperitoneal Fibromatosis-Associated Herpesvirus Reveals a Close Similarity in Gene Sequence and Genome Organization to Kaposi's Sarcoma-Associated Herpesvirus." *J. Virol.* **77**(9): 5084-5097.

<http://jvi.asm.org/cgi/content/abstract/77/9/5084>

We previously identified retroperitoneal fibromatosis-associated herpesvirus (RFHV) as a simian homolog of Kaposi's sarcoma-associated herpesvirus (KSHV) in a fibroproliferative malignancy of macaques that has similarities to Kaposi's sarcoma. In this report, we cloned 4.3 kb of divergent

locus B (DL-B) flanking the DNA polymerase gene from two variants of RFHV from different species of macaque with a consensus degenerate hybrid oligonucleotide primer approach. Within the DL-B region of RFHV, viral homologs of the cellular interleukin-6, dihydrofolate reductase, and thymidylate synthase genes were identified, along with a homolog of the gammaherpesvirus open reading frame (ORF) 10. In addition, a homolog of the KSHV ORF K3, the modulator of immune recognition-1, was identified. Our data show a close similarity in sequence conservation, gene content, and genomic structure between RFHV and KSHV which strongly supports the grouping of these viral species within the same RV-1 rhadinovirus lineage and the hypothesis that RFHV is the macaque homolog of KSHV.

Roux, M. M., A. Pain, et al. (2002). "The Lipopolysaccharide and {beta}-1,3-Glucan Binding Protein Gene Is Upregulated in White Spot Virus-Infected Shrimp (*Penaeus stylirostris*)."
J. Virol. **76**(14): 7140-7149.

<http://jvi.asm.org/cgi/content/abstract/76/14/7140>

Pattern recognition proteins such as lipopolysaccharide and {beta}-1,3-glucan binding protein (LGBP) play an important role in the innate immune response of crustaceans and insects. Random sequencing of cDNA clones from a hepatopancreas cDNA library of white spot virus (WSV)-infected shrimp provided a partial cDNA (PsEST-289) that showed similarity to the LGBP gene of crayfish and insects. Subsequently full-length cDNA was cloned by the 5'-RACE (rapid amplification of cDNA ends) technique and sequenced. The shrimp LGBP gene is 1,352 bases in length and is capable of encoding a polypeptide of 376 amino acids that showed significant similarity to homologous genes from crayfish, insects, earthworms, and sea urchins. Analysis of the shrimp LGBP deduced amino acid sequence identified conserved features of this gene family including a potential recognition motif for {beta}-(1->3) linkage of polysaccharides and putative RGD cell adhesion sites. It is known that LGBP gene expression is upregulated in bacterial and fungal infection and that the binding of lipopolysaccharide and {beta}-1,3-glucan to LGBP activates the prophenoloxidase (proPO) cascade. The temporal expression of LGBP and proPO genes in healthy and WSV-challenged *Penaeus stylirostris* shrimp was measured by real-time quantitative reverse transcription-PCR, and we showed that LGBP gene expression in shrimp was upregulated as the WSV infection progressed. Interestingly, the proPO expression was upregulated initially after infection followed by a downregulation as the viral infection progressed. The downward trend in the expression of proPO coincided with the detection of WSV in the infected shrimp. Our data suggest that shrimp LGBP is an inducible acute-phase protein that may play a critical role in shrimp-WSV interaction and that the WSV infection regulates the activation and/or activity of the proPO cascade in a novel way.

Rovira, A., M. Balasch, et al. (2002). "Experimental Inoculation of Conventional Pigs with Porcine Reproductive and Respiratory Syndrome Virus and Porcine Circovirus 2." *J. Virol.* **76**(7): 3232-3239.

<http://jvi.asm.org/cgi/content/abstract/76/7/3232>

Postweaning multisystemic wasting syndrome (PMWS) is a disease of nursery and fattening pigs characterized by growth retardation, paleness of the skin, dyspnea, and increased mortality rates. Porcine circovirus 2 (PCV2) has been demonstrated to be the cause of PMWS. However, other factors are needed for full development of the syndrome, and porcine reproductive and respiratory syndrome virus (PRRSV) infection has been suggested to be one of them. Twenty-four conventional 5-week-old pigs were distributed in four groups: control (n = 5), PRRSV inoculated (n = 5), PCV2 inoculated (n = 7), and PRRSV and PCV2 inoculated (n = 7). The two groups inoculated with PRRSV showed growth retardation. Pigs inoculated with both PRRSV and

PCV2 had increased rectal temperature. One of these pigs developed wasting, had severe respiratory distress, and died. The most important microscopic lesion in pigs inoculated with PCV2 was lymphocyte depletion with histiocytic infiltration of the lymphoid organs, more severe and in a wider range of tissues in doubly inoculated pigs. Interstitial pneumonia was observed in the three inoculated groups. PCV2 nucleic acid was found by in situ hybridization in larger amounts and in a wider range of lymphoid tissues in PRRSV- and PCV2-inoculated than in PCV2-inoculated pigs. TaqMan PCR was performed to quantify the PCV2 loads in serum during the experiment. PCV2 loads were higher in doubly inoculated pigs than in pigs inoculated with PCV2 alone. These findings indicate that severe disease can be reproduced in conventional 5-week-old pigs by inoculation of PRRSV and PCV2. Moreover, these results support the hypothesis that PRRSV infection enhances PCV2 replication.

Schafer, A., D. Lengenfelder, et al. (2003). "The Latency-Associated Nuclear Antigen Homolog of Herpesvirus Saimiri Inhibits Lytic Virus Replication." *J. Virol.* **77**(10): 5911-5925.

<http://jvi.asm.org/cgi/content/abstract/77/10/5911>

Herpesvirus saimiri (HVS), a T-lymphotropic tumor virus of neotropical primates, and the Kaposi's sarcoma-associated human herpesvirus 8 (KSHV) belong to the gamma-2-herpesvirus (Rhadinovirus) subfamily and share numerous features of genome structure and organization. The KSHV latency-associated nuclear antigen (LANA) protein appears to be relevant for viral persistence, latency, and transformation. It binds to DNA, colocalizes with viral episomal DNA, and presumably mediates efficient persistence of viral genomes. LANA further represses the transcriptional and proapoptotic activities of the p53 tumor suppressor protein. Here we report on the ORF73 gene of HVS strain C488, which is the positional and structural homolog of KSHV LANA. The ORF73 gene in OMK cells can encode a 62-kDa protein that localizes to the nucleus in a pattern similar to that of LANA. We show that the ORF73 gene product can regulate viral gene expression by acting as a transcriptional modulator of latent and lytic viral promoters. To define the HVS ORF73 function in the background of a replication-competent virus, we constructed a viral mutant that expresses ORF73 under the transcriptional control of a mifepristone (RU-486)-inducible promoter. The HVS ORF73 gene product efficiently suppresses lytic viral replication in permissive cells, indicating that it defines a critical control point between viral persistence and lytic replication.

Scholtissek, C., J. Stech, et al. (2002). "Cooperation between the Hemagglutinin of Avian Viruses and the Matrix Protein of Human Influenza A Viruses." *J. Virol.* **76**(4): 1781-1786.

<http://jvi.asm.org/cgi/content/abstract/76/4/1781>

To analyze the compatibility of avian influenza A virus hemagglutinins (HAs) and human influenza A virus matrix (M) proteins M1 and M2, we doubly infected Madin-Darby canine kidney cells with amantadine (1-aminoadamantane hydrochloride)-resistant human viruses and amantadine-sensitive avian strains. By using antisera against the human virus HAs and amantadine, we selected reassortants containing the human virus M gene and the avian virus HA gene. In our system, high virus yields and large, well-defined plaques indicated that the avian HAs and the human M gene products could cooperate effectively; low virus yields and small, turbid plaques indicated that cooperation was poor. The M gene products are among the primary components that determine the species specificities of influenza A viruses. Therefore, our system also indicated whether the avian HA genes effectively reassorted into the genome and replaced the HA gene of the prevailing human influenza A viruses. Most of the avian HAs that we tested efficiently cooperated with the M gene products of the early human A/PR/8/34 (H1N1) virus; however, the avian HAs did not effectively cooperate with the most recently isolated human virus

that we tested, A/Nanchang/933/95 (H3N2). Cooperation between the avian HAs and the M proteins of the human A/Singapore/57 (H2N2) virus was moderate. These results suggest that the currently prevailing human influenza A viruses might have lost their ability to undergo antigenic shift and therefore are unable to form new pandemic viruses that contain an avian HA, a finding that is of great interest for pandemic planning.

Scobie, L., S. Taylor, et al. (2004). "Absence of Replication-Competent Human-Tropic Porcine Endogenous Retroviruses in the Germ Line DNA of Inbred Miniature Swine." *J. Virol.* **78**(5): 2502-2509.

<http://jvi.asm.org/cgi/content/abstract/78/5/2502>

The potential transmission of porcine endogenous retroviruses (PERVs) has raised concern in the development of porcine xenotransplantation products. Our previous studies have resulted in the identification of animals within a research herd of inbred miniature swine that lack the capacity to transmit PERV to human cells in vitro. In contrast, other animals were capable of PERV transmission. The PERVs that were transmitted to human cells are recombinants between PERV-A and PERV-C in the post-VRA region of the envelope (B. A. Oldmixon, J. C. Wood, T. A. Ericsson, C. A. Wilson, M. E. White-Scharf, G. Andersson, J. L. Greenstein, H. J. Schuurman, and C. Patience, *J. Virol.* 76:3045-3048, 2002); these viruses we term PERV-A/C. This observation prompted us to determine whether these human-tropic replication-competent (HTRC) PERV-A/C recombinants were present in the genomic DNA of these miniature swine. Genomic DNA libraries were generated from one miniature swine that transmitted HTRC PERV as well as from one miniature swine that did not transmit HTRC PERV. HTRC PERV-A/C proviruses were not identified in the germ line DNAs of these pigs by using genomic mapping. Similarly, although PERV-A loci were identified in both libraries that possessed long env open reading frames, the Env proteins encoded by these loci were nonfunctional according to pseudotype assays. In the absence of a germ line source for HTRC PERV, further studies are warranted to assess the mechanisms by which HTRC PERV can be generated. Once identified, it may prove possible to generate animals with further reduced potential to produce HTRC PERV.

Silverman, L. R., A. J. Phipps, et al. (2004). "Human T-Cell Lymphotropic Virus Type 1 Open Reading Frame II-Encoded p30II Is Required for In Vivo Replication: Evidence of In Vivo Reversion." *J. Virol.* **78**(8): 3837-3845.

<http://jvi.asm.org/cgi/content/abstract/78/8/3837>

Human T-cell lymphotropic virus type 1 (HTLV-1) causes adult T-cell leukemia/lymphoma and exhibits high genetic stability in vivo. HTLV-1 contains four open reading frames (ORFs) in its pX region. ORF II encodes two proteins, p30II and p13II, both of which are incompletely characterized. p30II localizes to the nucleus or nucleolus and has distant homology to the transcription factors Oct-1, Pit-1, and POU-M1. In vitro studies have demonstrated that at low concentrations, p30II differentially regulates cellular and viral promoters through an interaction with CREB binding protein/p300. To determine the in vivo significance of p30II, we inoculated rabbits with cell lines expressing either a wild-type clone of HTLV-1 (ACH.1) or a clone containing a mutation in ORF II, which eliminated wild-type p30II expression (ACH.30.1). ACH.1-inoculated rabbits maintained higher HTLV-1-specific antibody titers than ACH.30.1-inoculated rabbits, and all ACH.1-inoculated rabbits were seropositive for HTLV-1, whereas only two of six ACH.30.1-inoculated rabbits were seropositive. Provirus could be consistently PCR amplified from peripheral blood mononuclear cell (PBMC) DNA in all ACH.1-inoculated rabbits but in only three of six ACH.30.1-inoculated rabbits. Quantitative competitive PCR indicated higher PBMC proviral loads in ACH.1-inoculated rabbits. Interestingly, sequencing of ORF II from PBMC of provirus-

positive ACH.30.1-inoculated rabbits revealed a reversion to wild-type sequence with evidence of early coexistence of mutant and wild-type sequence. Our data provide evidence that HTLV-1 must maintain its key accessory genes to survive in vivo and that in vivo pressures select for maintenance of wild-type ORF II gene products during the early course of infection.

Silvestri, G., A. Fedanov, et al. (2005). "Divergent Host Responses during Primary Simian Immunodeficiency Virus SIVsm Infection of Natural Sooty Mangabey and Nonnatural Rhesus Macaque Hosts." *J. Virol.* **79**(7): 4043-4054.

<http://jvi.asm.org/cgi/content/abstract/79/7/4043>

To understand how natural sooty mangabey hosts avoid AIDS despite high levels of simian immunodeficiency virus (SIV) SIVsm replication, we inoculated mangabeys and nonnatural rhesus macaque hosts with an identical inoculum of uncloned SIVsm. The unpassaged virus established infection with high-level viral replication in both macaques and mangabeys. A species-specific, divergent immune response to SIV was evident from the first days of infection and maintained in the chronic phase, with macaques showing immediate and persistent T-cell proliferation, whereas mangabeys displayed little T-cell proliferation, suggesting subdued cellular immune responses to SIV. Importantly, only macaques developed CD4+-T-cell depletion and AIDS, thus indicating that in mangabeys limited immune activation is a key mechanism to avoid immunodeficiency despite high levels of SIVsm replication. These studies demonstrate that it is the host response to infection, rather than properties inherent to the virus itself, that causes immunodeficiency in SIV-infected nonhuman primates.

Sitki-Green, D., M. Covington, et al. (2003). "Compartmentalization and Transmission of Multiple Epstein-Barr Virus Strains in Asymptomatic Carriers." *J. Virol.* **77**(3): 1840-1847.

<http://jvi.asm.org/cgi/content/abstract/77/3/1840>

Infection with the Epstein-Barr virus (EBV) is often subclinical in the presence of a healthy immune response; thus, asymptomatic infection is largely uncharacterized. This study analyzed the nature of EBV infection in 20 asymptomatic immunocompetent hosts over time through the identification of EBV strain variants in the peripheral blood and oral cavity. A heteroduplex tracking assay specific for the EBV gene LMP1 precisely identified the presence of multiple EBV strains in each subject. The strains present in the peripheral blood and oral cavity were often completely discordant, indicating the existence of distinct infections, and the strains present and their relative abundance changed considerably between time points. The possible transmission of strains between the oral cavity and peripheral blood compartments could be tracked within subjects, suggesting that reactivation in the oral cavity and subsequent reinfection of B lymphocytes that reenter the periphery contribute to the maintenance of persistence. In addition, distinct virus strains persisted in the oral cavity over many time points, suggesting an important role for epithelial cells in the maintenance of persistence. Asymptomatic individuals without tonsillar tissue, which is believed to be an important source of virus for the oral cavity, also exhibited multiple strains and a cyclic pattern of transmission between compartments. This study revealed that the majority of patients with infectious mononucleosis were infected with multiple strains of EBV that were also compartmentalized, suggesting that primary infection involves the transmission of multiple strains. Both the primary and carrier states of infection with EBV are more complex than previously thought.

Sitki-Green, D., R. H. Edwards, et al. (2002). "Identification of Epstein-Barr Virus Strain Variants in Hairy Leukoplakia and Peripheral Blood by Use of a Heteroduplex Tracking Assay." J. Virol. **76**(19): 9645-9656.

<http://jvi.asm.org/cgi/content/abstract/76/19/9645>

Epstein-Barr virus (EBV) strains can be distinguished by specific sequence variations in the LMP1 gene. In this study, a heteroduplex tracking assay (HTA) specific for LMP1 was developed to precisely identify the prototypic undeleted strain B958, other undeleted strains (Ch2, AL, NC, and Med-), and strains with the 30-bp deletion (Med+ and Ch1). This technique also provides an estimate of the relative abundance of strains in patient samples. In this study, EBV strains were identified in 25 hairy leukoplakia (HLP) biopsies and six matched peripheral blood samples and throat washes with the LMP1-HTA. To investigate the relationship of the virus found in the peripheral blood to that in the HLP lesion, the strain variants in the peripheral blood B lymphocytes and those present within the epithelial cells in the HLP lesion and in throat washes were identified. In many of the subjects, compartmental differences in the EBV strain profiles in the oral cavity and peripheral blood were readily apparent. The throat wash specimens usually had a strain profile similar to that within the corresponding HLP sample, which was distinct from the strain profile detected in the peripheral blood. These analyses reveal that the nature of EBV infection can be very dynamic, with changes in relative strain abundance over time as well as the appearance of new strains. The patterns of abundance in the blood and oral cavity provide evidence for compartmentalization and for the transmission of strains between the blood and oropharynx.

Smith, M. Z., C. J. Dale, et al. (2005). "Analysis of Pigtail Macaque Major Histocompatibility Complex Class I Molecules Presenting Immunodominant Simian Immunodeficiency Virus Epitopes." J. Virol. **79**(2): 684-695.

<http://jvi.asm.org/cgi/content/abstract/79/2/684>

Successful human immunodeficiency virus (HIV) vaccines will need to induce effective T-cell immunity. We studied immunodominant simian immunodeficiency virus (SIV) Gag-specific T-cell responses and their restricting major histocompatibility complex (MHC) class I alleles in pigtail macaques (*Macaca nemestrina*), an increasingly common primate model for the study of HIV infection of humans. CD8⁺ T-cell responses to an SIV epitope, Gag164-172KP9, were present in at least 15 of 36 outbred pigtail macaques. The immunodominant KP9-specific response accounted for the majority (mean, 63%) of the SIV Gag response. Sequencing from six macaques identified 7 new Mane-A and 13 new Mane-B MHC class I alleles. One new allele, Mane-A*10, was common to four macaques that responded to the KP9 epitope. We adapted reference strand-mediated conformational analysis (RSCA) to MHC class I genotype *M. nemestrina*. Mane-A*10 was detected in macaques presenting KP9 studied by RSCA but was absent from non-KP9-presenting macaques. Expressed on class I-deficient cells, Mane-A*10, but not other pigtail macaque MHC class I molecules, efficiently presented KP9 to responder T cells, confirming that Mane-A*10 restricts the KP9 epitope. Importantly, naive pigtail macaques infected with SIVmac251 that respond to KP9 had significantly reduced plasma SIV viral levels (log₁₀ 0.87 copies/ml; *P* = 0.025) compared to those of macaques not responding to KP9. The identification of this common *M. nemestrina* MHC class I allele restricting a functionally important immunodominant SIV Gag epitope establishes a basis for studying CD8⁺ T-cell responses against AIDS in an important, widely available nonhuman primate species.

Stadnick, E., M. Dan, et al. (2004). "Attenuating Mutations in Coxsackievirus B3 Map to a Conformational Epitope That Comprises the Puff Region of VP2 and the Knob of VP3." J. Virol. **78**(24): 13987-

14002.

<http://jvi.asm.org/cgi/content/abstract/78/24/13987>

Ten antibody escape mutants of coxsackievirus B3 (CVB3) were used to identify nucleotide substitutions that determine viral virulence for the heart and pancreas. The P1 region, encoding the structural genes of each mutant, was sequenced to identify mutations associated with the lack of neutralization. Eight mutants were found to have a lysine-to arginine mutation in the puff region of VP2, while two had a glutamate-to-glycine substitution in the knob of VP3. Two mutants, EM1 and EM10, representing each of these mutations, were further analyzed, initially by determining their entire sequence. In addition to the mutations in P1, EM1 was found to have two mutations in the 3D polymerase, while EM10 had a mutation in stem-loop II of the 5' nontranslated region (5'NTR). The pathogenesis of the mutants relative to that of CVB3 strain RK [CVB3(RK)] then was examined in A/J mice. Both mutants were found to be less cardiotropic than the parental strain, with a 40-fold (EM1) or a 100- to 1,000-fold (EM10) reduction in viral titers in the heart relative to the titers of CVB3(RK). The mutations in VP2, VP3, and the 5'NTR were introduced independently into the RK infectious clone, and the phenotypes of the progeny viruses were determined. The results substantiated that the VP2 and VP3 mutations reduced cardiovirulence, while the 5'NTR mutation in EM10 was associated with a more virulent phenotype when expressed on its own. Stereographic imaging of the two mutations in the capsomer showed that they lie in close proximity on either side of a narrow cleft between the puff and the knob, forming a conformational epitope that is part of the putative binding site for coreceptor DAF.

Stonebraker, J. R., D. Wagner, et al. (2004). "Glycocalyx Restricts Adenoviral Vector Access to Apical Receptors Expressed on Respiratory Epithelium In Vitro and In Vivo: Role for Tethered Mucins as Barriers to Luminal Infection." J. Virol. **78**(24): 13755-13768.

<http://jvi.asm.org/cgi/content/abstract/78/24/13755>

Inefficient adenoviral vector (AdV)-mediated gene transfer to the ciliated respiratory epithelium has hindered gene transfer strategies for the treatment of cystic fibrosis lung disease. In part, the inefficiency is due to an absence of the coxsackie B and adenovirus type 2 and 5 receptor (CAR) from the apical membranes of polarized epithelia. In this study, using an in vitro model of human ciliated airway epithelium, we show that providing a glycosylphosphatidylinositol (GPI)-linked AdV receptor (GPI-CAR) at the apical surface did not significantly improve AdV gene transfer efficiency because the luminal surface glycocalyx limited the access of AdV to apical GPI-CAR. The highly glycosylated tethered mucins were considered to be significant glycocalyx components that restricted AdV access because proteolytic digestion and inhibitors of O-linked glycosylation enhanced AdV gene transfer. To determine whether these in vitro observations are relevant to the in vivo situation, we generated transgenic mice expressing GPI-CAR at the surface of the airway epithelium, crossbred these mice with mice that were genetically devoid of tethered mucin type 1 (Muc1), and tested the efficiency of gene transfer to murine airways expressing apical GPI-human CAR (GPI-hCAR) in the presence and absence of Muc1. We determined that AdV gene transfer to the murine airway epithelium was inefficient even in GPI-hCAR transgenic mice but that the gene transfer efficiency improved in the absence of Muc1. However, the inability to achieve a high gene transfer efficiency, even in mice with a deletion of Muc1, suggested that other glycocalyx components, possibly other tethered mucin types, also provide a significant barrier to AdV interacting with the airway luminal surface.

Streblow, D. N., C. Kreklywich, et al. (2003). "Cytomegalovirus-Mediated Upregulation of Chemokine Expression Correlates with the Acceleration of Chronic Rejection in Rat Heart Transplants." J. Virol. **77**(3): 2182-2194.

<http://jvi.asm.org/cgi/content/abstract/77/3/2182>

Cytomegalovirus (CMV) infections have been shown to dramatically affect solid organ transplant graft survival in both human and animal models. Recently, it was demonstrated that rat CMV (RCMV) infection accelerates the development of transplant vascular sclerosis (TVS) in both rat heart and small bowel graft transplants. However, the mechanisms involved in this process are still unclear. In the present study, we determined the kinetics of RCMV-accelerated TVS in a rat heart transplant model. Acute RCMV infection enhances the development of TVS in rat heart allografts, and this process is initiated between 21 and 24 days posttransplantation. The virus is consistently detected in the heart grafts from day 7 until day 35 posttransplantation but is rarely found at the time of graft rejection (day 45 posttransplantation). Grafts from RCMV-infected recipients had upregulation of chemokine expression compared to uninfected controls, and the timing of this increased expression paralleled that of RCMV-accelerated neointimal formation. In addition, graft vessels from RCMV-infected grafts demonstrate the increased infiltration of T cells and macrophages during periods of highest chemokine expression. These results suggest that CMV-induced acceleration of TVS involves the increased graft vascular infiltration of inflammatory cells through enhanced chemokine expression.

Sung, V. M. H., S. Shimodaira, et al. (2003). "Establishment of B-Cell Lymphoma Cell Lines Persistently Infected with Hepatitis C Virus In Vivo and In Vitro: the Apoptotic Effects of Virus Infection." J. Virol. **77**(3): 2134-2146.

<http://jvi.asm.org/cgi/content/abstract/77/3/2134>

Hepatitis C virus (HCV) is a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Studies of HCV replication and pathogenesis have so far been hampered by the lack of an efficient tissue culture system for propagating HCV in vitro. Although HCV is primarily a hepatotropic virus, an increasing body of evidence suggests that HCV also replicates in extrahepatic tissues in natural infection. In this study, we established a B-cell line (SB) from an HCV-infected non-Hodgkin's B-cell lymphoma. HCV RNA and proteins were detectable by RNase protection assay and immunoblotting. The cell line continuously produces infectious HCV virions in culture. The virus particles produced from the culture had a buoyant density of 1.13 to 1.15 g/ml in sucrose and could infect primary human hepatocytes, peripheral blood mononuclear cells (PBMCs), and an established B-cell line (Raji cells) in vitro. The virus from SB cells belongs to genotype 2b. Single-stranded conformational polymorphism and sequence analysis of the viral RNA quasispecies indicated that the virus present in SB cells most likely originated from the patient's spleen and had an HCV RNA quasispecies pattern distinct from that in the serum. The virus production from the infected primary hepatocytes showed cyclic variations. In addition, we have succeeded in establishing several Epstein-Barr virus-immortalized B-cell lines from PBMCs of HCV-positive patients. Two of these cell lines are positive for HCV RNA as detected by reverse transcriptase PCR and for the nonstructural protein NS3 by immunofluorescence staining. These observations unequivocally establish that HCV infects B cells in vivo and in vitro. HCV-infected cell lines show significantly enhanced apoptosis. These B-cell lines provide a reproducible cell culture system for studying the complete replication cycle and biology of HCV infections.

Suzuki, N. and D. L. Nuss (2002). "Contribution of Protein p40 to Hypovirus-Mediated Modulation of Fungal Host Phenotype and Viral RNA Accumulation." J. Virol. **76**(15): 7747-7759.

<http://jvi.asm.org/cgi/content/abstract/76/15/7747>

The papain-like protease p29, derived from the N-terminal portion of the hypovirus CHV1-EP713-

encoded open reading frame (ORF) A polyprotein, p69, was previously shown to contribute to reduced pigmentation and sporulation by the infected host, the chestnut blight fungus *Cryphonectria parasitica*, while being dispensable for virus replication and attenuation of fungal virulence (hypovirulence). We now report that deletion of the C-terminal portion of p69, which encodes the highly basic protein p40, resulted in replication-competent mutant viruses that were, however, significantly reduced in RNA accumulation. While the Δ p40 mutants retained the ability to confer hypovirulence, Δ p40-infected fungal strains produced more asexual spores than strains infected with either wild-type CHV1-EP713 or a Δ p29 mutant virus. As observed for Δ p29-infected colonies, pigment production was significantly increased in Δ p40-infected fungal strains relative to that in CHV1-EP713-infected strains. Virus-mediated suppression of laccase production was not affected by p40 deletion. A gain-of-function analysis was employed to map the p40 symptom determinant to the N-terminal domain, encompassing p69 amino acid residues Thr(288) to Arg(312). Evidence that the gain of function was due to the encoded protein rather than the corresponding RNA sequence element was provided by introducing frameshift mutations on either side of the activity determinant domain. Moreover, restoration of symptoms correlated with increased accumulation of viral RNA. These results suggest that p40 indirectly contributes to virus-mediated suppression of fungal pigmentation and conidiation by providing an accessory function in hypovirus RNA amplification. A possible role for p40 in facilitating ORF B expression and the relationship between hypovirus RNA accumulation and symptom expression are discussed.

Taddeo, B., A. Esclatine, et al. (2003). "The Stress-Inducible Immediate-Early Responsive Gene IEX-1 Is Activated in Cells Infected with Herpes Simplex Virus 1, but Several Viral Mechanisms, Including 3' Degradation of Its RNA, Preclude Expression of the Gene." *J. Virol.* **77**(11): 6178-6187.

<http://jvi.asm.org/cgi/content/abstract/77/11/6178>

The accumulation of cellular transcripts from cells infected with herpes simplex virus 1 (HSV-1) as measured with the aid of Affymetrix microchips has been reported elsewhere. Among these transcripts were genes that respond to stress and that could have a noxious effect on viral replication. We have selected the stress-inducible cellular gene encoding the immediate-early response protein IEX-1 to verify and determine the significance of the accumulation of these transcripts in infected cells. We report that we verified the increase in accumulation of IEX-1 transcripts after infection by Northern analyses and real-time PCR. These transcripts reach peak levels between 3 and 7 h after infection and decrease thereafter. However, IEX-1 protein was detected in cells 1 h after infection but not at later intervals. Studies designed to elucidate the failure of IEX-1 protein to be synthesized revealed the following points. (i) IEX-1 RNA transported to the cytoplasm after 1 h of infection consisted of at least two populations, a partially degraded population and a population consisting of unspliced IEX-1 RNA. Neither of these RNAs could translate the authentic IEX-1 protein. (ii) The partially degraded IEX-1 RNA was not detected in the cytoplasm of cells infected with a mutant virus lacking the UL41 gene encoding the virion host shutoff protein (vhs). Although degradation of RNA mediated by vhs was reported to be 5' to 3', the partially degraded IEX-1 RNA lacked the 3' sequences rather than the 5' sequences. (iii) The unspliced pre-RNA form containing the IEX-1 intron sequences was detected in the cytoplasm of cell infected with wild-type virus but not in those infected with a mutant lacking the α 27 gene encoding the infected cell protein No. 27. (iv) Overexpression of IEX-1 protein by transduction of the gene prior to infection with 1 PFU of HSV-1 per cell had no effect on the accumulation of late genes and virus yield. We conclude that the failure of IEX-1 to express its protein reflects the numerous mechanisms by which the virus thwarts the cells from expressing its genes after infection.

Taddeo, B., W. Zhang, et al. (2004). "Cells Lacking NF- κ B or in Which NF- κ B Is Not

Activated Vary with Respect to Ability To Sustain Herpes Simplex Virus 1 Replication and Are Not Susceptible to Apoptosis Induced by a Replication-Incompetent Mutant Virus." *J. Virol.* **78**(21): 11615-11621.

<http://jvi.asm.org/cgi/content/abstract/78/21/11615>

Earlier we reported that NF- κ B is activated by protein kinase R (PKR) in herpes simplex virus 1-infected cells. Here we report that in PKR $^{-/-}$ cells the yields of wild-type virus are 10-fold higher than in PKR $^{+/+}$ cells. In cells lacking NF- κ B p50 (nfb1), p65 (relA), or both p50 and p65, the yields of virus were reduced 10-fold. Neither wild-type nor mutant cells undergo apoptosis following infection with wild-type virus. Whereas PKR $^{+/+}$ and NF- κ B $^{+/+}$ control cell lines undergo apoptosis induced by the d120 ($\Delta\alpha$ 4) mutant of HSV-1, the mutant PKR $^{-/-}$ and NF- κ B $^{-/-}$ cell lines were resistant. The evidence suggests that the stress-induced apoptosis resulting from d120 infection requires activation of NF- κ B and that this proapoptotic pathway is blocked in cells in which NF- κ B is not activated or absent. Activation of NF- κ B in the course of viral infection may have dual roles of attempting to curtail viral replication by rendering the cell susceptible to apoptosis induced by the virus and by inducing the synthesis of proteins that enhance viral replication.

Tam, P. E., M. L. Weber-Sanders, et al. (2003). "Multiple Viral Determinants Mediate Myopathogenicity in Coxsackievirus B1-Induced Chronic Inflammatory Myopathy." *J. Virol.* **77**(21): 11849-11854.

<http://jvi.asm.org/cgi/content/abstract/77/21/11849>

Mice infected with myopathic coxsackievirus B1 Tucson (CVB1T) develop chronic inflammatory myopathy (CIM) consisting of hind limb weakness and inflammation. Amyopathic virus variants are infectious but attenuated for CIM. In this report, viral clones, chimeras, and sequencing were used to identify viral determinants of CIM. Chimeras identified several regions involved in CIM and localized a weakness determinant to nucleotides 2493 to 3200 of VP1. Sequencing of multiple clones and viruses identified five candidate determinants that were strictly conserved in myopathic viruses with one located in the 5' untranslated region (UTR), three in the VP1 capsid, and one in the 3C protease. Taken together, these studies implicate Tyr-87 and/or Val-136 as candidate determinants of weakness. They also indicate that there are at least two determinants of inflammation and one additional determinant of weakness encoded by myopathic CVB1T.

Tang, Y., F. Villinger, et al. (2002). "Slowly Declining Levels of Viral RNA and DNA in DNA/Recombinant Modified Vaccinia Virus Ankara-Vaccinated Macaques with Controlled Simian-Human Immunodeficiency Virus SHIV-89.6P Challenges." *J. Virol.* **76**(20): 10147-10154.

<http://jvi.asm.org/cgi/content/abstract/76/20/10147>

In a recent vaccine trial, we showed efficient control of a virulent simian-human immunodeficiency virus SHIV-89.6P challenge by priming with a Gag-Pol-Env-expressing DNA and boosting with a Gag-Pol-Env-expressing recombinant-modified vaccinia virus Ankara. Here we show that long-term control has been associated with slowly declining levels of viral RNA and DNA. In the vaccinated animals both viral DNA and RNA underwent an initial rapid decay, which was followed by a lower decay rate. Between 12 and 70 weeks postchallenge, the low decay rates have had half-lives of about 20 weeks for viral RNA in plasma and viral DNA in peripheral blood mononuclear cells and lymph nodes. In vaccinated animals the viral DNA has been mostly unintegrated and has appeared to be largely nonfunctional as evidenced by a poor ability to recover infectious virus in cocultivation assays, even after CD8 depletion. In contrast, in control

animals, which have died, viral DNA was mostly integrated and a larger proportion appeared to be functional as evidenced by the recovery of infectious virus. Thus, to date, control of the challenge infection has appeared to improve with time, with the decay rates for viral DNA being at the lower end of values reported for patients on highly active antiretroviral therapy.

Thomsen, D. R., N. L. Oien, et al. (2003). "Amino Acid Changes within Conserved Region III of the Herpes Simplex Virus and Human Cytomegalovirus DNA Polymerases Confer Resistance to 4-Oxo-Dihydroquinolines, a Novel Class of Herpesvirus Antiviral Agents." *J. Virol.* **77**(3): 1868-1876.

<http://jvi.asm.org/cgi/content/abstract/77/3/1868>

The 4-oxo-dihydroquinolines (PNU-182171 and PNU-183792) are nonnucleoside inhibitors of herpesvirus polymerases (R. J. Brideau et al., *Antiviral Res.* 54:19-28, 2002; N. L. Oien et al., *Antimicrob. Agents Chemother.* 46:724-730, 2002). In cell culture these compounds inhibit herpes simplex virus type 1 (HSV-1), HSV-2, human cytomegalovirus (HCMV), varicella-zoster virus (VZV), and human herpesvirus 8 (HHV-8) replication. HSV-1 and HSV-2 mutants resistant to these drugs were isolated and the resistance mutation was mapped to the DNA polymerase gene. Drug resistance correlated with a point mutation in conserved domain III that resulted in a V823A change in the HSV-1 or the equivalent amino acid in the HSV-2 DNA polymerase. Resistance of HCMV was also found to correlate with amino acid changes in conserved domain III (V823A+V824L). V823 is conserved in the DNA polymerases of six (HSV-1, HSV-2, HCMV, VZV, Epstein-Barr virus, and HHV-8) of the eight human herpesviruses; the HHV-6 and HHV-7 polymerases contain an alanine at this amino acid. In vitro polymerase assays demonstrated that HSV-1, HSV-2, HCMV, VZV, and HHV-8 polymerases were inhibited by PNU-183792, whereas the HHV-6 polymerase was not. Changing this amino acid from valine to alanine in the HSV-1, HCMV, and HHV-8 polymerases alters the polymerase activity so that it is less sensitive to drug inhibition. In contrast, changing the equivalent amino acid in the HHV-6 polymerase from alanine to valine alters polymerase activity so that PNU-183792 inhibits this enzyme. The HSV-1, HSV-2, and HCMV drug-resistant mutants were not altered in their susceptibilities to nucleoside analogs; in fact, some of the mutants were hypersensitive to several of the drugs. These results support a mechanism where PNU-183792 inhibits herpesviruses by interacting with a binding determinant on the viral DNA polymerase that is less important for the binding of nucleoside analogs and deoxynucleoside triphosphates.

Towner, J. S., P. E. Rollin, et al. (2004). "Rapid Diagnosis of Ebola Hemorrhagic Fever by Reverse Transcription-PCR in an Outbreak Setting and Assessment of Patient Viral Load as a Predictor of Outcome." *J. Virol.* **78**(8): 4330-4341.

<http://jvi.asm.org/cgi/content/abstract/78/8/4330>

The largest outbreak on record of Ebola hemorrhagic fever (EHF) occurred in Uganda from August 2000 to January 2001. The outbreak was centered in the Gulu district of northern Uganda, with secondary transmission to other districts. After the initial diagnosis of Sudan ebolavirus by the National Institute for Virology in Johannesburg, South Africa, a temporary diagnostic laboratory was established within the Gulu district at St. Mary's Lacor Hospital. The laboratory used antigen capture and reverse transcription-PCR (RT-PCR) to diagnose Sudan ebolavirus infection in suspect patients. The RT-PCR and antigen-capture diagnostic assays proved very effective for detecting ebolavirus in patient serum, plasma, and whole blood. In samples collected very early in the course of infection, the RT-PCR assay could detect ebolavirus 24 to 48 h prior to detection by antigen capture. More than 1,000 blood samples were collected, with multiple samples obtained from many patients throughout the course of infection. Real-time quantitative

RT-PCR was used to determine the viral load in multiple samples from patients with fatal and nonfatal cases, and these data were correlated with the disease outcome. RNA copy levels in patients who died averaged 2 log₁₀ higher than those in patients who survived. Using clinical material from multiple EHF patients, we sequenced the variable region of the glycoprotein. This Sudan ebolavirus strain was not derived from either the earlier Boniface (1976) or Maleo (1979) strain, but it shares a common ancestor with both. Furthermore, both sequence and epidemiologic data are consistent with the outbreak having originated from a single introduction into the human population.

Traina-Dorge, V. L., R. Lorino, et al. (2005). "Molecular Epidemiology of Simian T-Cell Lymphotropic Virus Type 1 in Wild and Captive Sooty Mangabeys." *J. Virol.* **79**(4): 2541-2548.

<http://jvi.asm.org/cgi/content/abstract/79/4/2541>

A study was conducted to evaluate the prevalence and diversity of simian T-cell lymphotropic virus (STLV) isolates within the long-established Tulane National Primate Research Center (TNPRC) colony of sooty mangabeys (SMs; *Cercocebus atys*). Serological analysis determined that 22 of 39 animals (56%) were positive for STLV type 1 (STLV-1). A second group of thirteen SM bush meat samples from Sierra Leone in Africa was also included and tested only by PCR. Twenty-two of 39 captive animals (56%) and 3 of 13 bush meat samples (23%) were positive for STLV-1, as shown by testing with PCR. Nucleotide sequencing and phylogenetic analysis of viral strains obtained demonstrated that STLV-1 strains from SMs (STLV-1sm strains) from the TNPRC colony and Sierra Leone formed a single cluster together with the previously reported STLV-1sm strain from the Yerkes National Primate Research Center. These data confirm that Africa is the origin for TNPRC STLV-1sm and suggest that Sierra Leone is the origin for the SM colonies in the United States. The TNPRC STLV-1sm strains further divided into two subclusters, suggesting STLV-1sm infection of two original founder SMs at the time of their importation into the United States. STLV-1sm diversity in the TNPRC colony matches the high diversity of SIVsm in the already reported colony. The lack of correlation between the lineage of the simian immunodeficiency virus from SMs (SIVsm) and the STLV-1sm subcluster distribution of the TNPRC strains suggests that intracolony transmissions of both viruses were independent events.

Troyer, J. L., J. Pecon-Slaterry, et al. (2004). "Patterns of Feline Immunodeficiency Virus Multiple Infection and Genome Divergence in a Free-Ranging Population of African Lions." *J. Virol.* **78**(7): 3777-3791.

<http://jvi.asm.org/cgi/content/abstract/78/7/3777>

Feline immunodeficiency virus (FIV) is a lentivirus that causes AIDS-like immunodeficiency disease in domestic cats. Free-ranging lions, *Panthera leo*, carry a chronic species-specific strain of FIV, FIV-Ple, which so far has not been convincingly connected with immune pathology or mortality. FIV-Ple, harboring the three distinct strains A, B, and C defined by pol gene sequence divergences, is endemic in the large outbred population of lions in the Serengeti ecosystem in Tanzania. Here we describe the pattern of variation in the three FIV genes gag, pol-RT, and pol-RNase among lions within 13 prides to assess the occurrence of FIV infection and coinfection. Genome diversity within and among FIV-Ple strains is shown to be large, with strain divergence for each gene approaching genetic distances observed for FIV between different species of cats. Multiple infections with two or three strains were found in 43% of the FIV-positive individuals based on pol-RT sequence analysis, which may suggest that antiviral immunity or interference evoked by one strain is not consistently protective against infection by a second. This comprehensive study of FIV-Ple in a free-ranging population of lions reveals a dynamic transmission of virus in a social species that has historically adapted to render the virus benign.

Tsai, C.-T., J.-W. Ting, et al. (2005). "Complete Genome Sequence of the Grouper Iridovirus and Comparison of Genomic Organization with Those of Other Iridoviruses." J. Virol. **79**(4): 2010-2023.

<http://jvi.asm.org/cgi/content/abstract/79/4/2010>

The complete DNA sequence of grouper iridovirus (GIV) was determined using a whole-genome shotgun approach on virion DNA. The circular form genome was 139,793 bp in length with a 49% G+C content. It contained 120 predicted open reading frames (ORFs) with coding capacities ranging from 62 to 1,268 amino acids. A total of 21% (25 of 120) of GIV ORFs are conserved in the other five sequenced iridovirus genomes, including DNA replication, transcription, nucleotide metabolism, protein modification, viral structure, and virus-host interaction genes. The whole-genome nucleotide pairwise comparison showed that GIV virus was partially colinear with counterparts of previously sequenced ranaviruses (ATV and TFV). Besides, sequence analysis revealed that GIV possesses several unique features which are different from those of other complete sequenced iridovirus genomes: (i) GIV is the first ranavirus-like virus which has been sequenced completely and which infects fish other than amphibians, (ii) GIV is the only vertebrate iridovirus without CpG sequence methylation and lacking DNA methyltransferase, (iii) GIV contains a purine nucleoside phosphorylase gene which is not found in other iridoviruses or in any other viruses, (iv) GIV contains 17 sets of repeat sequence, with basic unit sizes ranging from 9 to 63 bp, dispersed throughout the whole genome. These distinctive features of GIV further extend our understanding of molecular events taking place between ranavirus and its hosts and the iridovirus evolution.

Waris, G., J. Turkson, et al. (2005). "Hepatitis C Virus (HCV) Constitutively Activates STAT-3 via Oxidative Stress: Role of STAT-3 in HCV Replication." J. Virol. **79**(3): 1569-1580.

<http://jvi.asm.org/cgi/content/abstract/79/3/1569>

The hepatitis C virus (HCV) causes chronic hepatitis, which often results in liver cirrhosis and hepatocellular carcinoma. We have previously shown that HCV nonstructural proteins induce activation of STAT-3 via oxidative stress and Ca²⁺ signaling (G. Gong, G. Waris, R. Tanveer, and A. Siddiqui, Proc. Natl. Acad. Sci. USA 98:9599-9604, 2001). In this study, we focus on the signaling pathway leading to STAT-3 activation in response to oxidative stress induced by HCV translation and replication activities. Here, we demonstrate the constitutive activation of STAT-3 in HCV replicon-expressing cells. The HCV-induced STAT-3 activation was inhibited in the presence of antioxidant (pyrrolidine dithiocarbamate) and Ca²⁺ chelators (BAPTA-AM and TMB-8). Previous studies have shown that maximum STAT-3 transactivation requires Ser727 phosphorylation in addition to tyrosine phosphorylation. Using a series of inhibitors and dominant negative mutants, we show that HCV-induced activation of STAT-3 is mediated by oxidative stress and influenced by the activation of cellular kinases, including p38 mitogen-activated protein kinase, JNK, JAK-2, and Src. Our results also suggest a potential role of STAT-3 in HCV RNA replication. We also observed the constitutive activation of STAT-3 in the liver biopsy of an HCV-infected patient. These studies provide an insight into the mechanisms by which HCV induces intracellular events relevant to liver pathogenesis associated with the viral infection.

Warke, R. V., K. Khaja, et al. (2003). "Dengue Virus Induces Novel Changes in Gene Expression of Human Umbilical Vein Endothelial Cells." J. Virol. **77**(21): 11822-11832.

<http://jvi.asm.org/cgi/content/abstract/77/21/11822>

Endothelial cells are permissive to dengue virus (DV) infection in vitro, although their importance as targets of DV infection in vivo remains a subject of debate. To analyze the virus-host interaction, we studied the effect of DV infection on gene expression in human umbilical vein endothelial cells (HUVECs) by using differential display reverse transcription-PCR (DD-RT-PCR), quantitative RT-PCR, and Affymetrix oligonucleotide microarrays. DD identified eight differentially expressed cDNAs, including inhibitor of apoptosis-1, 2'-5' oligoadenylate synthetase (OAS), a 2'-5' OAS-like (OASL) gene, galectin-9, myxovirus protein A (MxA), regulator of G-protein signaling, endothelial and smooth muscle cell-derived neuropilin-like protein, and phospholipid scramblase 1. Microarray analysis of 22,000 human genes confirmed these findings and identified an additional 269 genes that were induced and 126 that were repressed more than fourfold after DV infection. Broad functional responses that were activated included the stress, defense, immune, cell adhesion, wounding, inflammatory, and antiviral pathways. These changes in gene expression were seen after infection of HUVECs with either laboratory-adapted virus or with virus isolated directly from plasma of DV-infected patients. Tumor necrosis factor alpha, OASL, and MxA and h-IAP1 genes were induced within the first 8 to 12 h after infection, suggesting a direct effect of DV infection. These global analyses of DV effects on cellular gene expression identify potentially novel mechanisms involved in dengue disease manifestations such as hemostatic disturbance.

Widjaja, L., S. L. Krauss, et al. (2004). "Matrix Gene of Influenza A Viruses Isolated from Wild Aquatic Birds: Ecology and Emergence of Influenza A Viruses." *J. Virol.* **78**(16): 8771-8779.

<http://jvi.asm.org/cgi/content/abstract/78/16/8771>

Wild aquatic birds are the primary reservoir of influenza A viruses, but little is known about the viruses' gene pool in wild birds. Therefore, we investigated the ecology and emergence of influenza viruses by conducting phylogenetic analysis of 70 matrix (M) genes of influenza viruses isolated from shorebirds and gulls in the Delaware Bay region and from ducks in Alberta, Canada, during >18 years of surveillance. In our analysis, we included 61 published M genes of isolates from various hosts. We showed that M genes of Canadian duck viruses and those of shorebird and gull viruses in the Delaware Bay shared ancestors with the M genes of North American poultry viruses. We found that North American and Eurasian avian-like lineages are divided into sublineages, indicating that multiple branches of virus evolution may be maintained in wild aquatic birds. The presence of non-H13 gull viruses in the gull-like lineage and of H13 gull viruses in other avian lineages suggested that gulls' M genes do not preferentially associate with the H13 subtype or segregate into a distinct lineage. Some North American avian influenza viruses contained M genes closely related to those of Eurasian avian viruses. Therefore, there may be interregional mixing of the two clades. Reassortment of shorebird M and HA genes was evident, but there was no correlation among the HA or NA subtype, M gene sequence, and isolation time. Overall, these results support the hypothesis that influenza viruses in wild waterfowl contain distinguishable lineages of M genes.

Wilkinson, J., A. Cope, et al. (2002). "Identification of Kaposi's Sarcoma-Associated Herpesvirus (KSHV)-Specific Cytotoxic T-Lymphocyte Epitopes and Evaluation of Reconstitution of KSHV-Specific Responses in Human Immunodeficiency Virus Type 1-Infected Patients Receiving Highly Active Antiretroviral Therapy." *J. Virol.* **76**(6): 2634-2640.

<http://jvi.asm.org/cgi/content/abstract/76/6/2634>

Following the introduction of highly active antiretroviral therapy (HAART), the incidence of Kaposi's sarcoma (KS) has significantly declined in human immunodeficiency virus type 1 (HIV-1)-positive (HIV-1+) individuals and clinical remission is often observed. We hypothesize that these effects are partly due to anti-KS-associated herpesvirus (KSHV) immune restoration. Here, 15-mer overlapping peptides from proteins K12 and K8.1 were used to identify novel KSHV-specific cytotoxic T-lymphocyte epitopes. Three immunogenic peptides, two lytic and one latent, were subsequently used to monitor the anti-KSHV CD8+ T-cell responses in a cohort of 19 HIV-1+ KSHV+/- KS+/- individuals during 52 weeks of HAART. KSHV and HIV-1 loads, KSHV antibody titers, and both CD4+ and CD8+ T-lymphocyte counts were enumerated. Prior to HAART, the total number of spot-forming cells (SFC) for all three peptides correlated with both CD4+ and CD8+ T-lymphocyte counts ($P \leq 0.05$) in the KSHV-positive KS-positive cohort ($n = 11$). Following 52 weeks of HAART, significant decreases in HIV-1 and KSHV loads were associated with significant increases in CD4+ T-lymphocyte counts and number of SFC for the three KSHV-specific peptides. Although these increases were modest in comparison to the number of SFC observed with the HIV-1 gag peptide SLYNTVATL, they represented a fourfold increase from the baseline, continuing an upward trend to week 52.

Wilkinson, R. C., C. K. Murrell, et al. (2003). "Persistence and Dissemination of Simian Retrovirus Type 2 DNA in Relation to Viremia, Seroreponse, and Experimental Transmissibility in *Macaca fascicularis*." *J. Virol.* **77**(20): 10751-10759.

<http://jvi.asm.org/cgi/content/abstract/77/20/10751>

Endemic simian retrovirus (SRV) infection can cause fatal simian AIDS in *Macaca fascicularis*, but many individuals survive with few clinical signs. To further clarify the parameters of SRV pathogenesis, we investigated the persistence of viral DNA forms in relation to active viremia, antibody response, and transmissibility of infection. In *M. fascicularis* from endemically SRV-2-infected colonies, viral DNA was present in both linear and unintegrated long terminal repeat circular forms in peripheral blood mononuclear cells of all viremic and many nonviremic animals. Long-term followup of three individuals with distinct infection patterns demonstrated persistence of linear and circular forms of viral DNA in peripheral blood mononuclear cells and tissues, irrespective of viremia or antibody status, but reactivation of latent infections was not observed. The role of viral DNA in transmission and early pathogenesis of SRV-2 was investigated by inoculation of SRV-2 DNA-positive blood into groups of naive *M. fascicularis* from either a viremic or nonviremic donor and subsequent analysis of the virological and serological status of the recipients. Transmission of SRV and development of anti-SRV antibodies were only observed in recipients of blood from the viremic donor; transfer of SRV provirus and unintegrated circular DNA in blood from the nonviremic donor did not lead to infection of the recipients. These results indicate that a proportion of *M. fascicularis* are able to effectively control the replication and infectivity of SRV despite long-term persistence of viral DNA forms in infected lymphocytes.

Wu, E., L. Pache, et al. (2003). "Flexibility of the Adenovirus Fiber Is Required for Efficient Receptor Interaction." *J. Virol.* **77**(13): 7225-7235.

<http://jvi.asm.org/cgi/content/abstract/77/13/7225>

The adenovirus (Ad) fiber protein mediates Ad binding to the coxsackievirus and Ad receptor (CAR) and is thus a major determinant of viral tropism. The fiber contains three domains: an N-terminal tail that anchors the fiber to the viral capsid, a central shaft region of variable length and flexibility, and a C-terminal knob domain that binds to cell receptors. Ad type 37 (Ad37), a subgroup D virus associated with severe ocular infections, is unable to use CAR efficiently to infect host cells, despite containing a CAR binding site in its fiber knob. We hypothesized that the

relatively short, inflexible Ad37 fiber protein restricts interactions with CAR at the cell surface. To test this hypothesis, we analyzed the infectivity and binding of recombinant Ad particles containing modified Ad37 or Ad5 fiber proteins. Ad5 particles equipped with a truncated Ad5 fiber or with a chimeric fiber protein comprised of the Ad5 knob fused to the short, rigid Ad37 shaft domain had significantly reduced infectivity and attachment. In contrast, placing the Ad37 knob onto the long, flexible Ad5 shaft allowed CAR-dependent virus infection and cell attachment, demonstrating the importance of the shaft domain in receptor usage. Increasing fiber rigidity by substituting the predicted flexibility modules in the Ad5 shaft with the corresponding regions of the rigid Ad37 fiber dramatically reduced both virus infection and cell attachment. Cryoelectron microscopy (cryo-EM) single-particle analysis demonstrated the increased rigidity of this chimeric fiber. These studies demonstrate that both length and flexibility of the fiber shaft regulate CAR interaction and provide a molecular explanation for the use of alternative receptors by subgroup D Ad with ocular tropism. We present a molecular model for Ad-CAR interactions at the cell surface that explains the significance of fiber flexibility in cell attachment.

Wu, E., S. A. Trauger, et al. (2004). "Membrane Cofactor Protein Is a Receptor for Adenoviruses Associated with Epidemic Keratoconjunctivitis." J. Virol. **78**(8): 3897-3905.

<http://jvi.asm.org/cgi/content/abstract/78/8/3897>

Subgroup D adenovirus (Ad) types 8, 19, and 37 (Ad8, -19, and -37, respectively) are causative agents of epidemic keratoconjunctivitis and genital tract infections. Previous studies showed that Ad37 binds to a 50-kDa membrane glycoprotein expressed on human ocular (conjunctival) cells. To identify and characterize the role of the 50-kDa glycoprotein in Ad37 infection, we partially purified this molecule from solubilized Chang C conjunctival cell membranes by using lentil lectin chromatography and preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Liquid chromatography coupled to nano-electrospray ionization-tandem mass spectrometry was subsequently used to identify four Ad37 receptor candidates: CD46, CD87, CD98, and CD147. Immunodepletion analyses demonstrated that the 50-kDa protein is identical to CD46 (also known as membrane cofactor protein). The Ad37, but not Ad5, fiber knob bound to the extracellular domain of CD46, demonstrating a direct interaction of an Ad37 capsid protein with CD46. An antibody specific for the N-terminal 19 amino acids of CD46 also blocked Ad37 infection of human cervical carcinoma and conjunctival cells, indicating a requirement for CD46 in infection. Finally, expression of a 50-kDa isoform of human CD46 in a CD46-null cell line increased cell binding by wild-type Ad37 and gene delivery by an Ad vector pseudotyped with the Ad37 fiber, but not by a vector bearing the Ad5 fiber. Together, these studies demonstrate that CD46 serves as an attachment receptor for Ad37 and shed further light on the cell entry pathway of subgroup D Ads.

Xu, Y., D. P. AuCoin, et al. (2005). "A Kaposi's Sarcoma-Associated Herpesvirus/Human Herpesvirus 8 ORF50 Deletion Mutant Is Defective for Reactivation of Latent Virus and DNA Replication." J. Virol. **79**(6): 3479-3487.

<http://jvi.asm.org/cgi/content/abstract/79/6/3479>

Kaposi's sarcoma-associated herpesvirus (also called human herpesvirus type 8 [HHV8]) latently infects a number of cell types. Reactivation of latent virus can occur by treatment with the phorbol ester tetradecanoyl phorbol acetate (TPA) or with the transfection of plasmids expressing the lytic switch activator protein K-Rta, the gene product of ORF50. K-Rta expression is sufficient for the activation of the entire lytic cycle and the transactivation of viral genes necessary for DNA replication. In addition, recent evidence has suggested that K-Rta may participate directly in the initiation of lytic DNA synthesis. We have now generated a recombinant HHV8 bacterial artificial

chromosome (BAC) with a large deletion within the ORF50 locus. This BAC, BAC36{Delta}50, failed to produce infectious virus upon treatment with TPA and was defective for DNA synthesis. Expression of K-Rta in trans in BAC36{Delta}50-containing cells was able to abolish both defects. Real-time PCR revealed that K-bZIP, ORF40/41, and K8.1 were not expressed when BAC36{Delta}50-containing cells were induced with TPA. However, the mRNA levels of ORF57 were over fivefold higher in TPA-treated BAC36{Delta}50-containing cells than those observed in similarly treated wild-type BAC-containing cells. In addition, immunohistochemical analysis showed that while the latency-associated nuclear antigen (LANA) was expressed in the mutant BAC-containing cells, ORF59 and K8.1 expression was not detected in TPA-induced BAC36{Delta}50-containing cells. These results showed that K-Rta is essential for lytic viral reactivation and transactivation of viral genes contributing to DNA replication.

Yamamoto, T., S. Litwin, et al. (2002). "Mutations of the Woodchuck Hepatitis Virus Polymerase Gene That Confer Resistance to Lamivudine and 2'-Fluoro-5-Methyl-{beta}-L-Arabinofuranosyluracil." J. Virol. **76**(3): 1213-1223.

<http://jvi.asm.org/cgi/content/abstract/76/3/1213>

Administration of either lamivudine (2'-deoxy-3'-thiacytidine) or L-FMAU (2'-fluoro-5-methyl-{beta}-L-arabinofuranosyluracil) to woodchucks chronically infected with woodchuck hepatitis virus (WHV) induces a transient decline in virus titers. However, within 6 to 12 months, virus titers begin to increase towards pretreatment levels. This is associated with the emergence of virus strains with mutations of the B and C regions of the viral DNA polymerase (T. Zhou et al., *Antimicrob. Agents Chemother.* 43:1947-1954, 1999; Y. Zhu et al., *J. Virol.* 75:311-322, 2001). The present study was carried out to determine which of the mutants that we have identified conferred resistance to lamivudine and/or to L-FMAU. When inserted into a laboratory strain of WHV, each of the mutations, or combinations of mutations, of regions B and C produced a DNA replication-competent virus and typically conferred resistance to both nucleoside analogs in cell culture. Sequencing of the polymerase active site also occasionally revealed other mutations, but these did not appear to contribute to drug resistance. Moreover, in transfected cells, most of the mutants synthesized viral DNA nearly as efficiently as wild-type WHV. Computational models suggested that persistence of several of the WHV mutants as prevalent species in the serum and, by inference, liver for up to 6 months following drug withdrawal required a replication efficiency of at least 10 to 30% of that of the wild type. However, their delayed emergence during therapy suggested replication efficiency in the presence of the drug that was still well below that of wild-type WHV in the absence of the drug.

Yao, Z. Q., A. Eisen-Vandervelde, et al. (2004). "Direct Binding of Hepatitis C Virus Core to gC1qR on CD4+ and CD8+ T Cells Leads to Impaired Activation of Lck and Akt." J. Virol. **78**(12): 6409-6419.

<http://jvi.asm.org/cgi/content/abstract/78/12/6409>

Complement plays a pivotal role in the regulation of innate and adaptive immunity. It has been shown that the binding of C1q, a natural ligand of gC1qR, on T cells inhibits their proliferation. Here, we demonstrate that direct binding of the hepatitis C virus (HCV) core to gC1qR on T cells leads to impaired Lck/Akt activation and T-cell function. The HCV core associates with the surface of T cells specifically via gC1qR, as this binding is inhibited by the addition of either anti-gC1qR antibody or soluble gC1qR. The binding affinity constant of core protein for gC1qR, as determined by BIAcore analysis, is 3.8×10^{-7} M. The specificity of the HCV core-gC1qR interaction is confirmed by reduced core binding on Molt-4 T cells treated with gC1qR-silencing small interfering RNA and enhanced core binding on GPC-16 guinea pig cells transfected with

human gC1qR. Interestingly, gC1qR is expressed at higher levels on CD8+ than on CD4+ T cells, resulting in more severe core-induced suppression of the CD8+-T-cell population. Importantly, T-cell receptor-mediated activation of the Src kinases Lck and ZAP-70 but not Fyn and the phosphorylation of Akt are impaired by the HCV core, suggesting that it inhibits the very early events of T-cell activation.

Ye, R., C. Montalto-Morrison, et al. (2004). "Genetic Analysis of Determinants for Spike Glycoprotein Assembly into Murine Coronavirus Virions: Distinct Roles for Charge-Rich and Cysteine-Rich Regions of the Endodomain." *J. Virol.* **78**(18): 9904-9917.

<http://jvi.asm.org/cgi/content/abstract/78/18/9904>

The coronavirus spike protein (S) forms the distinctive virion surface structures that are characteristic of this viral family, appearing in negatively stained electron microscopy as stems capped with spherical bulbs. These structures are essential for the initiation of infection through attachment of the virus to cellular receptors followed by fusion to host cell membranes. The S protein can also mediate the formation of syncytia in infected cells. The S protein is a type I transmembrane protein that is very large compared to other viral fusion proteins, and all except a short carboxy-terminal segment of the S molecule constitutes the ectodomain. For the prototype coronavirus mouse hepatitis virus (MHV), it has previously been established that S protein assembly into virions is specified by the carboxy-terminal segment, which comprises the transmembrane domain and the endodomain. We have genetically dissected these domains in the MHV S protein to localize the determinants of S incorporation into virions. Our results establish that assembly competence maps to the endodomain of S, which was shown to be sufficient to target a heterologous integral membrane protein for incorporation into MHV virions. In particular, mutational analysis indicated a major role for the charge-rich carboxy-terminal region of the endodomain. Additionally, we found that the adjacent cysteine-rich region of the endodomain is critical for fusion of infected cells, confirming results previously obtained with S protein expression systems.

Yi, Y., F. Shaheen, et al. (2005). "Preferential Use of CXCR4 by R5X4 Human Immunodeficiency Virus Type 1 Isolates for Infection of Primary Lymphocytes." *J. Virol.* **79**(3): 1480-1486.

<http://jvi.asm.org/cgi/content/abstract/79/3/1480>

Coreceptor specificity of human immunodeficiency virus type 1 (HIV-1) strains is generally defined in vitro in cell lines expressing CCR5 or CXCR4, but lymphocytes and macrophages are the principal targets in vivo. CCR5-using (R5) variants dominate early in infection, but strains that use CXCR4 emerge later in a substantial minority of subjects. Many or most CXCR4-using variants can use both CXCR4 and CCR5 (R5X4), but the pathways that are actually used to cause infection in primary cells and in vivo are unknown. We examined several R5X4 prototype and primary isolates and found that they all were largely or completely restricted to CXCR4-mediated entry in primary lymphocytes, even though lymphocytes are permissive for CCR5-mediated entry by R5 strains. In contrast, in primary macrophages R5X4 isolates used both CCR5 and CXCR4. The R5X4 strains were also more sensitive than R5 strains to CCR5 blocking, suggesting that interactions between the R5X4 strains and CCR5 are less efficient. These results indicate that coreceptor phenotyping in transformed cells does not necessarily predict utilization in primary cells, that variability exists among HIV-1 isolates in the ability to use CCR5 expressed on lymphocytes, and that many or most strains characterized as R5X4 are functionally X4 in primary lymphocytes. Less efficient interactions between R5X4 strains and CCR5 may be responsible for the inability to use CCR5 on lymphocytes, which express relatively low CCR5 levels. Since isolates that acquire CXCR4 utilization retain the capacity to use CCR5

on macrophages despite their inability to use it on lymphocytes, these results also raise the possibility that a CCR5-mediated macrophage reservoir is required for sustained infection in vivo.

Zhang, P. F., P. Bouma, et al. (2002). "A Variable Region 3 (V3) Mutation Determines a Global Neutralization Phenotype and CD4-Independent Infectivity of a Human Immunodeficiency Virus Type 1 Envelope Associated with a Broadly Cross-Reactive, Primary Virus-Neutralizing Antibody Response." *J. Virol.* **76**(2): 644-655.

<http://jvi.asm.org/cgi/content/abstract/76/2/644>

The human serum human immunodeficiency virus type 1 (HIV-1)-neutralizing serum 2 (HNS2) neutralizes many primary isolates of different clades of HIV-1, and virus expressing envelope from the same donor, clone R2, is neutralized cross-reactively by HIV-immune human sera. The basis for this cross-reactivity was investigated. It was found that a rare mutation in the proximal limb of variable region 3 (V3), 313-4 PM, caused virus pseudotyped with the R2 envelope to be highly sensitive to neutralization by monoclonal antibodies (MAbs) directed against conformation-sensitive epitopes at the tip of the V3 loop, such as 19b, and moderately sensitive to MAbs against CD4 binding site (CD4bs) and CD4-induced (CD4i) epitopes, soluble CD4 (sCD4), and HNS2. In addition, introduction of this sequence by mutagenesis caused enhanced sensitivity to neutralization by 19b, anti-CD4i MAb, and HNS2 in three other primary HIV-1 envelopes and by anti-CD4bs MAb and sCD4 in one of the three. The 313-4 PM sequence also conferred increased infectivity for CD4+ CCR5+ cells and the ability to infect CCR5+ cells upon all of these four and two of these four HIV-1 envelopes, respectively. Neutralization of R2 by HNS2 was substantially inhibited by the cyclized R2 V3 35-mer synthetic peptide. Similarly, the peptide also had some lesser efficacy in blocking neutralization of R2 by other sera or of neutralization of other primary viruses by HNS2. Together, these results indicate that the unusual V3 mutation in the R2 clone accounts for its uncommon neutralization sensitivity phenotype and its capacity to mediate CD4-independent infection, both of which could relate to immunogenicity and the neutralizing activity of HNS2. This is also the first primary HIV-1 isolate envelope glycoprotein found to be competent for CD4-independent infection.

Zhu, M., J. A. Bristol, et al. (2005). "Linked Tumor-Selective Virus Replication and Transgene Expression from E3-Containing Oncolytic Adenoviruses." *J. Virol.* **79**(9): 5455-5465.

<http://jvi.asm.org/cgi/content/abstract/79/9/5455>

Historically, the adenoviral E3 region was found to be nonessential for viral replication in vitro. In addition, adenoviruses whose genome was more than approximately 105% the size of the native genome were inefficiently packaged. These profound observations were used experimentally to insert transgenes into the adenoviral backbone. More recently, however, the reintroduction of the E3 region into oncolytic adenoviruses has been found to positively influence antitumor efficacy in preclinical models and clinical trials. In the studies reported here, the granulocyte-macrophage colony-stimulating factor (GM-CSF) cDNA sequence has been substituted for the E3-gp19 gene in oncolytic adenoviruses that otherwise retained the E3 region. Five viruses that differed slightly in the method of transgene insertion were generated and compared to Ar6pAE2fGmF (E2F/GM/{Delta}E3), a previously described E3-deleted oncolytic adenovirus encoding GM-CSF. In all of the viruses, the human E2F-1 promoter regulated E1A expression and GM-CSF expression was under the control of the adenoviral E3 promoter and the packaging signal was relocated immediately upstream from the right terminal repeat. The E3-gp19-deleted viruses had similar cytolytic properties, as measured in vitro by cytotoxicity assays, but differed markedly in their capacity to express and secrete GM-CSF. Ar15pAE2fGmF (E2F/GM/E3b), the virus that produced the highest levels of GM-CSF and retained the native GM-CSF leader sequence, was

selected for further analysis. The E2F/GM/E3b and E2F/GM/{Delta}E3 viruses exhibited similar cytotoxic activity and GM-CSF production in several tumor cell lines in vitro. However, when compared in vivo in nude mouse xenograft tumor models, E2F/GM/E3b spread through tumors to a greater extent, resulted in higher peak GM-CSF and total exposure levels in both tumor and serum, and was more efficacious than the E3-deleted virus. Using the matched WI-38 (parental) and WI-38-VA13 (simian virus 40 large T antigen transformed) cell pair, GM-CSF was shown to be selectively produced in cells expressing high levels of E2F, indicating that the tumor-selective E2F promoter controlled E1A and GM-CSF expression.

J. Wildl. Dis. (6)

Divina, B. P., E. Wilhelmsson, et al. (2002). "Molecular identification and prevalence of *Dictyocaulus* spp. (Trichostrongyloidea: Dictyocaulidae) in Swedish semi-domestic and free-living cervids." J. Wildl. Dis. **38**(4): 769-775.

<http://www.jwildlifedis.org/cgi/content/abstract/38/4/769>

Lungs of 102 roe deer (*Capreolus capreolus*), 136 moose (*Alces alces*), 68 fallow deer (*Dama dama*), and six red deer (*Cervus elaphus*) were examined during hunting seasons from 16 September 1997 to 1 March 2000. The aim was to determine the species composition and prevalence of *Dictyocaulus* lungworms in these hosts in Sweden. Worms were identified following polymerase chain reaction (PCR) amplification of the internal transcribed spacer of ribosomal DNA (ITS2), followed by hybridization with four species-specific oligonucleotides. In addition, 50 lungworms from five reindeer (*Rangifer tarandus*) from Norway were similarly analyzed. A total of 399 worms were recovered and analyzed representing a range of 29-128 worms per host species. All specimens from roe deer were identified as *Dictyocaulus capreolus*, whereas those from red deer and reindeer were identical with *D. eckerti*. From moose, 73 (81.1%) of the worms were identified as *D. capreolus* whereas 17 (18.9%) were *D. eckerti*. The ITS2 sequence of fallow deer lungworms differed significantly when compared with the ITS2 of *D. viviparus*, *D. capreolus*, and *D. eckerti*. This indicated that fallow deer in Sweden may be infected with a new genotype of *Dictyocaulus* spp. Consequently, a specific probe designed for the ITS2 from this *Dictyocaulus* sp. hybridized exclusively with samples from lungworms of fallow deer. Interestingly, no *D. viviparus* were found in any of these hosts. The prevalence of infection in each host was as follows: *D. capreolus* in roe deer (14.7%) and moose (10.6%); *D. eckerti* in moose (0.7%) and red deer (33.3%); and *Dictyocaulus* sp. in fallow deer (10.3%). Regardless of lungworm species, the overall prevalence of *Dictyocaulus* spp. in these hosts was 12.2%. Prevalence between male and female animals and among the different age groups did not differ significantly. Finally an enzyme linked immunosorbent assay (ELISA) specific for patent *D. viviparus* infection in cattle was utilized to analyze lung tissue fluids from infected animals. All samples from roe deer, red deer, and fallow deer were negative in the ELISA. However, three out of twelve (25%) samples from moose and 17 of 40 (43%) samples from cattle were positive. This indicated that moose anti-*D. capreolus* antibodies recognized the *D. viviparus* antigen and that anti-cattle immunoglobulin cross-reacted with moose antibodies.

Ketz-Riley, C. J., M. V. Reichard, et al. (2003). "An intraerythrocytic small piroplasm in wild-caught Pallas's cats (*Otocolobus manul*) from Mongolia." J. Wildl. Dis. **39**(2): 424-430.

<http://www.jwildlifedis.org/cgi/content/abstract/39/2/424>

During the quarantine examination of four Pallas's cats (*Otocolobus manul*) imported from Mongolia in October and December 2000, intraerythrocytic piroplasms were detected on Wright-Giemsa stained blood films that were morphologically indistinguishable from other small piroplasms of felids. Further characterization of this unknown organism via polymerase chain reaction amplification, sequencing of a portion of the 18S nuclear small subunit rRNA gene, and comparisons with orthologous sequences from other piroplasms, revealed similarity to *Cytauxzoon felis*. This is the first report of naturally occurring erythroparasitemia in Pallas's cats and the first documented case of naturally occurring piroplasm infections in a free-ranging felid from Mongolia.

Olsen, S. C., J. Rhyan, et al. (2004). "SAFETY OF BRUCELLA ABORTUS STRAIN RB51 IN BLACK BEARS." *J. Wildl. Dis.* **40**(3): 429-433.

<http://www.jwildlifedis.org/cgi/content/abstract/40/3/429>

In two studies conducted from October 1999 to March 2000 and December 2000 to April 2001, adult black bears (*Ursus americanus*) were orally inoculated with 1.4-3.1x10¹⁰ colony-forming units (CFU) of *Brucella abortus* strain RB51 (SRB51, n=12) or 2 ml of 0.15 M NaCl solution (saline, n=11). We did not detect a difference ($P>0.05$) in antibody titers to SRB51 in serum obtained before vaccination, at 8 wk after vaccination, or at necropsy at 21 or 23 wk after vaccination between SRB51-vaccinated and nonvaccinated bears. The SRB51 vaccine strain was recovered from tissues obtained at necropsy from one of six SRB51-vaccinated bears in study 1, but none of the six SRB51-vaccinated bears in study 2. Vaccination of black bears with SRB51 did not appear to influence ($P>0.05$) reproductive performance.

Rotstein, D. S., T. R. Schoeb, et al. (2002). "Detection by microsatellite analysis of early embryonic mortality in an alligator population in Florida." *J. Wildl. Dis.* **38**(1): 160-165.

<http://www.jwildlifedis.org/cgi/content/abstract/38/1/160>

In the 1980s, alligators (*Alligator mississippiensis*) of Lake Apopka (Florida, USA) underwent a population decline associated with decreased egg viability, effects that have been associated with endocrine-disrupting, persistent organochlorine pesticides. It is currently unknown whether the decreased egg viability is due to fertilization failure or early embryonic death. Therefore, we conducted a preliminary study to evaluate the use of microsatellite DNA loci to determine the fertilization status of nonviable eggs. Using microsatellite analysis, we compared genotypes from blasto-disks and embryos with the genotypes from females trapped at the nest. Four of five nonviable egg samples tested yielded evidence of fertilization. No evidence of unfertilized eggs was obtained, but amplifiable DNA could not be obtained from one entirely nonviable clutch. Thus, we demonstrate that early embryonic mortality in alligators can be detected by microsatellite analysis, but also suggest substantial effort is needed to improve the recovery of DNA and amplification of alligator microsatellite loci.

Schettler, E., J. Fickel, et al. (2003). "Newcastle disease virus and *Chlamydia psittaci* in free-living raptors from eastern Germany." *J. Wildl. Dis.* **39**(1): 57-63.

<http://www.jwildlifedis.org/cgi/content/abstract/39/1/57>

Organ samples from free-living raptors from the federal states of Berlin and Brandenburg in eastern Germany were tested for Newcastle disease virus (NDV; n = 331) and *Chlamydia psittaci* (n = 39) by polymerase chain reaction (PCR). In 18 individuals NDV nucleic acids were detected. These samples originated from barn owls (*Tyto alba*; n = 15, 28%), tawny owl (*Strix aluco*; n = 1, 5%), common buzzard (*Buteo buteo*, n = 1, 1%), and European kestrel (*Falco tinnunculus*; n = 1, 4%). In 29 (74%) of 39 samples *C. psittaci* was detected. *Chlamydia psittaci* is common in free-living birds of prey in the investigated area.

Stabel, J. R., M. V. Palmer, et al. (2003). "Immune responses after oral inoculation of weanling bison or beef calves with a bison or cattle isolate of *Mycobacterium avium* subsp. *paratuberculosis*." J. Wildl. Dis. **39**(3): 545-555.

<http://www.jwildlifedis.org/cgi/content/abstract/39/3/545>

Paratuberculosis is endemic in domestic and wild ruminants worldwide. We designed the following study to compare host immune responses and pathologic changes in beef calves and bison calves after challenge with either a cattle or bison (*Bison bison*) strain of *Mycobacterium avium* subsp. *paratuberculosis*. In the first part of the study, six bison and six beef calves were orally inoculated with a cattle isolate of *M. avium* subsp. *paratuberculosis* over a 2 wk period. In the second part, an additional six bison and six beef calves were similarly inoculated with a bison strain of *M. avium* subsp. *paratuberculosis*. Throughout each of the studies, blood and fecal samples were taken monthly for a 6 mo infection period. Tissue samples were obtained at necropsy for culture and histopathologic analyses. Results from this study demonstrated that bison calves were more susceptible to tissue colonization than beef calves after challenge with the cattle isolate and, conversely, that beef calves were more susceptible to the bison strain of *M. avium* subsp. *paratuberculosis*. Although lesions were minimal they were most apparent in the jejunum and distal ileum. Interferon-gamma (IFN-gamma) responses were noted in some calves by 1 mo postinoculation and were sustained longer in beef calves after challenge with the bison isolate. Antibody was not detected in either beef or bison calves during the 6 mo infection period. These results indicate that the host response to strains of *M. avium* subsp. *paratuberculosis* may differ between ruminant species.

JAMA (2)

Kim, J.-H., S. J. Skates, et al. (2002). "Osteopontin as a Potential Diagnostic Biomarker for Ovarian Cancer." JAMA **287**(13): 1671-1679.

<http://jama.ama-assn.org/cgi/content/abstract/287/13/1671>

Context Development of new biomarkers for ovarian cancer is needed for early detection and disease monitoring. Analyses involving complementary DNA (cDNA) microarray data can be used to identify up-regulated genes in cancer cells, whose products may then be further validated as potential biomarkers. Objective To describe validation studies of an up-regulated gene known as osteopontin, previously identified using a cDNA microarray system. Design, Setting, and Participants Experimental and cross-sectional studies were conducted involving ovarian cancer and healthy human ovarian surface epithelial cell lines and cultures, archival paraffin-embedded ovarian tissue collected between June 1992 and June 2001, and fresh tissue and preoperative

plasma from 144 patients evaluated for a pelvic mass between June 1992 and June 2001 in gynecologic oncology services at 2 US academic institutions. Plasma samples from 107 women selected from an epidemiologic study of ovarian cancer initiated between May 1992 and March 1997 were used as healthy controls. Main Outcome Measures Relative messenger RNA expression in cancer cells and fresh ovarian tissue, measured by real-time polymerase chain reaction as $2^{-\Delta\Delta CT}$ (a quantitative value representing the amount of osteopontin expression); osteopontin production, localized and scored in ovarian healthy and tumor tissue with immunohistochemical studies; and amount of osteopontin in patient vs control plasma, measured using an enzyme-linked immunoassay. Results The geometric mean for $2^{-\Delta\Delta CT}$ for osteopontin expression in 5 healthy ovarian epithelial cell cultures was 4.1 compared with 270.4 in 14 ovarian cancer cell lines ($P = .03$). The geometric mean $2^{-\Delta\Delta CT}$ for osteopontin expression in tissue from 2 healthy ovarian epithelial samples was 9.0 compared with 164.0 in 27 microdissected ovarian tumor tissue samples ($P = .06$). Immunolocalization of osteopontin showed that tissue samples from 61 patients with invasive ovarian cancer and 29 patients with borderline ovarian tumors expressed higher levels of osteopontin than tissue samples from 6 patients with benign tumors and samples of healthy ovarian epithelium from 3 patients ($P = .03$). Osteopontin levels in plasma were significantly higher ($P < .001$) in 51 patients with epithelial ovarian cancer (486.5 ng/mL) compared with those of 107 healthy controls (147.1 ng/mL), 46 patients with benign ovarian disease (254.4 ng/mL), and 47 patients with other gynecologic cancers (260.9 ng/mL). Conclusions Our findings provide evidence for an association between levels of a biomarker, osteopontin, and ovarian cancer and suggest that future research assessing its clinical usefulness would be worthwhile.

Kulasingam, S. L., J. P. Hughes, et al. (2002). "Evaluation of Human Papillomavirus Testing in Primary Screening for Cervical Abnormalities: Comparison of Sensitivity, Specificity, and Frequency of Referral." *JAMA* 288(14): 1749-1757.

<http://jama.ama-assn.org/cgi/content/abstract/288/14/1749>

Context Human papillomavirus (HPV) DNA testing of women having Papanicolaou (Pap) smears showing atypical squamous cells of undetermined significance (ASCUS) has clinical usefulness. Whether HPV DNA testing alone is useful in primary screening remains to be determined. Objective To determine the accuracy of HPV DNA testing for detecting cervical intraepithelial neoplasia (CIN) grade 3 or cancer (the criterion standard). Design, Setting, and Participants Between December 1997 and October 2000, 4075 women who attended Planned Parenthood clinics in Washington State were screened simultaneously using thin-layer Pap and HPV DNA testing by a polymerase chain reaction (PCR)-based method and by a liquid-based RNA-DNA hybridization capture with signal amplification assay (signal amplification). Women who were positive for high-risk HPV types, or had Pap results of ASCUS or higher, were considered to have positive screening test results and were referred for colposcopy and biopsy. Additionally, a random sample of women with negative screening test results was referred for colposcopy. Based on individual and combined thin-layer Pap, HPV PCR, and HPV signal amplification test results from the screening and the colposcopy visits, 7 colposcopy triage strategies were defined and evaluated. Main Outcome Measure Sensitivity and specificity for detecting cervical lesions graded CIN 3 or higher for each of the 7 triage strategies. Results The estimated prevalence of CIN 3 or higher was 3.2%. The sensitivity (95% confidence interval) of thin-layer Pap (with a result of $\geq$ ASCUS) for identifying women with CIN 3 or higher was only 61.3% (48.5%-70.9%) compared with 88.2% (78.9%-93.8%) for HPV testing by PCR and 90.8% (83.1%-95.8%) by signal amplification. Differences in specificities were also observed: 82.4% (81.8%-83.1%) for thin-layer Pap (with a result of $\geq$ ASCUS), 78.8% (77.9%-79.7%) for PCR, and 72.6% (69.4%-75.0%) for signal amplification. Compared with referral for colposcopy of all women with ASCUS or higher, signal amplification testing of women with ASCUS and referral of those with a positive result was about as sensitive (61.3% vs 60.3%, respectively) and significantly more specific (82.4% vs 88.9%, respectively). The strategy requiring repeat positive PCR tests on 2

visits had a sensitivity of 84.2% (75.3%-91.0%) and a specificity of 86.2% (85.1%-87.3%). All tests were more specific and less sensitive in older (≥ 30 years) vs younger women. Conclusions Testing for HPV has higher sensitivity but lower specificity than thin-layer Pap screening. In some settings, particularly where screening intervals are long or haphazard, screening for HPV DNA may be a reasonable alternative to cytology-based screening of reproductive-age women.

Japanese Journal of Ophthalmology (1)

Saitoh-Ishibashi, K., K. Ishibashi, et al. (2003). "Analysis of Cytokine mRNAs in Murine Herpes Simplex Virus Type 1 Retinitis." Japanese Journal of Ophthalmology **47**(2): 166.

<http://www.sciencedirect.com/science/article/B6VJ7-48H218P-8/2/7516af1e1363e818e1aa3558586847bb>

Purpose: To investigate cytokine mRNA expression during the inflammatory process induced in the contralateral eyes by unocular inoculation of herpes simplex virus type 1 (HSV-1) via the anterior chamber. **Methods:** BALB/c mice were inoculated in the anterior chamber with 5×10^4 plaque-forming units of HSV-1 (KOS). mRNA was extracted from the inflamed posterior segments of the uninoculated eyes at 0 (control), 9, 11, 14, and 21 days postinoculation (p.i.). Reverse transcription-polymerase chain reaction was performed for semiquantitative analysis of mRNA expression of interleukin (IL)-1[beta], IL-2, IL-4, IL-10, IL-12p35, IL-12p40, interferon (IFN)[gamma], tumor necrosis factor (TNF)[alpha], transforming growth factor (TGF)[beta]2 and induced nitric oxide synthase (iNOS). **Results:** Peak mRNA expression of iNOS was observed at day 14 p.i. The time profiles of mRNA expression for IL-1[beta], IL-2, IL-4, IL-10, IFN-[gamma], TNF[alpha] were similar to that of iNOS, while TGF[beta]2, IL-12p35, and IL-12p40 demonstrated a reverse pattern. **Conclusions:** The kinetics of the analyzed cytokines synchronized with the clinicopathological activity of the experimental murine HSV-1 retinitis. The immunosuppressive cytokines TGF[beta]2 and IL-10 demonstrated different peaks of mRNA expressions suggesting that the down-regulation phase of the inflammatory process was controlled by several factors working at different phases.

Journal of Affective Disorders (2)

Levitan, R. D., M. Masellis, et al. (2002). "Polymorphism of the serotonin-2A receptor gene (HTR2A) associated with childhood attention deficit hyperactivity disorder (ADHD) in adult women with seasonal affective disorder." Journal of Affective Disorders **71**(1-3): 229.

<http://www.sciencedirect.com/science/article/B6T2X-46G3SNW-11/2/2c42dea4e95eec1494615917938b6122>

Introduction: Several lines of research point to a possible overlap between seasonal affective disorder (SAD) and attention deficit hyperactivity disorder (ADHD), particularly in females. There

is also emerging evidence that variation of the 5-HT_{2A} receptor gene (HTR2A) contributes to both SAD and ADHD. The current study investigated whether variation in HTR2A was associated with symptoms of childhood ADHD in adult women with SAD. Method: Sixty-six women with SAD were administered the Wender-Utah Rating Scale (WURS), which retrospectively assesses childhood ADHD, as part of an ongoing genetic study of SAD. WURS scores were compared across the three genotypic groups defined by the T102C polymorphism of HT2RA. Results: Analysis of variance indicated a significant difference in mean 25-item WURS scores across the three genotypic groups ($p=0.035$). Post-hoc tests revealed that the C/C genotypic group had a significantly higher mean score than both the T/T group and T/C group. Based on previously established WURS criteria, 38% of subjects with the C/C genotype, and none with the T/T genotype, had scores consistent with childhood ADHD. Limitations: The current sample size is small, and childhood ADHD diagnoses were based on retrospective recall. Conclusion: These preliminary results suggest a possible association between variation in HTR2A, childhood ADHD, and the later development of SAD in women.

Mitchell, P., B. Waters, et al. (1993). "Exclusion of close linkage of bipolar disorder to the dopamine D3 receptor gene in nine Australian pedigrees." *Journal of Affective Disorders* **27**(4): 213.

<http://www.sciencedirect.com/science/article/B6T2X-46040F6-D2/2/f75179efda6dc9a007d69b47606532cf>

The recently cloned dopamine D3 receptor (DRD3) gene is of potential relevance to the aetiology of bipolar disorder because of an almost exclusive expression in limbic tissue, the region of the brain putatively responsible for control of emotion. We therefore aimed to determine whether bipolar disorder in nine pedigrees (with 171 members) was linked to this receptor gene, which has been mapped to chromosomal region 3q 13.3. Linkage of bipolar disorder and recurrent depression to the DRD3 gene was tested using a series of autosomal dominant and recessive models with varying penetrance levels. Additionally, linkage was examined using a series of levels of definitions of affective illness (ranging from bipolar I alone to all affective disorders). Close linkage to the DRD3 gene was strongly excluded using each model and definition, and these conclusions persisted when a wide range of rates of 'sporadic' (non-genetic) presentations of illness were incorporated in the analysis.

Journal of Allergy and Clinical Immunology (7)

Jogie-Brahim, S., H.-K. Min, et al. (2004). "Expression of [alpha]-tryptase and [beta]-tryptase by human basophils." *Journal of Allergy and Clinical Immunology* **113**(6): 1086.

<http://www.sciencedirect.com/science/article/B6WH4-4CMHF7S-1G/2/b4e803c222ee16aeee4b4e40dd51fd35>

Background[alpha] and [beta]-Tryptase levels in serum are clinical tools for the evaluation of systemic anaphylaxis and systemic mastocytosis. Basophils and mast cells are known to produce these proteins.
ObjectiveThe current study examines the effect of the [alpha],[beta]-tryptase genotype on basophil tryptase levels and the type of tryptase stored in these cells.
MethodsTryptase extracted from purified peripheral blood basophils from 20 subjects was examined by using ELISAs measuring mature and total tryptase and by using an enzymatic

assay with tosyl-Gly-Pro-Lys-p-nitroanilide. Tryptase genotypes (4:0, 3:1, and 2:2 [beta]/[alpha] ratios) were assessed by using a hot-stop PCR technique with [alpha],[beta]-tryptase-specific primers. Total [alpha],[beta]-tryptase mRNA was measured by means of competitive RT-PCR, and ratios of [alpha] to [beta]-tryptase mRNA were measured by means of hot-stop RT-PCR. Results Tryptase in all but one of the basophil preparations was mature and enzymatically active. Tryptase quantities in basophils were less than 1% of those in tissue mast cells. Tryptase genotypes ([beta]/[alpha]) among the 20 donors were 4:0 in 7, 3:1 in 7, and 2:2 in 6. Tryptase protein and mRNA levels per basophil were not affected by the tryptase genotype. Conclusion Basophils from healthy subjects contain modest amounts of mature and enzymatically active tryptase unaffected by the tryptase genotype.

Leung, T.-f., N. L. S. Tang, et al. (2004). "Association between TARC C-431T and atopy and asthma in children." Journal of Allergy and Clinical Immunology **114**(1): 199.

<http://www.sciencedirect.com/science/article/B6WH4-4CS46C4-29/2/9dce29a7116c1c1ccdee88f9c9788ee9>

Min, H.-K., G. Moxley, et al. (2004). "Effect of sex and haplotype on plasma tryptase levels in healthy adults." Journal of Allergy and Clinical Immunology **114**(1): 48.

<http://www.sciencedirect.com/science/article/B6WH4-4CS46C4-19/2/6287b60b49ecc666c1442e4fba184209>

Background The total level of [alpha]-tryptase and [ss]-tryptase in serum or plasma is used as a clinical indicator of the mast cell burden. Objective The effect of the tryptase haplotype and of sex on the total tryptase level of healthy individuals was determined. Methods A novel hot-stop PCR technique was used to determine the tryptase genotype, and a standard fluoroenzyme immunoassay was used to measure total plasma tryptase levels in 106 healthy subjects. Mx modeling and the QTL association routine of Mendel 5.0 were used to analyze the data. Results Tryptase haplotypes exhibit a 1 ([ss][alpha]/[ss][alpha]):2 ([ss][ss]/[ss][alpha]):1 ([ss][ss]/[ss][ss]) distribution, monomorphic for [ss] at 1 position and allelic for [ss] and [alpha] at the other position. The [ss][alpha] haplotype has a frequency of 0.49. The [ss][alpha] haplotype increases total tryptase levels by 0.5 ng/mL from the overall mean, whereas female sex increases the level by 0.2 ng/mL from the mean. Conclusion The tryptase haplotype and sex each have a statistically significant effect on the total plasma tryptase level of healthy subjects.

Morwood, K., H. Bourne, et al. (2004). "Phenotypic variability: clinical presentation between the 6th year and the 60th year in a family with X-linked agammaglobulinemia." Journal of Allergy and Clinical Immunology **113**(4): 783.

<http://www.sciencedirect.com/science/article/B6WH4-4C5YH61-1X/2/4ea2ecd85a3f91408ecd5f59d5083b0e>

Ou, L.-S., E. Goleva, et al. (2004). "T regulatory cells in atopic dermatitis and subversion of their activity by superantigens." Journal of Allergy and Clinical Immunology **113**(4): 756.

<http://www.sciencedirect.com/science/article/B6WH4-4C5YH61->

1S/2/bb20ef226e632a3e772d1bb264dff411

Background Atopic dermatitis (AD) is a chronic inflammatory skin disease involving colonization by superantigen (SAg)-secreting *Staphylococcus aureus*. CD4+CD25+ T regulatory (Treg) cells are thought to play an important role in controlling inflammatory responses. **Objective** In this study we examined whether Treg cells might be deficient in patients with AD. **Methods** CD4+CD25+ and CD4+CD25- T cells were isolated from PBMCs by using immunomagnetic beads. Cells were cultured with anti-CD3 or SAg, staphylococcal enterotoxin B (SEB), for 72 hours. Proliferation was measured by means of tritiated thymidine incorporation. CD4, CD8, CD25, and cutaneous lymphocyte-associated antigen expression on PBMCs was assessed by means of flow cytometry. RNA was extracted from isolated subsets of T cells, and the results of real-time PCR for FoxP3 mRNA were determined. **Results** Surprisingly, CD4+CD25+ T cells were significantly ($P < 0.05$) in skin-homing, CD4+, cutaneous lymphocyte-associated antigen-positive T cells than asthmatic and nonatopic subjects, with values of 35.95% versus 22.44% versus 23.03%, respectively ($P < 0.05$). CD4+CD25+ cells expressed FoxP3, whereas CD4+CD25- T cells and CD4- cells did not. Consistent with known properties of Treg cells, CD4+CD25+ cells were anergic to anti-CD3 stimulation. When CD4+CD25+ cells from each study group were mixed with CD4+CD25- cells, proliferative responses were equally suppressed after anti-CD3 stimulation. In contrast, after SEB stimulation, CD4+CD25+ cells were no longer anergic. Furthermore, when CD4+CD25+ cells were mixed with CD4+CD25- cells and stimulated with SEB, the suppressive function of Treg cells was reversed. **Conclusion** Patients with AD have significantly increased numbers of peripheral blood Treg cells with normal immunosuppressive activity. However, after SAg stimulation, Treg cells lose their immunosuppressive activity. These data suggest a novel mechanism by which SAg could augment T-cell activation in patients with AD.

Roy, S. R., A. M. Schiltz, et al. (2003). "Bacterial DNA in house and farm barn dust." *Journal of Allergy and Clinical Immunology* **112**(3): 571.

<http://www.sciencedirect.com/science/article/B6WH4-49W6061-1B/2/aed95e490b7cf57540a02596335ac880>

Background Early in life, natural exposure to microbial components (eg, endotoxin) may mitigate allergy and asthma development in childhood. Bacterial DNA is a potent stimulus for the innate immune system; its immune stimulatory potential in dust is unknown. **Objectives** We sought to quantify bacterial DNA and endotoxin content in dust from urban homes, rural homes, farm homes, and farm barns and to determine if dust DNA is immune-stimulatory. **Methods** Total DNA, bacterial DNA, and endotoxin were measured in 32 dust samples. To measure bacterial DNA content, a quantitative polymerase chain reaction assay specific for bacterial ribosomal DNA was developed. Peripheral blood mononuclear cells from 5 adults were stimulated with endotoxin-free dust DNA with/without lipopolysaccharide (LPS) from selected dust samples. IL-12p40, IL-10, and tumor necrosis factor- α were measured in cell supernatants by enzyme-linked immunosorbent assay. **Results** Bacterial DNA in dust correlated with endotoxin ($r = 0.56$, $P = 0.003$). The highest bacterial DNA levels were measured in farm barns (mean, 22.1 $\mu\text{g/g}$ dust; range, 1.3 to 56.2), followed by rural homes (6.3 $\mu\text{g/g}$; 0.2 to 20), farm homes (2.2 $\mu\text{g/g}$; 0.1 to 9.1), and urban homes (0.6 $\mu\text{g/g}$; 0.1 to 1.2). Farm barn DNA significantly potentiated ($P < 0.05$) endotoxin-induced IL-12p40 and IL-10 production. **Conclusions** Endotoxin is a marker for bacterial DNA, which is also higher in locales of lower asthma and allergy prevalence. DNA from farm barn dust augments the immune modulatory effects of endotoxin and may combine with exposure to other such naturally occurring microbial components to mitigate allergy and asthma development.

Srivastava, K., A. A. Teper, et al. (2004). "Immunomodulatory effect of the antiasthma Chinese herbal formula MSSM-002 on TH2 cells." *Journal of Allergy and Clinical Immunology* **113**(2): 268.

<http://www.sciencedirect.com/science/article/B6WH4-4BMC07G-1C/2/c3d030c51782d264d6d825b47c3fe86c>

Background TH2 cytokines play a central role in the pathogenesis of allergic asthma. We previously showed that the "antiasthma" Chinese herbal formula MSSM-002 exhibited therapeutic effects on established allergic airway responses in a murine model of allergic asthma. However, the mechanisms underlying these effects are largely unknown. **Objective** The objective of this study was to determine whether and how MSSM-002 modulates an established TH2 response and whether the actions of MSSM-002 on TH2 cell differs from corticosteroids. **Methods** TH2 polarized splenocytes (TH2-SPCs) from mice with antigen-induced airway hyperresponsiveness and TH2 cloned cells, D10 G4.1 (D10), were cultured in the presence or absence of antigen with or without MSSM-002 and dexamethasone, and the proliferative responses and cytokine profiles were determined. Apoptosis and TH2 transcription factor GATA-3 expression and binding to IL-4 gene promoter and VA enhancer in MSSM-002-treated D10 cells were also determined. **Results** MSSM-002 significantly decreased antigen-induced proliferation and IL-4 and IL-5 production but increased IFN- γ production by TH2-SPCs, whereas dexamethasone suppressed IFN- γ as well as IL-4 and IL-5. Anti-IL-12 antibody, although abrogating MSSM-002 induction of IFN- γ , had no significant effect on MSSM-002 suppression of IL-4 and IL-5 secretion. MSSM-002 also suppressed TH2 cytokine secretion by D10 cells, and in contrast to dexamethasone, MSSM-002 did not induce apoptosis of D10 cells. MSSM-002 markedly suppressed GATA-3 mRNA and protein expression and the binding to IL-4 gene promoter and VA enhancer in D10 cells. **Conclusion** MSSM-002, in contrast to the overall suppression of T cells by dexamethasone, exhibits immunomodulatory actions on TH2 cells caused, at least partially, by downregulation of GATA-3.

Journal of Autoimmunity (4)

Ide, A., E. Kawasaki, et al. (2004). "Association between IL-18 gene promoter polymorphisms and CTLA-4 gene 49A/G polymorphism in Japanese patients with type 1 diabetes." Journal of Autoimmunity **22**(1): 73.

<http://www.sciencedirect.com/science/article/B6WHC-4B22WHV-1/2/7d78cada8c5b7fa05f7c932b7ccc2270>

Interleukin-18 (IL-18) is a potent proinflammatory cytokine which is strongly associated with the development of diabetes in NOD mice. To test the putative involvement of IL-18 gene polymorphism in predisposition to human type 1 diabetes, the SNPs at position -607 (C/A) and -137 (G/C) in the promoter region of IL-18 gene were analyzed by sequence-specific PCR in 116 patients with type 1 diabetes and 114 normal controls. A linkage disequilibrium found only three of the four possible haplotypes defined by these SNPs. The distribution of the IL-18 gene genotypes at position -607 was significantly different between patients with type 1 diabetes and normal controls ($P=0.023$). Furthermore, there was a significant increase in haplotype 1 (-607C/-137G) in the patients compared with controls ($P=0.006$). The association study of the susceptible CTLA-4 genotype (GG at nucleotide position 49 in exon 1) or HLA-DR4-DQB1*0401 and type 1 diabetes showed that the predisposing IL-18 gene haplotype modulates the risk on CTLA-4 GG genotype, but not on HLA-DR4-DQB1*0401 haplotype. Among subjects carrying the CTLA-4 GG genotype, the frequency of IL-18 haplotype 1 in patients with type 1 diabetes was significantly higher than that in controls (91% vs. 71%, $P=0.012$). However, IL-18 haplotype 1 was not frequent in patients who do not exhibit the CTLA-4 high-risk genotype. These results suggest that the IL-18 gene polymorphism is associated with a type 1 diabetes susceptibility, and there might

be a gene-gene interaction between IL-18 gene with susceptible CTLA-4 gene.

Neumann, E., S. Lechner, et al. (2003). "Evaluation of differentially expressed genes by a combination of cDNA array and RAP-PCR using the AtlasImage(TM) 2.0 software." Journal of Autoimmunity **21**(2): 161.

<http://www.sciencedirect.com/science/article/B6WHC-494HM8F-1/2/69e6bdb8a3967fbd3ab540909ecccc04>

Evaluation of genes regulated differentially is essential for the development of therapeutic approaches in multifactorial diseases. To characterize gene expression profiles in multifactorial inflammatory and malignant diseases such as rheumatoid arthritis (RA) or colon adenoma (CA), RNA arbitrarily primed PCR (RAP-PCR) combined with cDNA array hybridization were performed and evaluated using an array-specific software. RNA of synovial fibroblasts from patients with RA and osteoarthritis (OA), and laser microdissected normal and colon adenoma tissue was used. RAP-PCR reactions were hybridized to cDNA array membranes. Arrays were analyzed by phosphor imaging, and the AtlasImage(TM) 2.0 software with different normalization settings. The AtlasImage(TM) 2.0 software was a useful tool to evaluate differentially expressed genes. However, software settings were needed to be optimized for every experimental approach and should be used without changes for all experiments. To compare RA vs. OA synovial fibroblasts and normal vs. CA expression patterns, global normalization using the sum method is recommended.

Wapenaar, M. C., M. J. van Belzen, et al. (2004). "The interferon gamma gene in celiac disease: augmented expression correlates with tissue damage but no evidence for genetic susceptibility." Journal of Autoimmunity **23**(2): 183.

<http://www.sciencedirect.com/science/article/B6WHC-4CWBJRN-2/2/21172f2666ae69962a7442d7a58395a8>

Celiac disease (CD) is a complex genetic disorder characterized by gluten intolerance. The Th1 immune response, with a key position for interferon gamma (IFN-[gamma]), is an important determinant of intestinal remodeling in CD. We aimed at further ascertaining the role of IFN-[gamma], either as a genetic factor in the etiology, or as a facilitator of disease initiation/progression. Duodenal biopsies were sampled across distinct histopathological stages of the disease, including refractory CD (RCD), and used to determine IFN-[gamma] gene (IFNG) expression by real-time RT-PCR. INFG expression correlated with the extent of tissue restructuring, reaching a 240-fold higher expression in total villous atrophy compared to healthy tissue. CD and RCD patients with similar lesions had comparable expression levels. Interestingly, patients in complete remission still had 7.6-fold residual over-expression. An INFG marker was tested in three cohorts of Dutch patients for both genetic linkage and association. Linkage analysis yielded no significant scores for IFNG or its flanking markers. In addition, IFNG allele frequencies were not differently distributed between cases and controls. Likewise, all alleles were randomly transmitted to affected children in parents-case trios. There is no evidence for IFNG as a predisposing gene in CD, despite its enhanced expression in patients in complete remission.

Zhang, J., C. Vandevyver, et al. (1995). "Activation and clonal expansion of human Myelin Basic Protein-reactive T cells by bacterial superantigens." Journal of Autoimmunity **8**(4): 615.

<http://www.sciencedirect.com/science/article/B6WHC-4DB57WR-D/2/7caf4be3e45267f0477464ad143bd923>

Autoreactive T cells specific for myelin basic protein (MBP) are part of the normal T cell repertoire and are present both in patients with multiple sclerosis (MS) and healthy individuals. There is evidence suggesting in vivo activation and persistent clonal expansion of MBP-reactive T cells in MS. This study was undertaken to investigate the potential role of bacterial superantigens (SA) in the activation of MBP-reactive T cells. Twenty-seven MBP-reactive T cell clones generated from 10 MS patients and one normal individual were examined for reactivity to SA, in association with their T cell receptor V[beta] gene usage. The majority of the clones responded to at least one of the SA tested, staphylococcal enterotoxins (SEA and SEB) and toxic shock syndrome toxin-1 (TSST-1). The clones reactive to SEA and SEB expressed various V[beta] genes while T cell reactivity to TSST-1 correlated with the V[beta]2 expression. Furthermore, circulating MBPreactive T cells could be expanded from lymphocyte cultures primarily exposed to respective SA in more than 50% of MS patients and normal individuals tested. However, activation and expansion of circulating MBPreactive T cells by SA was not directly associated with the disease. This study lends support to the potential role of SA in the activation of MBP-reactive T cells and suggests that an altered regulatory mechanism may account for further expansion and persistence of MBP-reactive T cells in MS.

Journal of Biochemical and Biophysical Methods (14)

Burkhart, C. A., M. D. Norris, et al. (2002). "A simple method for the isolation of genomic DNA from mouse tail free of real-time PCR inhibitors." Journal of Biochemical and Biophysical Methods **52**(2): 145.

<http://www.sciencedirect.com/science/article/B6T28-46HJMPJ-2/2/d8f639256a9e5983ff8fceed0137e308>

Although real-time PCR is a rapid, quantitative method for the analysis of gene and RNA levels, the presence of inhibitors in samples is an obstacle to its successful use. We have found that genomic DNA isolated from mouse tail tips using a standard proteinase K digestion method caused marked inhibition of real-time PCR. Inhibition was specific for mouse tail DNA since genomic DNA isolated from other tissue sources using the same methodology was readily amplified. We have therefore developed a nonproteinase K DNA isolation method involving the use of Chelex(R) 100 resin. This method produces mouse tail DNA that is free of real-time PCR inhibitors.

Falzon, L., C. Kirk, et al. (1994). "PCR generation of large amounts of purified DNA." Journal of Biochemical and Biophysical Methods **29**(3-4): 251.

<http://www.sciencedirect.com/science/article/B6T28-47NVM5J-6/2/cd2c380957833a38c4147fcddac6673e>

The preparation and purification of PCR generated DNA fragments suitable for footprinting and classical drug binding studies is described. One of the fragments, a 214-mer derived from pBR322 DNA exhibits a biphasic melting profile. This behavior appears to be due to a non-

random distribution of base pairs within the fragment causing a region rich in AT base pairs to melt prior to a segment having a high concentration of GC base pairs. The usefulness of large amounts of PCR generated DNA for footprinting and optical binding studies involving drugs is also presented and discussed.

Giordano, M., M. Mellai, et al. (2001). "Determination of SNP allele frequencies in pooled DNAs by primer extension genotyping and denaturing high-performance liquid chromatography." Journal of Biochemical and Biophysical Methods **47**(1-2): 101.

<http://www.sciencedirect.com/science/article/B6T28-429Y53Y-C/2/6fe14bea71f71b178da1b9f60cc4b7df>

By testing DNA pools rather than single samples the number of tests for a case-control association study can be decreased to only two for each marker: one on the patient and one on the control pool. A fundamental requirement is that each pool represents the frequency of the markers in the corresponding population beyond the influence of experimental errors. Consequently the latter must be carefully determined. To this aim, we prepared pools of different size (49-402 individuals) with accurately quantified DNAs, estimated the allelic frequencies in the pools of two SNPs by primer extension genotyping followed by DHPLC analysis and compared them with the real frequencies determined in the single samples. Our data show that (1) the method is highly reproducible: the standard deviation of repeated determinations was ± 0.014 ; (2) the experimental error (i.e., the discrepancy between the estimated and real frequencies) was ± 0.013 (95% C.I.: 0.0098-0.0165). The magnitude of this error was not correlated to the pool size or to the type of SNP. The effect of the observed experimental error on the power of the association test was evaluated. We conclude that this method constitutes an efficient tool for high-throughput association screenings provided that the experimental error is low. We therefore recommend that before a pool is used for extensive association studies, its quality, i.e., the experimental error, is verified by determining the difference between estimated and real frequencies for at least one marker.

Gullicksen, P. S., R. G. Dean, et al. (2004). "Detection of DNA fragmentation and apoptotic proteins, and quantification of uncoupling protein expression by real-time RT-PCR in adipose tissue." Journal of Biochemical and Biophysical Methods **58**(1): 1.

<http://www.sciencedirect.com/science/article/B6T28-49JHR0K-4/2/cb03d8f49eb3270582a36a35c7fde837>

Better understanding of the mechanisms involved in adipose tissue growth and metabolism is critical for the development of more effective treatments for obesity. However, because of its high lipid and low protein content, adipose tissue can present unique problems in some experimental procedures. We describe three protocols that provide new or improved methods for analysis of DNA, RNA, and protein from different adipose tissues. The first protocol provides a simple and rapid method for separation of fragmented DNA and visualization of apoptotic DNA laddering without the need for radioisotopes. This technique allows for an estimate of the amount of DNA fragmentation, and hence, apoptosis. The second protocol details subcellular fractionation of adipose tissue for the extraction of protein in the mitochondrial and cytosol fractions and the measurement of apoptotic protein (Bcl-2 and Bax) levels in each fraction. The last protocol involves extraction of total RNA from adipose tissue and the measurement of uncoupling protein mRNA using real-time RT-PCR, a method that has not previously been used to measure expression of uncoupling proteins in adipose tissue.

Huber, C. G., A. Premstaller, et al. (2001). "Mutation detection by capillary denaturing high-performance liquid chromatography using monolithic columns." Journal of Biochemical and Biophysical Methods **47**(1-2): 5.

<http://www.sciencedirect.com/science/article/B6T28-429Y53Y-2/2/8b095b042b00341d566dae95d00f5604>

The high resolving power of the chromatographic separation of single- and double-stranded nucleic acids in 200 [μm] i.d. monolithic poly(styrene-divinylbenzene) capillary columns was utilized for mutation screening in polymerase chain reaction amplified polymorphic loci. Recognition of mutations is based on the separation of homo- and heteroduplex species by ion-pair reversed-phase high-performance liquid chromatography (IP-RP-HPLC) under partially denaturing conditions, resulting in characteristic peak patterns both for homozygous and heterozygous samples. Six different single nucleotide substitutions and combinations thereof were confidently identified in 413 bp amplicons from six heterozygous individuals each of which yielded a different unique chromatographic profile. Alternatively, mutations were identified in short, 62 bp PCR products upon their complete on-line denaturation at 75[deg]C taking advantage of the ability of IP-RP-HPLC to resolve single-stranded nucleic acids of identical length that differ in a single nucleotide. Separations in monolithic capillary columns can be readily hyphenated to electrospray ionization mass spectrometry and promise increased sample throughput by operating in arrays similar to those already used in capillary electrophoresis.

Katsahambas, S. and M. T. W. Hearn (1996). "Application of an optimised reverse transcriptase-polymerase chain reaction method to determine the cDNA nucleotide sequence of porcine basic fibroblast growth factor." Journal of Biochemical and Biophysical Methods **33**(3): 231.

<http://www.sciencedirect.com/science/article/B6T28-3Y2FV5D-8/2/ee6a7e7dcb66b95b444a76937936a61d>

In the present investigation, oligonucleotide primers of high hybridisation stringency have been used in combination with optimised reverse transcriptase-polymerase chain reaction (RT-PCR) methods for the determination of the cDNA sequence corresponding to porcine FGF-2 mRNA present in brain and uterine tissue. Application of these optimised methods have overcome previous limitations associated with the low abundance of the porcine FGF-2 mRNA, and allowed as little as 100 [μg] of tissue to be employed to generate the complete cDNA nucleotide sequences as well as to provide specific template fragments selected for their suitability in subsequent ligation and mutagenesis studies with conventional expression vectors. Comparisons of the cDNA nucleotide and the deduced amino-acid sequence of porcine FGF-2 and the known FGF-2s from other species have indicated nucleotide sequence homologies of 95.5% with the bovine, 94.7% with the human and 88.7% with the rat FGF-2 cDNA whilst amino-acid sequence homologies of 100% with the bovine, 98.7% with the human and 96.8% with the rat FGF-2, respectively, were found. Based on these investigations, application of analogous strategies and methods with low abundance mRNAs related to other members of this family of growth factors, as well as very low abundance mRNAs of other protein growth factor, in the pig should now be readily realised.

Kosaki, K., H. Yoshihashi, et al. (2001). "Fluorescence-based DHPLC for allelic quantification by single-nucleotide primer extension." Journal of Biochemical and Biophysical Methods **47**(1-2): 111.

<http://www.sciencedirect.com/science/article/B6T28-429Y53Y-D/2/bdeb926e5ac4cb9aa646cd408b5ba262>

We have investigated the possibility of determining quantitatively the alleles of binary DNA polymorphisms by single-nucleotide primer extension (SNUPE) and fluorescence-based DHPLC. Using a polymorphism of interest to our group, ROX-labeled dideoxy CTP (ROX-ddCTP) was incorporated at the 3' end of the primer annealed to the template adjacent to the polymorphic site. The primer extension product was then resolved from the unincorporated dye terminator by ion-pair reversed-phase liquid chromatography. The signal intensity of incorporated ROX-ddCTP correlated well over one order of magnitude with the relative amount of the C-allele present in the genomic DNA template. We conclude that SNUPE, when combined with fluorescence-based DHPLC, can accurately determine the relative molar proportion of one allele in total DNA.

Lombardi, M. P., M. J. B. van den Hoff, et al. (2003). "Expression analysis of subtractively enriched libraries (EASEL): a widely applicable approach to the identification of differentially expressed genes." Journal of Biochemical and Biophysical Methods **57**(1): 17.

<http://www.sciencedirect.com/science/article/B6T28-48JK5S4-2/2/dc5e5536d80fb6c6e9d899399170b7e5>

A variety of methods for high throughput analysis of differential gene expression has been developed over the past years. We have implemented the EASEL technique that adds flexibility, efficiency and wide-applicability to these methods. The EASEL procedure is unique as it integrates several well established techniques and thereby offers a combination of subtractive hybridization of 3' cDNA ends with macroarrays analysis and Serial Analysis of Gene Expression (SAGE). In addition, once a set of interesting, differentially expressed genes is identified, the material required for follow up studies to test the hypothesis that the gene is truly involved in the process of interest is readily available. In this report, we first present a step-by-step validation of the procedure, since several of the incorporated steps had to be tailored to meet specific requirements and implied drastic modifications of the original methods. Secondly, we applied EASEL to the identification of up-regulated gene products in the outflow tract region of the embryonic rat heart. Here we provide evidence that at least two among the differentially expressed genes detected, follistatin-like protein gene and membrane type 1-metallo proteinase gene, are selectively up-regulated in the outflow tract, suggesting their involvement in the development of this region during embryogenesis.

Nath, K., J. W. Sarosy, et al. (2000). "Effects of ethidium bromide and SYBR(R) Green I on different polymerase chain reaction systems." Journal of Biochemical and Biophysical Methods **42**(1-2): 15.

<http://www.sciencedirect.com/science/article/B6T28-3Y15WC0-2/2/f503da156ac52de77a9f31e22913133e>

In an in-gel polymerase chain reaction (PCR), the generation of a 1750-bp yeast DNA fragment was inhibited when yeast DNA gel-stabs or gel-slices stained with ethidium bromide (EtBr) or SYBR(R) Green I were used. Similar inhibition occurred to a varying degree in the reamplification of PCR fragments in prokaryotic systems. Inclusion of the dyes in PCR resulted in an inhibition at about 10 [mu]g/ml EtBr and at 10,000-20,000-fold dilution of SYBR(R) Green I in all systems. The effect remained unchanged despite increasing the PCR cycles to 40. However, increasing the magnesium chloride concentration did reverse the inhibitory actions, although the PCR specificity was lost. In an unusual observation, we find that, at higher dye concentrations (50 [mu]g/ml EtBr,

or thousand fold dilution of SYBR(R) Green I), the input yeast DNA electrophoretic profile is maintained following 25 PCR cycles (despite a denaturation temperature of 94[deg]C). It varied significantly in different DNA systems and was readily reversed by high Mg++ concentrations. It is concluded that, at low Mg++ concentrations, different PCR systems are inhibited to varying extents by intercalating dyes and, in some PCR systems, intercalating dyes at unusually high concentrations maintain input DNA electrophoretic profile.

Nordstrom, T., A. Alderborn, et al. (2002). "Method for one-step preparation of double-stranded DNA template applicable for use with Pyrosequencing(TM) technology." Journal of Biochemical and Biophysical Methods **52**(2): 71.

<http://www.sciencedirect.com/science/article/B6T28-46HJMPJ-1/2/a26d61dd3fd4580c69894558dfd3f6a6>

A new one-step method for fast and efficient preparation of double-stranded DNA template, suitable for use with Pyrosequencing(TM) technology, has been developed. In the new method, two different types of oligonucleotides were used to prevent reannealing of remaining PCR primers to the template: oligonucleotides complementary to the PCR primers and 3'-end modified oligonucleotides with the same sequence as the PCR primers. Advantages with the new strategy are: (i) faster and simpler template preparation procedure (one-step); (ii) no need for exonuclease I treatment; and (iii) less problem with unspecific priming from loop structures and dimers. By careful oligonucleotide design, and/or by addition of single-stranded DNA-binding protein, problems with unspecific sequence signals due to mispriming can be reduced. The new method was used for analysis of genotype variations within the renin-angiotensin-aldosterone system.

Roberts, P. S., S. Jozwiak, et al. (2001). "Denaturing high-performance liquid chromatography (DHPLC) is a highly sensitive, semi-automated method for identifying mutations in the TSC1 gene." Journal of Biochemical and Biophysical Methods **47**(1-2): 33.

<http://www.sciencedirect.com/science/article/B6T28-429Y53Y-4/2/94f0c3b5a800e14a5b834f4c502b0815>

Sensitive and automated methods for the detection of DNA sequence variation are required for a wide variety of genetic studies. Diagnostic testing in human genetic disorders is one application of such methods. Tuberous sclerosis complex (TSC) is an autosomal dominant familial tumor syndrome characterized by the development of benign tumors (hamartomas) in multiple organs (OMIM # 19110, #191092). There is a high frequency of sporadic cases and significant demand from patients and families for genetic testing information. Two TSC genes have been identified (TSC1 and TSC2) and together account for all cases [1 and 2]. Here we report our methods for DHPLC analysis of the TSC1 gene and demonstrate the high sensitivity of this method in a blinded analysis of 21 TSC patients with known TSC1 mutations. In this series, DHPLC detected 27/28 (96%) known TSC1 sequence variations. The only sequence variation not identified by DHPLC in this study is a mosaic case.

Seth, D., M. D. Gorrell, et al. (2003). "SMART amplification maintains representation of relative gene expression: quantitative validation by real time PCR and application to studies of alcoholic liver disease in primates." Journal of Biochemical and Biophysical Methods **55**(1): 53.

<http://www.sciencedirect.com/science/article/B6T28-47MSRBD->

1/2/e903600f32d9314a752839b8ea02cad7

The small quantities of tissue available for most studies of human disease are a significant limitation for meaningful gene expression profiling. The Atlas Switch Mechanism At the 5' end of Reverse Transcript (SMART(TM)) probe amplification kit uses as little as 50 ng of total RNA to generate complex cDNA probes for DNA array and other analyses. However, the extent to which this attractive methodology maintains representation of relative gene expression has not been quantified. In this study, we demonstrate using real-time quantitative PCR analysis that the relative expression levels of a range of low- to high-abundance mRNAs are retained after SMART amplification independent of transcript abundance and full-length transcript, coding region and PCR product size. Using this technology, a mean amplification of 3800-fold was achieved in human liver samples, greatly enhancing the ability to perform replicate DNA array experiments. Probes generated with the SMART amplification method were used to detect increased expression of genes involved with inflammation, fibrosis, xenobiotic metabolism, immune function, oxidant stress and endothelium in liver from the baboon model of alcoholic liver disease.

Suzuki, T., M. Akimoto, et al. (2005). "A new PCR-based approach for the preparation of RNA probe." Journal of Biochemical and Biophysical Methods **62**(3): 251.

<http://www.sciencedirect.com/science/article/B6T28-4F473MF-1/2/12e5c2652c39908f0fa0af90410868f0>

A number of PCR-based in situ hybridization (ISH) techniques have been reported to facilitate the procedure. However, those techniques require additional gene specific primers with RNA polymerase binding site. We developed a new PCR-based ISH technique without extra gene-specific primers. We amplified gene specific PCR products with regular gene-specific primer pairs. Special linker, including T7 RNA polymerase binding site, was adapted to amplified PCR products. Secondary PCR was performed with T7 primer, and forward or reverse primer, used for the first PCR to prepare template DNA for RNA transcription. We were able to generate sense and anti-sense probes for ISH in a day. Recently, real-time PCR and ISH are required to validate microarray results quantitatively and qualitatively. This technique can be expected to facilitate the high-throughput validation of transcripts detected by microarrays.

van den Boom, D., A. Ruppert, et al. (1997). "Combined amplification and sequencing in a single reaction using two DNA polymerases with differential incorporation rates for dideoxynucleotides." Journal of Biochemical and Biophysical Methods **35**(2): 69.

<http://www.sciencedirect.com/science/article/B6T28-3RC4TD2-1/2/bb6e63c5486309c3e3e295f45a1eb451>

We describe an approach, which combines the process of DNA amplification and sequence determination by using a pair of primers and two DNA polymerases with different incorporation rates for dideoxynucleotides. The process of target sequence amplification is carried out by the DNA polymerase with a low dideoxynucleotide incorporation rate while its polymerase counterpart with a high incorporation rate generates a sequence ladder. The needs for separate amplification via polymerase chain reaction (PCR) or cloning into plasmids including the respective purification steps therefore can be avoided. In addition, the use of dye terminator chemistry enables the simultaneous generation of forward and reverse sequence ladders, which can be separated based on the streptavidin-biotin system when one amplification primer is biotinylated.

Tanaka, S. M., J. Li, et al. (2003). "Effects of broad frequency vibration on cultured osteoblasts." Journal of Biomechanics **36**(1): 73.

<http://www.sciencedirect.com/science/article/B6T82-47DGWG4-9/2/52920f4949c343217d2d48064e4b36f2>

Bone is subjected in vivo to both high amplitude, low frequency strain, incurred by locomotion, and to low amplitude, broad frequency strain. The biological effects of low amplitude, broad frequency strain are poorly understood. To evaluate the effects of low amplitude strains ranging in frequency from 0 to 50 Hz on osteoblastic function, we seeded MC3T3-E1 cells into collagen gels and applied the following loading protocols for 3 min per day for either 3 or 7 days: (1) sinusoidal strain at 3 Hz, with 0-3000 μ strain peak-to-peak followed by 0.33 s resting time, (2) "broad frequency vibration" of low amplitude strain (standard deviation of 300 μ strain) including frequency components from 0 to 50 Hz, and (3) sinusoidal strain combined with broad frequency vibration (S+V). The cells were harvested on day 4 or 8. We found that the S+V stimulation significantly repressed cell proliferation by day 8. Osteocalcin mRNA was up-regulated 2.6-fold after 7 days of S+V stimulation, and MMP-9 mRNA was elevated 1.3-fold after 3 days of vibration alone. Sinusoidal stimulation alone did not affect the cell responses. No differences due to loading were observed in alkaline phosphatase activity and in mRNA levels of type I collagen, osteopontin, connexin 43, MMPs-1A, -3, -13. These results suggest that osteoblasts are more sensitive to low amplitude, broad frequency strain, and this kind of strain could sensitize osteoblasts to high amplitude, low frequency strain. This suggestion implies a potential contribution of stochastic resonance to the mechanical sensitivity of osteoblasts.

Ahmed, K., S. Chohnan, et al. (2003). "Purification, bacteriolytic activity, and specificity of [beta]-lytic protease from *Lysobacter* sp. IB-9374." Journal of Bioscience and Bioengineering **95**(1): 27.

<http://www.sciencedirect.com/science/article/B6VSD-49GYVN6-1S/2/a1db729715796e2ced6ed23648e92595>

Lysobacter sp. IB-9374, which was isolated from soil as a high lysyl endopeptidase-producing strain (Chohnan et al., FEMS Microbiol. Lett., 213, 13-20, 2002), was found to produce a [beta]-lytic protease capable of lysing gram-positive bacteria such as *Staphylococcus aureus*, *Micrococcus eus*, and *Bacillus subtilis*. The *Lysobacter* strain secreted the [beta]-lytic protease into the culture medium at a 2.4-fold higher level than *Achromobacter lyticus*. The enzyme was highly purified through a series of six steps with a high yield. The enzyme was strongly inhibited by tetraethylene-pentamine and 1,10-phenanthroline. The purified enzyme lysed more efficiently almost all the gram-positive bacteria tested than lysozyme, lysostaphin, and mutanolysin. The enzyme was very similar to *Achromobacter* [beta]-lytic protease containing one zinc atom in terms of amino acid composition and N-terminal sequence. The nucleotide sequence revealed that the mature enzyme was composed of 179 amino acid residues with additional 198 amino

acids at the amino-terminal end of the enzyme. The deduced amino acid sequence of the mature enzyme coincided with that of the *Achromobacter* enzyme, although the prepro-region showed a 41% sequence identity with the counterpart. These results indicate that *Lysobacter* sp. is a useful strain for an efficient large-scale preparation of [beta]-lytic protease capable of lysing bacteria.

Fujii, T., H. Sugino, et al. (2002). "Characterization of the microbial community in an anaerobic ammonium-oxidizing biofilm cultured on a nonwoven biomass carrier." Journal of Bioscience and Bioengineering **94**(5): 412.

<http://www.sciencedirect.com/science/article/B6VSD-48KCSM4-7/2/3e71266ed9da71523ce58d428ed912d9>

The enrichment and characterization of anaerobic ammonium-oxidizing biofilm cultures are ongoing in our laboratories. Biomass, with a predominately red color, demonstrating simultaneous removal of ammonium and nitrite under autotrophic and anoxic conditions, which is characteristic of anaerobic ammonium-oxidizing planctomycetes, was enriched and maintained for an extended period on a polyester nonwoven carrier. To investigate the bacterial composition of the mature biofilm community, 16S rDNA sequences were amplified by PCR and comparative analyses using DNA databases were conducted. Only one sequence had a notable similarity (92.2%) to that of the first discovered anaerobic ammonium-oxidizing planctomycete and lesser, yet significant, similarities to the 16S rDNA sequences of other recently reported anaerobic ammonium-oxidizing strains. The newly discovered strain (designated KSU-1) reported here was dominant among detectable members of the biofilm community. By fluorescence imaging, KSU-1 was shown to form spherical clusters wrapped in a thin layer of *Zoogloea* sp. Possible interactions and interdependencies of these two species are discussed with regard to the putative unculturability of the anaerobic ammonium-oxidizing planctomycetes.

Kato, T., M. Haruki, et al. (2001). "Isolation and characterization of long-chain-alkane degrading *Bacillus thermoleovorans* from deep subterranean petroleum reservoirs." Journal of Bioscience and Bioengineering **91**(1): 64.

<http://www.sciencedirect.com/science/article/B6VSD-43CTKX4-D/2/929454cd3e5a3820325c2ebbae3ed357>

Two extremely thermophilic alkane-degrading bacterial strains, B23 and H41, were respectively isolated from deep subterranean petroleum reservoirs in the Minami-aga (Niigata) and Yabase (Akita) oil fields. Both strains were able to grow at temperatures ranging from 50 to 80[deg]C, with optimal growth at 70[deg]C for B23 and 65[deg]C for H41. From 16S rRNA gene sequence analysis and physiological characterization, both strains were identified as *Bacillus thermoleovorans* (identities of 99.5% and 99.6% to strain DSM 5366, and 98.3% and 98.7% to the type strain LEH-1TS, respectively). Strains B23 and H41 effectively (60%) degraded n-alkanes longer than C12 and C15, respectively, at 70[deg]C, while strain LEH-1TS degraded undecane (C11) most effectively. When B23 and H41 were cultivated in the presence of heptadecane, heptadecanoate and pentadecanoate were specifically accumulated in the cells. These results strongly suggest that the two strains degraded n-alkanes by a terminal oxidation pathway, followed by a [beta]-oxidation pathway.

Kawagoshi, Y., S. Nakamura, et al. (2004). "Isolation of aryl-phosphate ester-degrading bacterium from leachate of a sea-based waste disposal site." Journal of Bioscience and Bioengineering **98**(6):

<http://www.sciencedirect.com/science/article/B6VSD-4FB66DB-B/2/6c84c70a46d677c72124dd0522a0aa39>

An aryl-phosphate ester (APE)-degrading bacterium was isolated from the leachate of a sea-based waste disposal site. The isolated APE-degrading bacterial strain YS-57 grew well in a medium containing glucose and NaCl, and degraded two types of APE: tricresyl phosphate and triphenyl phosphate. The optimal temperature, pH, and NaCl concentration for the growth of strain YS-57 were 30[deg]C, 7.0, and 1.0%, respectively. Strain YS-57 grew at an APE concentration of 25 mg/l without being inhibited. APEs were degraded by the supernatant of the medium in which strain YS-57 was cultured, suggesting that the APE-degrading enzyme was released into the extracellular space in the logarithmic growth phase. The 16S rDNA sequence of strain YS-57 showed 95.6% similarity to that of *Roseobacter gallaeciensis* and the morphological properties were also comparable. Consequently, strain YS-57 was closely related to the genus *Roseobacter*.

Matsubara, Y., R. Kawada, et al. (2000). "Cloning and sequence analysis of a gene (aly PG) encoding poly([alpha]-guluronate) lyase from *Corynebacterium* sp. strain ALY-1." Journal of Bioscience and Bioengineering **89**(2): 199.

<http://www.sciencedirect.com/science/article/B6VSD-409W3NW-J/2/ad5d3b710b04dbf583031e77feedd0ad>

The aly PG gene, coding for a poly [alpha]-guluronate lyase (PG lyase) of *Corynebacterium* strain ALY-1, was cloned and sequenced. The gene consists of 768 bp encoding a signal peptide of 32 amino acids and a mature protein of 224 amino acids. Two disulfide bond cross-linkages were found to be formed between Cys-4 and Cys-51 and between Cys-200 and Cys-206 in the native PG lyase molecule. The deduced amino acid sequence of the *Corynebacterium* sp. aly PG gene exhibited 29% homology toward that of the *Klebsiella pneumoniae*, subsp. *aerogenes* aly A gene, with two conserved regions (the amino acid sequences from Y-102 to M-110 and from Y-221 to Q-229).

Minamida, K., I. N. Sujaya, et al. (2004). "The effects of Di--fructofuranose-1,2':2,3'-dianhydride (DFA III) administration on human intestinal microbiota." Journal of Bioscience and Bioengineering **98**(4): 244.

<http://www.sciencedirect.com/science/article/B6VSD-4DTSGN1-3/2/ccdfa0294e10555a9ff58320dfda8cd4>

Di--fructofuranose-1,2':2,3'-dianhydride (DFA III) was shown to enhance Ca absorption in rat and human intestine. The effects of DFA III administration (9 g per day for 4 weeks that corresponded to 3-fold the optimal dosage of DFA III) on human intestinal microbiota were studied using denaturing gradient gel electrophoresis (DGGE). The major groups of human intestinal microbiota reported previously: the *Bacteroides*, the *Clostridium* *coccoides* group (*Clostridium* cluster XIVa), the *Clostridium* *leptum* group (*Clostridium* cluster IV), and the *Bifidobacterium* group were detected. The similarity of 30 DGGE profiles based on the V3 region (before and after administration to the 15 subjects) of the 16S rDNA were calculated using Pearson's correlation based on numbers, positions and intensity of bands, and then a dendrogram of DGGE profiles was constructed by the unweighted pair group method using arithmetic average (UPGMA) clustering method. By these analyses, no difference in DGGE profiles after DFA III administration

was observed in healthy subjects, while two subjects with chronic constipation showed different profiles, namely on numbers, positions and the intensity of some bands. Their stools were softer and stool frequencies increased and they obtained relief from constipation.

Mori, K., M. Hatsu, et al. (2000). "Effect of heavy metals on the growth of a methanogen in pure culture and coculture with a sulfate-reducing bacterium." Journal of Bioscience and Bioengineering **90**(3): 260.

<http://www.sciencedirect.com/science/article/B6VSD-41XVBHW-6/2/1ac265263170e5445202f4e0e2271b65>

The sensitivity of a methanogen and sulfate-reducing bacterium isolated from a sea-based landfill site to Cd²⁺ and Cu²⁺ was studied. Methanogens and sulfate-reducing bacteria in leachates of the waste disposal site were enumerated using the MPN method. Methanobacterium thermoautotrophicum KHT-2, isolated from the leachate, could not grow at 0.5 mM Cd²⁺ or 1.0 mM Cu²⁺. Desulfotomaculum sp. RHT-3, isolated from the same leachate, was able to insolubilize 3.0 mM Cd²⁺ or 2.0 mM Cu²⁺ by production of hydrogen sulfide. When strains KHT-2 and RHT-3 were cultured together in the presence of the heavy metals, strain KHT-2 could grow at high heavy metal concentrations after insolubilization of the metals by strain RHT-3.

Nakano, H., K. Kobayashi, et al. (2000). "Single-step single-molecule PCR of DNA with a homo-priming sequence using a single primer and hot-startable DNA polymerase." Journal of Bioscience and Bioengineering **90**(4): 456.

<http://www.sciencedirect.com/science/article/B6VSD-426YS50-N/2/b7e99e44285b101e467aded8c40d3e66>

We have previously reported that a protein library can be constructed by directly combining PCR amplification of a single DNA molecule and cell-free protein synthesis. To specifically amplify single DNA molecules, however, two-step PCR with nested primers was used. Here we describe a simpler method for single-step amplification of a single molecule. The method involves the use of both hot-startable DNA polymerase and a DNA template that has homo-priming sequences at both ends for amplification using a single primer. These two modifications greatly decreased the possibility of formation and subsequent accumulation, respectively, of primer-dimers that inhibit the amplification of target template. In addition, a high-fidelity DNA polymerase was successfully used, resulting in the significant reduction of the accumulation of mutations during amplification.

Pedro, M. S., S. Haruta, et al. (2003). "Isolation and characterization of predominant microorganisms during decomposition of waste materials in a field-scale composter." Journal of Bioscience and Bioengineering **95**(4): 368.

<http://www.sciencedirect.com/science/article/B6VSD-49D202X-8/2/1bb08b5d0ffa9cdb9e4235d382c0a7bb>

A self-heating field-scale composter treating agro-industrial wastes within a period of 30 d was analyzed by denaturing gradient gel electrophoresis (DGGE) (Pedro et al., J. Biosci. Bioeng., 91, 159-165, 2001). Three major bands were derived from Propionibacterium acnes, Methylobacterium mesophilicum or M. radiotolerans, and Bacillus thermocloacae. Strains MSP09A and MSP06G with close affiliation to P. acnes and B. thermocloacae, respectively, were

successfully isolated. Based on quantitative-PCR results, the relative population of MSP09A increased towards the end of the composting process (mesophilic stage) while MSP06G seemed to predominate during the middle period (thermophilic stage). These results correlated highly with their growth temperatures. MSP09A and MSP06G had different metabolic profiles which were largely affected by culture conditions. MSP09A was able to utilize large complex molecules of lipids and proteins. An interspecies relationship in terms of metabolites such as propionic acid was expected between the two microorganisms.

Salvador Pedro, M., S. Haruta, et al. (2001). "Denaturing gradient gel electrophoresis analyses of microbial community from field-scale composter." Journal of Bioscience and Bioengineering **91**(2): 159.

<http://www.sciencedirect.com/science/article/B6VSD-431BBWD-9/2/25a8761c69c583fa0c0e8e515c6e065a>

The diversity of microbial community during the decomposition of waste in a field-scale composter (Hazaka system) was investigated by denaturing gradient gel electrophoresis (DGGE). The composter operates at a high temperature through a self-heating system, creating a thermophilic (60-76[deg]C) stage during the initial phase and a mesophilic (45[deg]C) stage towards the later phase of the composting period. The pH of the system (pH 7.75-8.10) did not vary significantly during the process while moisture content was reduced from 48.8% to 25.1%. DGGE and 16S rDNA analyses showed that the following genera were found throughout the process: *Propionibacterium* sp., *Methylobacterium* sp., *Pseudomonas* sp., and *Bradyrhizobium* sp. Different *Bacillus* spp. thrive at the thermophilic or the mesophilic stage while *Clostridium* sp. was only found at the initial phase of the process. *Staphylococcus* sp. and *Caulobacter* sp. or *Brevundimonas* sp. existed during the later phase of the composting period.

Sujaya, I. N., Y. Tamura, et al. (2003). "Development of internal transcribed spacer regions amplification restriction fragment length polymorphism method and its application in monitoring the population of *Zygosaccharomyces rouxii* M2 in miso fermentation." Journal of Bioscience and Bioengineering **96**(5): 438.

<http://www.sciencedirect.com/science/article/B6VSD-4BCWP93-5/2/baa6010b89632bd885fa91aadb7a62f5>

Recently, the use of the dry yeast of *Zygosaccharomyces rouxii* M2 for miso (soybean paste) fermentation has been established. A molecular monitoring method was developed and validated in this study to analyze the population of *Z. rouxii* M2 during the fermentation. The method was based on the restriction patterns of internal transcribed spacer (ITS) regions of the rDNA using *HaeIII* and *HhaI*. Among the homologous ITS regions of *Z. rouxii* strains, *Z. rouxii* M2 produced diagnostic bands by which it can be differentiated from the other strains used. The specific restriction bands were due to the difference in nucleotide sequence of two different copies of ITS of *Z. rouxii* M2. Both ITS copies showed 94% sequence similarity but a 13-bp nucleotide substitution and a 19-bp deletion were found in the ITS1 region. Phylogenetic trees were constructed based on ITS and 18S rDNA sequences and it was found that the ITS sequences provide better resolution for the classification of *Z. rouxii* M2. Since *Z. rouxii* M2 is a promising strain for use in miso fermentation as a dry starter, the method developed is significant in terms of industrial application in monitoring the growth of *Z. rouxii* M2 in miso fermentation.

Yin, Q. and Q. Zheng (2005). "Isolation and identification of the dominant *Lactobacillus* in gut and faeces of pigs using carbohydrate fermentation and 16S rDNA analysis." Journal of Bioscience and Bioengineering **99**(1): 68.

<http://www.sciencedirect.com/science/article/B6VSD-4FPN41C-C/2/bdba62d68269b852bb0901795855e6a1>

The gut contents and faeces of 9 adult pigs and 387 colonies growing on MRS culture (selective for *Lactobacillus*) were sampled to isolate and identify *Lactobacillus* species present. The results showed that the number of bacteria growing on MRS culture increased from the anterior to posterior of the gut and decreased in the faeces (e.g., stomach, 4.04×10^5 cfu/g; small intestine, 9.77×10^7 cfu/g; large intestine 3.85×10^{11} cfu/g; faeces, 1.22×10^{11} cfu/g). Fifty-two lactobacilli-like colonies were selected from 387 on the basis of their size and Gram staining, and used for the fermentation of 11 carbohydrates, from which 12 lactobacilli were selected for 16S rDNA analysis. The result showed that *Lactobacillus ruminis* was the dominant *Lactobacillus* in the stomach, small intestine, large intestine and faeces of pigs. The identification of most strains estimated by their 16S rDNA was 98-99% accurate.

Yoza, B., A. Arakaki, et al. (2003). "Fully automated DNA extraction from blood using magnetic particles modified with a hyperbranched polyamidoamine dendrimer." Journal of Bioscience and Bioengineering **95**(1): 21.

<http://www.sciencedirect.com/science/article/B6VSD-49GYVN6-1R/2/c472f5041ed10e830ce1c1e1c805ddbf>

Bacterial and artificial magnetic particles were modified using a polyamidoamine (PAMAM) dendrimer and outer shell amines determined. Bacterial magnetic particles were the most consistently modified. Transmission electron microscopic (TEM) analysis showed that the artificial magnetic particles were structurally damaged by the modification process including sonication. Furthermore, laser particle analysis of the magnetite also revealed damage. Small quantities of dendrimer-modified bacterial magnetic particles were used to extract DNA from blood. The efficiency of DNA recovery was consistently about 30 ng of DNA using 2-10 [μg] of dendrimer-modified bacterial magnetite. This technique was fully automated using newly developed liquid handling robots and bacterial magnetic particles.

Journal of Biotechnology (18)

Bangrak, P., P. Graidist, et al. (2004). "Molecular cloning and expression of a mammalian homologue of a translationally controlled tumor protein (TCTP) gene from *Penaeus monodon* shrimp." Journal of Biotechnology **108**(3): 219.

<http://www.sciencedirect.com/science/article/B6T3C-4BSVBH3-1/2/c4e9bc3a418367b798304506578fbdfa>

White spot syndrome, caused by white spot syndrome virus (WSSV), is a deadly disease of shrimps, causing a catastrophic loss in shrimp industries worldwide. In order to investigate molecular response of shrimp haemocyte to WSSV infection, we performed subtraction

hybridization of mRNAs from healthy and WSSV-infected haemocyte. One of the genes that were severely down-regulated in moribund WSSV-infected-haemocyte was translationally controlled tumor protein (TCTP) (or fortilin). Strikingly, while there was a slight difference in the amount of TCTP message between normal and early WSSV-infected shrimps, shrimps that exhibited severe symptoms uniformly had very little TCTP in their haemocyte. Taken together with the fact that TCTP functions as an anti-apoptotic protein in mammals, our data suggest that TCTP in shrimp protects WSSV-infected shrimps from death.

Barlaan, E. A., M. Sugimori, et al. (2005). "Electronic microarray analysis of 16S rDNA amplicons for bacterial detection." Journal of Biotechnology **115**(1): 11.

<http://www.sciencedirect.com/science/article/B6T3C-4DNB0SC-1/2/9b91c5ad283767c1ef860a53ee0ff38b>

Electronic microarray technology is a potential alternative in bacterial detection and identification. However, conditions for bacterial detection by electronic microarray need optimization. Using the NanoChip electronic microarray, we investigated eight marine bacterial species. Based on the 16S rDNA sequences of these species, we constructed primers, reporter probes, and species-specific capture probes. We carried out two separate analyses for longer (533 bp) and shorter (350 and 200 bp) amplified products (amplicons). To detect simultaneously the hybridization signals for the 350- and 200-bp amplicons, we designed a common reporter probe from an overlapping sequence within both fragments. We developed methods to optimize detection of hybridization signals for processing the DNA chips. A matrix analysis was performed for different bacterial species and complementary capture probes on electronic microarrays. Results showed that, when using the longer amplicon, not all bacterial targets hybridized with the complementary capture probes, which was characterized by the presence of false-positive signals. However, with the shorter amplicons, all bacterial species were correctly and completely detected using the constructed complementary capture probes.

Cox, M., G. Gerritse, et al. (2001). "Characterization of the promoter and upstream activating sequence from the *Pseudomonas alcaligenes* lipase gene." Journal of Biotechnology **86**(1): 9.

<http://www.sciencedirect.com/science/article/B6T3C-42D2C33-2/2/10035fdf5e07870efb6874c8619c7ce4>

Pseudomonas alcaligenes secretes a lipase with a high pH optimum, which has interesting properties for application in detergents. The expression of the lipase is strongly dependent on the presence of lipids in the growth medium such as soybean oil. The promoter of the gene was characterized and found to have resemblance to σ^{54} controlled promoters, which are known to be tightly regulated. The transcription start was mapped precisely downstream of a sequence with close similarity to the -12/-24 consensus sequence of σ^{54} controlled promoters. Interestingly, a hyperproducer mutant strain was isolated and found to have a C to T mutation in the -12/-24 promoter consensus region. In addition an Upstream Activating Sequence (UAS) with homology to σ^{54} UAS consensus sequences was identified. It was demonstrated that an increase of the distance from the UAS to the transcription start or the deletion of the UAS results in significantly lower expression levels of lipase. A systematic mutational analysis of the UAS sequence has resulted in a variant with an increased lipase expression.

Gerritse, G., R. Ure, et al. (1998). "The phenotype enhancement method identifies the Xcp outer membrane secretion machinery from *Pseudomonas alcaligenes* as a bottleneck for lipase production." Journal of Biotechnology **64**(1): 23.

<http://www.sciencedirect.com/science/article/B6T3C-3TXT1RT-4/2/087c52ad9fe7ed6bb35a2dc49dcad9a9>

Pseudomonas alcaligenes M-1 has been selected from an intensive screening for micro-organisms that can naturally produce a lipase active in detergent formulations. The lipase expression has been increased to allow high level secretion from *Pseudomonas alcaligenes*, via the introduction of multi-copy plasmids. In order to improve the lipase yield further, the phenotype enhancement method has been developed. This idea comprises the reintroduction of a cosmid library with random chromosomal fragments in a *P. alcaligenes* strain with already high lipase productivity. One of the strains which showed an enhanced lipase production appeared to contain a cosmid encoding the outer membrane secretion genes. These xcp-genes are clustered in two divergently transcribed operons similar to the situation in *Pseudomonas aeruginosa*. Remarkably and dissimilar to *P. aeruginosa*, in between the two xcp gene clusters, two reading frames of unknown function--OrfV and OrfX--are present. For OrfX no equivalent can be found in the known protein data bases. On the other hand, OrfV shows homology to the regulatory proteins MalT and AcoK. Some evidence is provided that suggests that OrfV acts as a regulator of the xcp operons. A model is proposed for the regulation of the secretion system from *P. alcaligenes*.

Hoshino, T., S. Tsuneda, et al. (2003). "In situ PCR for visualizing distribution of a functional gene "amoA" in a biofilm regardless of activity." Journal of Biotechnology **105**(1-2): 33.

<http://www.sciencedirect.com/science/article/B6T3C-497RD6K-5/2/ac6be9a7b587218391573b694fc36425>

In this study, ammonia-oxidizing bacteria present in biofilms resulting from a nitrifying reactor were detected by both a conventional FISH technique and an original in situ PCR technique. Both techniques showed that ammonia-oxidizing bacteria were found near the surface of the biofilms. However, after the biofilm had been exposed to 2 weeks of ammonia starvation, ammonia-oxidizing bacteria present in the biofilm could not be detected by fluorescence in situ hybridization (FISH) because they did not have sufficient copies of rRNA. In contrast, ammonia-oxidizing bacteria could be detected by in situ PCR with strong signal. It was thus demonstrated that a cell possessing a specific functional gene is detectable by in situ PCR regardless of its activity.

Lee, M.-H. and J.-W. Kwak (2003). "Expression and functional reconstitution of a recombinant antibody (Fab') specific for human apolipoprotein B-100." Journal of Biotechnology **101**(2): 189.

<http://www.sciencedirect.com/science/article/B6T3C-47P1TYB-3/2/037704017bc58504efc5473a0da9615d>

We have cloned and constructed plasmid vectors, pETB23H and pETB23L, for bacterial expression of heavy (H) and light (L) chain cDNAs of Fab' of mAbB23 a monoclonal antibody specific to human plasma apolipoprotein (apo) B-100. The H- and L-chains were expressed as insoluble inclusion bodies in the cytoplasm of *Escherichia coli*. The inclusion bodies of both chains were isolated from the cell lysate, solubilized in 6 M guanidium-HCl, and mixed in equal molar amounts. Refolding was performed in three stages of dialysis: first, dialysis against 3 M guanidium buffer, next, continuous decrement of guanidium in the dialysis buffer through slow addition of 1 M guanidium buffer, and finally, dialysis against a buffer without guanidium. After the

refolding, active Fab' (rFab') was purified through an apo B-100-coupled affinity column. When compared by ELISA, the rFab' had a slightly decreased antigen-binding activity (about 0.7-fold) compared with native Fab. The refolding yield was maximum (75%) when performed at the protein concentrations not more than 0.4 mg ml⁻¹, whereas the yield decreased exponentially at higher concentrations. The maximum recovery was obtained at the refolding concentration of 1.8 mg ml⁻¹, where the yield was about 45%. Overall, 2.4-3.0 mg of active rFab' specific to apo B-100 was successfully obtained from 1 l cultivation of E. coli cells.

Murakami, Y., K. Motohashi, et al. (1994). "Micromachined electroporation system for transgenic fish." Journal of Biotechnology **34**(1): 35.

<http://www.sciencedirect.com/science/article/B6T3C-47DKVN8-69/2/faf17e0f000e06bad7a6fb02f1634aab>

Luciferase gene was introduced into fertilized eggs of medaka by a localized electric field between thin film electrodes formed on a glass plate. Miniaturization of the electrodes enabled us to apply a localized electric field to the animal pole of the fertilized egg. Biochemical luminescence and an electrophoresis pattern showed expression and integration of the gene, respectively. The presented system had a higher ratio of gene introduction than the conventional electroporation method.

Nakagawa, T., T. Tanaka, et al. (2005). "Fabrication of amino silane-coated microchip for DNA extraction from whole blood." Journal of Biotechnology **116**(2): 105.

<http://www.sciencedirect.com/science/article/B6T3C-4DXBSVF-4/2/6e9182282cb57ba1937b65701215f8c6>

A simple microchip device for DNA extraction was constructed based on electrostatic interactions between surface amine groups and DNA. Microchannel was fabricated on silicon wafer by photolithography and coated with 3-aminopropyltriethoxysilane (APTES) or 3-[2-(2-aminoethylamino)-ethylamino]-propyltrimethoxysilane (AEEA) to introduce amine groups on the surface. Determination of the number of surface amine groups and optimization of DNA capture condition were demonstrated to characterize the microchip. Capacities of capturing DNA were approximately 97 ng/cm² in APTES and 194 ng/cm² in AEEA modified microchips, respectively. The amount of DNA captured in the microchip increased depending on surface amine density. Furthermore, DNA extraction using amine-coated microchip from whole blood was examined. Quantification of DNA and proteins in washing or eluting fraction indicates that proteins were removed at washing steps and only DNA was effectively eluted by changing alkalinity of buffer from pH 7.5 to 10.6. The amount of DNA extracted from whole blood was approximately 10 ng and its recovery ratio was 27-40%. Performance of PCR for the eluted fraction indicates that DNA extracted from whole blood was well purified using amine-coated microchip.

Neves-Petersen, M. T., E. I. Petersen, et al. (2001). "Engineering the pH-optimum of a triglyceride lipase: from predictions based on electrostatic computations to experimental results." Journal of Biotechnology **87**(3): 225.

<http://www.sciencedirect.com/science/article/B6T3C-42WP5JM-4/2/c4cc8a2fe818bc975a9bafa0dd287c4c>

The optimisation of enzymes for particular purposes or conditions remains an important target in virtually all protein engineering endeavours. Here, we present a successful strategy for altering the pH-optimum of the triglyceride lipase cutinase from *Fusarium solani* pisi. The computed electrostatic pH-dependent potentials in the active site environment are correlated with the experimentally observed enzymatic activities. At pH-optimum a distinct negative potential is present in all the lipases and esterases that we studied so far. This has prompted us to propose the 'The Electrostatic Catapult Model' as a model for product release after cleavage of the ester bond. The origin of the negative potential is associated with the titration status of specific residues in the vicinity of the active site cleft. In the case of cutinase, the role of Glu44 was systematically investigated by mutations into Ala and Lys. Also, the neighbouring Thr45 was mutated into Proline, with the aim of shifting the spatial location of Glu44. All the charge mutants displayed altered titration behaviour of active site electrostatic potentials. Typically, the substitution of the residue Glu44 pushes the onset of the active site negative potential towards more alkaline conditions. We, therefore, predicted more alkaline pH optima, and this was indeed the experimentally observed. Finally, it was found that the pH-dependent computed Coulombic energy displayed a strong correlation with the observed melting temperatures of native cutinase.

Nielsen, P. S., H. Ohlsson, et al. (2005). "Expression profiling by oligonucleotide microarrays spotted on coated polymer slides." *Journal of Biotechnology* **116**(2): 125.

<http://www.sciencedirect.com/science/article/B6T3C-4F0181B-1/2/9a0d5bb3b3b1e37686628b1fac5932af>

We have developed a ready-to-spot polymer microarray slide, which is coated with a uniform layer of reactive electrophilic groups using anthraquinone-mediated photo-coupling chemistry. The slide coating reduces the hydrophobicity of the native polymer significantly, thereby enabling robust and efficient one-step coupling of spotted 5' amino-linked oligonucleotides onto the polymer slide. The utility of the coated polymer slide in gene expression profiling was assessed by fabrication of spotted oligonucleotide microarrays using a collection of 5' amino-linked 70-mer oligonucleotide probes representing 96 yeast genes from Operon. Two-colour hybridizations with labelled cDNA target pools derived from standard grown and heat-shocked wild type yeast cells could reproducibly measure heat shock induced expression of seven different heat shock protein (HSP) genes. Moreover, the observed fold changes were comparable to those reported previously using spotted cDNA arrays and high-density 25-mer oligonucleotide arrays from Affymetrix. The low hybridization signals obtained from the Δ SSA4 mutant cDNA target, together with the high signal detected in two-colour hybridizations with heat-shocked wild type yeast relative to the Δ SSA4 mutant strain implies that unspecific binding of cDNA target to the SSA4-specific 70-mer oligonucleotide probes is negligible. Combined, our results indicate that the coated polymer microarray slide represents a robust and cost-effective array platform for pre-spotted oligonucleotide arrays.

Rishi, A. S., N. D. Nelson, et al. (2004). "Genome walking of large fragments: an improved method." *Journal of Biotechnology* **111**(1): 9.

<http://www.sciencedirect.com/science/article/B6T3C-4CC7WVX-6/2/267b1f79a8e1db483295394a168aceb3>

The PCR-based genome walking method has been commonly used to isolate upstream regions from known cDNA sequences. The limitation of this technique is based on the location of the restriction site upstream to the gene-specific primer in the genome; hence, different restriction enzymes have to be used to isolate larger upstream fragments. In this paper, we present the advantageous use of partial and size-selected DNA as templates for genome walking, in isolating

larger upstream fragments. We have successfully tested this approach to isolate larger upstream fragments using the FailSafe(TM) PCR System. Use of partial digestion and size selection can provide better chances in obtaining larger flanking regions of known DNA sequence, when compared to use of total digested DNA.

Sentinelli, F., S. Lovari, et al. (2000). "A simple method for non-radioactive PCR-SSCP using MDE(TM) gel solution and a midi gel format: Application for the detection of variants in the GLUT1 and CTLA-4 genes." Journal of Biotechnology **78**(2): 201.

<http://www.sciencedirect.com/science/article/B6T3C-3YTJKWB-C/2/2d633a9f26de1a00f8f9fc85542a92a3>

The need to identify disease-causing mutations and DNA polymorphisms has increased with the continuing identification of new candidate genes. PCR single-strand conformation polymorphism (PCR-SSCP) is one of the techniques most widely used to identify a mutant sequence or a polymorphism in a known gene. However, the original SSCP protocols using the incorporation of radioactive label and polyacrylamide gel electrophoresis on sequencing gels for detection were labour intensive and time-consuming. Here we describe a simple SSCP protocol using MDE(TM) gel solution and a midi gel format to detect SSCP variations in the glucose transporter gene GLUT1, that we have previously analysed with the standard radioactive SSCP protocol, and we have also tested this method on the previously described point mutation (A/G transition in exon 1) of the CTLA-4 (cytotoxic T lymphocyte associated-4) gene. All known variants were detected. Based on the results, this technique appears to be simple, with no use of radioactive labels and with easy handling of the gel. Furthermore, it needs little optimisation, is relatively rapid and highly sensitive. We propose this method for the first screening for candidate gene variants.

Song, J. K., M. K. Kim, et al. (1999). "Cloning and expression of the gene encoding phospholipase A1 from *Serratia* sp. MK1 in *Escherichia coli*." Journal of Biotechnology **72**(1-2): 103.

<http://www.sciencedirect.com/science/article/B6T3C-3WNNMH42-B/2/bda75e276cf2918f6ce72faa372ba56e>

The gene encoding extracellular phospholipase A1 of *Serratia* sp. MK1 was cloned from a genomic DNA library. Formation of transparent halos on the PCY agar plates was used to identify *E. coli* carrying the phospholipase A1 gene. A 4.2 kb *EcoRI* fragment was isolated and sequenced. From nucleotide sequences and expression of various plasmids, two open reading frames (*plaA* and *plaS*) involved in efficient expression of phospholipase A1 in natural and recombinant host were identified. Extracellular phospholipase A1 activity was identified as the gene product of *plaA* encoding 321 amino acids with a predicted MW of 33400. Analysis of the amino acid sequence revealed significant homology (around 70%) to phospholipase A1 of *Serratia liquefaciens* and *Yersinia enterocolitica*. The sequence, -Gly-X1-Ser-X2-Gly-, known as a lipase-specific consensus sequence was also found in the bacterial phospholipase A1. *PlaS* encoding a protein of 224 amino acids showed no enzymatic activity, but might be necessary for the efficient expression of phospholipase A1 in *E. coli*. To further improve the production of phospholipase A1 as a soluble and active form in *E. coli*, the effect of some parameters was examined. Surprisingly, a higher yield of soluble and active phospholipase A1 could be obtained under the combined conditions of a lower temperature, an enriched medium, and a lower-strength promoter.

Su, W., S. Song, et al. (2003). "Multiplex polymerase chain reaction/membrane hybridization assay for detection of genetically modified organisms." Journal of Biotechnology **105**(3): 227.

<http://www.sciencedirect.com/science/article/B6T3C-49P498R-1/2/7260c2aa61eaf59939996c6e0941db0e>

To improve detection efficiency and result accuracy, four screening primer pairs, four identifying primer pairs, one common primer pair and corresponding probes were designed for the development of multiplex polymerase chain reaction/membrane hybridization assay (MPCR-MHA) for detection of the foreign genes insert in genetically modified organisms (GMOs). After detecting condition and parameter were optimized and determined, MPCR reactions were developed for amplifying several target genes simultaneously in one tube. Primers were labeled with biotin at the 5'-end; biotinylated MPCR products were detected by hybridization to the oligonucleotide probes immobilized on a membrane with subsequent colorimetric detection to confirm hybridization. The testing of screening primers can judge whether the sample contains GMOs, and that of identifying primers can further judge what kinds of trait genes are contained in the sample. We detected nine soybean samples, six maize samples, seven potato samples and two rice samples by the MPCR-MHA method; at the same time we also detected them with single PCR-MHA method. The results between two methods have good consistency.

Tanaka, R., M. Mizukami, et al. (2003). "Cloning and expression of the ccdA-associated thiol-disulfide oxidoreductase (catA) gene from *Brevibacillus choshinensis*: stimulation of human epidermal growth factor production." Journal of Biotechnology **103**(1): 1.

<http://www.sciencedirect.com/science/article/B6T3C-48GVVH3-6/2/cf8c06067d9f1b51e7d6e746e4eb84f1>

Brevibacillus choshinensis (*Bacillus brevis*) is a protein-hyperproducing bacterium with a useful host-vector system for the production of recombinant proteins. Here, we cloned the ccdA-catA ("height="11" width="9">cdA associated thioredoxin-like thiol-disulfide oxidoreductase) locus of *B. choshinensis* HPD31-S5. CatA protein (molecular weight, 19664) contains a thioredoxin-like motif, Cys-Gly-Pro-Cys. It was successfully expressed in *B. choshinensis* extracellularly (~100 [μ]g ml⁻¹ culture) using the secretion vector pNCMO2, and in *Escherichia coli* intracellularly (~350 [μ]g ml⁻¹ culture) with an amino-terminal His-tag. Both recombinant proteins showed thiol-disulfide oxidoreductase activity. Incubation of non-native human epidermal growth factor (hEGF) containing incorrect disulfide bonds with *B. choshinensis* cells secreting CatA protein resulted in the stimulation of the conversion of non-native hEGF to the native form. Furthermore, co-expression of CatA protein with recombinant hEGF in the *B. choshinensis* production system increased the yield of native hEGF.

Uchide, T., J. Adur, et al. (2000). "Rapid quantification of murine endothelin-1 and vasoactive intestinal contractor gene expression levels by a real-time PCR system." Journal of Biotechnology **84**(2): 187.

<http://www.sciencedirect.com/science/article/B6T3C-41PNYNT-B/2/f46de637f24478cee1413c577f222161>

A rapid quantitative analysis method for murine endothelin-1 (ET-1) and vasoactive intestinal contractor (VIC) gene expression levels was established using a real-time polymerase chain reaction (PCR). We designed primer pairs and TaqMan probes specific for murine prepro-ET-1 (PPET-1) and prepro-VIC (PPVIC) genes, based on the cDNA sequence region common to both

mouse and rat. The dynamic range for detection in this system spanned 100000-fold of the starting molecule. The gene expression levels of PPET-1 and PPVIC were estimated as gene expression rates normalized by the expression of the house-keeping gene, glyceraldehyde-3-phosphate dehydrogenase. To examine the reproducibility of this assay system, we calculated the intra-assay and interassay coefficients of variation of the gene expression rate, which ranged from 16.2 to 55.0% and from 24.2 to 56.5%, respectively. Using this system, we examined gene expression levels of PPET-1 and PPVIC in mouse tissues. PPET-1 gene expression was found in all tissues at relatively high levels, whereas high levels of PPVIC gene expression were observed only in stomach, intestine, uterus, and ovary. The gene expression patterns agreed well with those determined by RNase protection assay and conventional PCR. These results show that this new rapid method is accurate and reproducible.

Wong, L. Y., A. Hafeman, et al. (2003). "Assessing gene expression variation in normal human tissues using GeneTag(TM), a novel, global, sensitive profiling method." Journal of Biotechnology **101**(3): 199.

<http://www.sciencedirect.com/science/article/B6T3C-47XSY7N-1/2/e186b5386c9d84fc14fc9fc7c5d8c98d>

GeneTag(TM) is a novel expression profiling method that allows the visualization, quantification and identification of expressed genes--whether known or novel--in any species, tissue or cell type, independent of knowledge of the underlying sequence. Here we describe the application of this method to determine variation of gene expression in individual human liver samples and the identification of tissue-specific genes by comparing expression patterns across several human organs. Expression data are stored in a database for future reference and data analysis relies on proprietary software, which allows complex comparisons to be performed. Differentially expressed genes are quickly identified through a link to a sequence database. The results from our study underscore the importance of knowledge of individual variation of gene expression for the design and interpretation of transcript profiling experiments in the context of any biological question.

Yoza, B., A. Arakaki, et al. (2003). "DNA extraction using bacterial magnetic particles modified with hyperbranched polyamidoamine dendrimer." Journal of Biotechnology **101**(3): 219.

<http://www.sciencedirect.com/science/article/B6T3C-47XFYR8-1/2/1f7a9843f20e768ea87edf2a7d008265>

A cascading hyperbranched polyamidoamine dendrimer was synthesized on the surface of bacterial magnetite from *Magnetospirillum magneticum* AMB-1 to allow enhanced extraction of DNA from fluid suspensions. Characterization of the synthesis revealed linear doubling of the surface amine charge from generations one through five starting with an amino silane initiator. Furthermore, transmission electron microscopy revealed clear dispersion of the single domain magnetite in aqueous solution. The dendrimer modified magnetic particles have been used to carry out magnetic separation of DNA. Binding and release efficiencies increased with the number of generations and those of bacterial magnetite modified with six generation dendrimer were 7 and 11 times respectively as many as those of bacterial magnetite modified with only amino silane.

Bausch, S. B., T. A. Patterson, et al. (1995). "Immunocytochemical localization of delta opioid receptors in mouse brain." Journal of Chemical Neuroanatomy **8**(3): 175.

<http://www.sciencedirect.com/science/article/B6T02-4031TYD-8/2/8567071b43de4653bd9dae2bcfde1333>

An affinity-purified anti-peptide antibody generated against the carboxy-terminal region of the [delta] opioid receptor was used to localize [delta] opioid receptors in mouse brain. [delta] Opioid receptor immunoreactivity was found in axons and nerve terminals in regions of the olfactory bulb, hippocampal formation, cerebral and cerebellar cortex, midbrain and hindbrain. The immunocytochemical distribution correlated well, though not completely with autoradiographic distribution of [delta] opioid receptors in mouse brain using either [3H][2--penicillamine, 5--penicillamine]-enkephalin (DPDPE) or [3H]naltrindole. Confocal microscopy of double-labeled tissue provided direct evidence that [delta] opioid receptors are principally expressed on GABAergic terminals in the hippocampus. These anatomical findings complement extensive physiological studies to provide a more detailed description of endogenous opioid circuitry.

Phillips, J. K., R. Dubey, et al. (2001). "Differential expression of the noradrenaline transporter in adrenergic chromaffin cells, ganglion cells and nerve fibres of the rat adrenal medulla." Journal of Chemical Neuroanatomy **21**(1): 95.

<http://www.sciencedirect.com/science/article/B6T02-429Y5BB-7/2/119d8d4a9815fa1f124dcb8153ef6c3e>

Expression of the noradrenaline transporter (NAT) was identified in various cell and fibre populations of the rat adrenal medulla, examined with immunohistochemistry and confocal microscopy. Immunoreactivity for the catecholamine biosynthetic enzymes tyrosine hydroxylase (TH), aromatic--amino-acid decarboxylase (AADC) and dopamine [beta]-hydroxylase (DBH) was present in all chromaffin cells, while phenylethanolamine N-methyltransferase (PNMT) was used to determine adrenergic chromaffin cell groups. Labelling with NAT antibody was predominantly cytoplasmic and colocalised with PNMT immunoreactivity. Noradrenergic chromaffin cells were not NAT immunoreactive. Additionally, NAT antibody labelling demonstrated clusters of ganglion cells (presumably Type I) and nerve fibres. Expression of TH, AADC, DBH, PNMT and NAT mRNA was examined using reverse transcription-polymerase chain reaction (RT-PCR) from adrenal medulla punches and single chromaffin cells, and results were consistent with those obtained with immunocytochemistry. Chromaffin cells and fibres labelled with antibodies against growth associated protein-43 (GAP-43) were not NAT immunoreactive, while ganglion cells were doubly labelled with the two antibodies. The presence of NAT in adrenergic chromaffin cells, and its absence from noradrenergic cells, suggests that the adrenergic cell type is primarily responsible for uptake of catecholamines in the adrenal medulla.

Ray, S. K., D. D. Matzelle, et al. (2003). "Calpain inhibitor prevented apoptosis and maintained transcription of proteolipid protein and myelin basic protein genes in rat spinal cord injury." Journal of Chemical Neuroanatomy **26**(2): 119.

<http://www.sciencedirect.com/science/article/B6T02-497RH1S-2/2/8b5f9df77927f895786c540839a25562>

Spinal cord injury (SCI) is associated with progressive neurodegeneration and dysfunction. Multiple cellular and molecular mechanisms are involved in this pathogenesis. In particular, the activation of proteases following trauma can cause apoptosis in the spinal cord. Calpain, a calcium-dependent cysteine protease, plays a major role in apoptosis following trauma. We identified apoptosis and decrease in transcription of the genes for proteolipid protein (PLP) and myelin basic protein (MBP) in five 1-cm long spinal cord segments (S1, distant rostral; S2, near rostral; S3, lesion; S4, near caudal; and S5, distant caudal) 24 h after induction of SCI (40 g.cm force) in rats by weight-drop method. Sham rats underwent laminectomy and did not receive injury. Internucleosomal DNA fragmentation occurred prominently in the lesion (S3), moderately in near segments (S2 and S4), and slightly in distant segments (S1 and S5) of injured rats, indicating the occurrence of apoptosis in the lesion and penumbra. Levels of transcription of PLP and MBP were reduced highly in the lesion and moderately in near segments, suggesting that apoptotic loss of cells impaired biosynthesis of two important structural components of myelin. Immediate administration of the calpain inhibitor E-64-d (1 mg/kg) to injured rats prevented apoptosis and restored transcription of these genes, indicating the therapeutic efficacy of calpain inhibitor for treatment of SCI.

Journal of Chromatography A (16)

Arakawa, H., S. Nakashiro, et al. (1996). "Analysis of single-strand DNA conformation polymorphism by capillary electrophoresis." Journal of Chromatography A **722**(1-2): 359.

<http://www.sciencedirect.com/science/article/B6TG8-3THM8DC-1G/2/72be2fc82a989a8001500bdaccf37886>

Analysis of single-strand conformation polymorphism (SSCP) by capillary electrophoresis (CE) was developed. The conformational change of single-strand DNA is caused by a mutation in a DNA fragment. The change is detected as mobility shift in CE. The effects of acrylamide gel concentration, running temperature and fragment size amplified by the polymerase chain reaction (PCR) were studied to develop the separation of SSCP. The model DNA used was the divE 42 gene carrying wild- and mutant-type (G->A point mutation at the 141 site). The results show that two single-strand DNA fragments that differ in one nucleotide can be separated by CE within minutes. This method was also applied to the separation of SSCP for N-ras gene including four kinds of mutations. All mutations tested in this study could be distinguished. CE is well suited for clinical analysis of SSCP because it is rapid and reproducible, allows on-line detection and is easy.

Arakawa, H., K. Uetanaka, et al. (1994). "Analysis of polymerase chain reaction product by capillary electrophoresis and its application to the detection of single base substitution in genes." Journal of Chromatography A **664**(1): 89.

<http://www.sciencedirect.com/science/article/B6TG8-44CPVGG-VF/2/9104831ae627cefb4b8c28e59bab3d2c>

Capillary gel electrophoresis (CGE) was studied for the direct analysis of polymerase chain reaction (PCR) amplified samples. A low cross-linked polyacrylamide gel (3%T, 0.5%C) was used for CGE with treated and untreated silica capillaries. CGE showed high reproducibility and

resolution in the separation of DNA fragments (ca. 100-1000 base pairs) produced by PCR. The CGE system was applied to the detection of an amplification refractory mutation system (ARMS) and PCR--restriction fragment length polymorphism (PCR-RFLP), which are detection methods of single base substitution in genes using PCR. With the CGE system, full automation of PCR product detection is feasible.

Arakawa, H., K. Uetanaka, et al. (1994). "Analysis of polymerase chain reaction-product by capillary electrophoresis with laser-induced fluorescence detection and its application to the diagnosis of medium-chain acyl-coenzyme A dehydrogenase deficiency." Journal of Chromatography A **680**(2): 517.

<http://www.sciencedirect.com/science/article/B6TG8-44CPS94-50/2/e05a0e94df4fc24ccd7db73cdb19d223>

Capillary gel electrophoresis with laser-induced fluorescence (CGE-LIF) has been developed to detect polymerase chain reaction (PCR) amplified samples. LIF detection was performed using Thiazole Orange as the fluorescent intercalating dye. This method was ca. 100X as sensitive as that with UV detection. The highly sensitive CGE-LIF was applied to the detection of the most prevalent mutation (lysine329- to-glutamic acid substitution) in medium-chain acyl-coenzyme A dehydrogenase (MCAD) deficiency. The disorder, which shows an autosomal recessive inheritance, is known to be highly prevalent among Caucasian population and often mimics as Reye-like syndrome or sudden infant death. A DNA fragment containing the mutation site was PCR-amplified with two sets of allele specific oligonucleotide primers, followed by CGE-LIF. The mutant allele produced a 175-base pairs DNA fragment, which the normal allele generated a 202-base pairs DNA fragment. CGE-LIF clearly distinguished these PCR products, facilitating rapid diagnosis of MCAD deficiency.

Bencina, M., K. Bencina, et al. (2005). "Immobilization of deoxyribonuclease via epoxy groups of methacrylate monoliths: Use of deoxyribonuclease bioreactor in reverse transcription-polymerase chain reaction." Journal of Chromatography A **1065**(1): 83.

<http://www.sciencedirect.com/science/article/B6TG8-4DTP23H-5/2/ec22cdec0f6a9f1930c2dc59f26f14b3>

A deoxyribonuclease bioreactor was prepared by immobilization of deoxyribonuclease I through epoxy groups inherently present on poly (glycidyl methacrylate-co-ethylene dimethacrylate) monoliths. Columns with various levels of DNase activity were prepared varying immobilization temperature, pH, time and method. The apparent Michaelis-Menten constant, and turnover number, for immobilized DNase determined by on-line frontal analysis method were, respectively, 0.28 g of DNA I-1 and 16 dA260nm min⁻¹ mg⁻¹ of immobilized DNase. The highest activity of immobilized DNase was detected at 1 mM calcium ions concentration and mirrored properties of free enzyme; however, reaction temperature in the range from 25 to 37 [deg]C has no significant effect on activity of immobilized DNase in contrary to free enzyme. The CIM DNase bioreactor was used for elimination of DNA contaminants in RNA samples prior to reverse transcription followed by PCR.

Branovic, K., D. Forcic, et al. (1999). "Introduction of the gene amplification technique to decrease the risk of hepatitis C virus transmission by plasma products." Journal of Chromatography A **852**(1): 305.

<http://www.sciencedirect.com/science/article/B6TG8-3YN2XK4-14/2/a48cb46561f28a669d6b1b183981074d>

The viral safety of plasma-derived products with respect to hepatitis C virus (HCV) is assured by selection of donors, screening of individual donations for antibodies to HCV and the incorporation of effective viral inactivation-removal steps into manufacturing processes. As antibody screening of single donations is not sufficient to completely eliminate HCV RNA positive plasmas from plasma pools, testing for HCV RNA by gene amplification techniques may be necessary to identify positive donations. Using modern molecular biology techniques, we developed a specific, sensitive and reproducible method for routine PCR screening for HCV RNA in plasma pools.

Chiang, L. W. (1998). "Detection of gene expression in single neurons by patch-clamp and single-cell reverse transcriptase polymerase chain reaction." Journal of Chromatography A **806**(1): 209.

<http://www.sciencedirect.com/science/article/B6TG8-3SR91XC-1V/2/bc65daf8b49813dfaf00423d43030c92>

Detection and quantitation of gene expression in single cells is especially important in the central nervous system where, at the cellular level, the synapse can be considered the single functional unit. For example, the consolidation of long-term memories may be mediated by persistent changes in the strength of synaptic transmission at individual synapses. In order to investigate the requirement for de novo RNA synthesis during long-term potentiation in individual neurons, we have combined single-cell electrophysiology with single-cell gene-expression methodology. Described are methods combining whole-cell patch-clamp and single-cell RT-PCR for the detection of a single mRNA species for nitric oxide synthase, or, through a multiplex strategy, for the simultaneous detection of several mRNAs including heme oxygenase 2, protein phosphatase inhibitor 1 protein, and several isoforms of the calcium/calmodulin dependent protein kinase II.

Deforce, D. L. D., R. E. M. Millecamps, et al. (1998). "Comparison of slab gel electrophoresis and capillary electrophoresis for the detection of the fluorescently labeled polymerase chain reaction products of short tandem repeat fragments." Journal of Chromatography A **806**(1): 149.

<http://www.sciencedirect.com/science/article/B6TG8-3SR91XC-1M/2/5c7b5e14d1606e80c9e344cb7380aaf1>

The sizing capability of slab gel electrophoresis for short tandem repeat (STR) fragments was compared to the sizing capability of capillary electrophoresis (CE). Both systems used automated laser fluorescence detection to detect four fluorescent dyes, enabling the use of an internal lane standard within each sample. The STR fragments were amplified using a multiplex polymerase chain reaction (PCR) in which the STR fragments Hum CD-4, Hum TH01, Hum D21S11 and Hum SE33 were amplified simultaneously. The reproducibility of the size calling was determined for both systems. The average standard deviation obtained for the slab gel system was 0.2, which was comparable to the standard deviation of 0.12 obtained for the CE system. The CE system produced results comparable to those obtained on the slab gel system, with a level of precision of +/-1.0 bp (between instruments).

Del Principe, D., M. Paola Iampieri, et al. (1993). "Analysis of a polymerase chain reaction-amplified product of the DXS 164 locus in the dystrophin gene: Detection by capillary electrophoresis of restriction fragment length polymorphism." Journal of Chromatography A **638**(2): 277.

<http://www.sciencedirect.com/science/article/B6TG8-44GP787-2K/2/e2445ee2e253337583374122eba5db2e>

Capillary electrophoresis (CE) was used to characterize restriction fragment length polymorphism (RFLP) in a polymerase chain reaction (PCR)-amplified product of a 740-base pairs DNA fragment from the DXS 164 locus of the dystrophin gene. The polymorphic alleles of 740 and 520/220 base pairs revealed by XmnI digestion were analysed from homozygous and heterozygous individuals by CE. Our studies show that extraction in phenol-chloroform may be useful in PCR-amplified product purification. Excellent separation was obtained in a short time. The data indicate that CE is suitable for genomic analysis such as carrier detection and prenatal diagnosis of X-linked recessive disorders after purification of PCR-amplified products.

Fung, E. N., H.-m. Pang, et al. (1998). "Fast DNA separations using poly(ethylene oxide) in non-denaturing medium with temperature programming." *Journal of Chromatography A* **806**(1): 157.

<http://www.sciencedirect.com/science/article/B6TG8-3SR91XC-1N/2/c86efdf80851206a0e60c2a71ae2f0ae>

We demonstrated fast DNA separations in low viscosity entangled solutions with a temperature gradient in a non-denaturing separation medium. The separations were carried out in a solution of commercially available poly(ethylene oxide) (PEO) [1 x Tris(hydroxymethyl)aminomethane borate buffer, without urea] with a temperature gradient of 2[deg]C/min. The performance was compared with that of a solution of PEO with urea at ambient temperature. We found that the former condition gives sufficient resolution for accurate base calling and that in general, it gave better separation for fragments larger than 450 base pairs (bp). Most importantly, the separation speed approaches 30 bp/min. In addition, we describe a simple yet reliable gel preparation protocol for such separations.

Hadd, A. G., M. P. Goard, et al. (2000). "Sub-microliter DNA sequencing for capillary array electrophoresis." *Journal of Chromatography A* **894**(1-2): 191.

<http://www.sciencedirect.com/science/article/B6TG8-41C28J6-T/2/ad27d1770fd7bd9f574853cb13bb85fe>

DNA sequencing from sub-microliter samples was demonstrated for capillary array electrophoresis by optimizing the analysis of 500 nl reaction aliquots of full-volume reactions and by preparing 500 nl reactions within fused-silica capillaries. Sub-microliter aliquots were removed from the pooled reaction products of 10 [mu]l dye-primer cycle-sequencing reactions and analyzed without modifying either the reagent concentrations or instrument workflow. The impact of precipitation methods, resuspension buffers, and injection times on electrokinetic injection efficiency for 500 nl aliquots were determined by peak heights, signal-to-noise ratios, and changes in base-called readlengths. For 500 nl aliquots diluted to 5 [mu]l in 60% formamide-1 mM EDTA and directly injected, a five-fold increase in signal-to-noise ratios was obtained by increasing injection times from 10 to 80 s without a corresponding increase in peak widths or reduction in readlengths. For 500 nl aliquots precipitated in alcohol, 80+/-5% template recovery and a two-fold decrease in conductivity was obtained, resulting in a two-fold increase in peak heights and 50 to 100 bases increase in readlengths. In a comparison of aliquot volumes and precipitation methods, equivalent readlengths were obtained for 500 nl, 4 [mu]l, and 8 [mu]l aliquots by simply adjusting the electrokinetic injection conditions. To ascertain the robustness of this methodology for genomic sequencing, 96 Arabidopsis thaliana subclones were sequenced, with a yield of 38624 bases obtained from 500 nl aliquots versus 30764 bases from standard

scale reactions. To demonstrate 500 nl sample preparation, reactions were performed in fused-silica capillary reaction chambers using air-based thermal cycling. A readlength of 690 bases was obtained for the polymerase chain reaction product of an Arabidopsis subclone without modifying the reagent concentrations, post-reaction processing or electrokinetic injection workflow. These results demonstrated the fundamental feasibility of small-volume DNA sequencing for high-throughput capillary electrophoresis.

Inoue, H., M. Tsuchiko, et al. (1998). "Enhanced separation of DNA sequencing products by capillary electrophoresis using a stepwise gradient of electric field strength." Journal of Chromatography A **802**(1): 179.

<http://www.sciencedirect.com/science/article/B6TG8-3SHJH9D-R/2/81c7e3866e95773745a9e5edb602a65a>

The effect of the electric field strength gradient on the separation of DNA sequencing fragments was investigated. We demonstrate that the stepwise gradient of electric field improves the separation of DNA sequencing fragments more than 500 bases in size and diminishes the analysis time for DNA sequencing of larger DNA fragments. The use of the electric field strength gradient induces an increase in the theoretical plate number as predicted by the theoretical formulation discussed in this paper.

Katsuragi, K., K. Kitagishi, et al. (1996). "Fluorescence-based polymerase chain reaction-single-strand conformation polymorphism analysis of p53 gene by capillary electrophoresis." Journal of Chromatography A **744**(1-2): 311.

<http://www.sciencedirect.com/science/article/B6TG8-3VB3P64-3P/2/11696f64a02b101efaae42a0f242bdf7>

Mutation of the p53 gene plays an important role in neoplastic progression in human tumorigenesis. Polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) techniques are now available for the detection of point mutations. The original method using polyacrylamide gel electrophoresis is disadvantageous, particularly for clinical tests and for analysis of large numbers of samples. Therefore, using an automated capillary electrophoresis (CE) technique with a molecular-sieving polymer solution, we have devised a completely automatic fluorescence-based PCR-SSCP system (CE-FSSCP) for the differential detection of point mutations that does not require SSCP with radioisotopes and polyacrylamide gels. This automatic CE-FSSCP system was developed for reproducible operations in the denaturation of double-stranded DNA and electrophoresis of single-stranded DNA. The detection system consists of a 100 W I2 lamp and photomultiplier. We performed CE-FSSCP with a 2% linear polyacrylamide polymer solution containing 5% glycerol. Four tissue specimens of lung tumors with mutations in exon 7 of the p53 gene were found to have mutant alleles; a six-base-pair deletion at codons 247-248, a one-base-pair deletion at codon 260, a one-base-pair deletion at codon 244 and a GGC to CGC substitution at codon 244. We expect this technique to prove useful for the clinical DNA diagnosis of human cancers, determination of the therapeutic effect of anticancer agents and for the study of the molecular aspects of the mechanisms involved in the pathogenesis of human cancers.

Kim, Y. and E. S. Yeung (1997). "Separation of DNA sequencing fragments up to 1000 bases by using poly(ethylene oxide)-filled capillary electrophoresis." Journal of Chromatography A **781**(1-2): 315.

<http://www.sciencedirect.com/science/article/B6TG8-3SDKTD6-17/2/0966f83ca92c48ae0b0634fbda55d3ce>

We have demonstrated that DNA bases up to 1000 base pairs (bp) in a sequencing ladder can be separated using poly(ethylene oxide)-filled capillary electrophoresis (resolution of raw data=0.5 at 966 bp). Separation performance of this sieving matrix has been tested under different experimental conditions. It was found that the electric field strength played a critical role in the onset of reptation and thus the separation efficiency. Optimized gel composition and concentration is required for good separation, but the total gel concentration should lie between 2.5 and 3.0%. We observed that the capillary length influences the number of theoretical plates and the maximum readable length of DNA. For sequencing up to 500 bp, relatively nonviscous solutions can be used, greatly facilitating the replacement of the sieving matrix in between runs.

Pigge, C. A., J. Muth, et al. (1997). "Capillary electrophoresis for the detection of known point mutations by single-nucleotide primer extension and laser-induced fluorescence detection." Journal of Chromatography A **781**(1-2): 367.

<http://www.sciencedirect.com/science/article/B6TG8-3SDKTD6-1D/2/8a8a6d6bfd0150bb895b3a2283321aba>

Capillary electrophoresis (CE) with laser-induced fluorescence (LIF) was used to detect known point mutations using the method of single-nucleotide primer extension (SNUPE). Three different point mutations in human mitochondrial DNA associated with Leber's hereditary optic neuropathy (LHON) were detected by annealing a primer immediately 5' to the mutation on the template and extending the primer by one fluorescently labeled dideoxy terminator complementary to the mutation. By using two or more differently labeled terminators, both the mutant and wild type could be simultaneously detected. The advantages of using CE-LIF for detecting SNUPE reactions include speed and ease of analysis, absence of radioactivity, and potential for automation.

Song, L., D. Liang, et al. (2001). "DNA sequencing by capillary electrophoresis using mixtures of polyacrylamide and poly(N,N-dimethylacrylamide)." Journal of Chromatography A **915**(1-2): 231.

<http://www.sciencedirect.com/science/article/B6TG8-42RDRV4-V/2/23554d243b90dd519165915fbc90a4ba>

The possibility of using polymer mixtures with different chemical compositions as a DNA sequencing matrix by capillary electrophoresis (CE) has been exploited. Polyacrylamide (PAM, 2.5%, w/v) having a molecular mass of 2.2.10⁶ has been mixed with poly(N,N-dimethylacrylamide) (PDMA) having molecular masses of 8000, 470000 and 2.1.10⁶ at concentrations of 0.2, 0.5 and 1% (w/v). Unlike polymer mixtures of the same polymer with different molecular masses, the use of polymer mixtures with different chemical compositions encounters an incompatibility problem. It was found that the incompatibility increased with increasing PDMA molecular mass and PDMA concentration, which resulted in decreased efficiency in DNA sequencing. Also, the incompatibility had a more pronounced effect on the efficiency as the base number was increased. However, by choosing a low-molecular-mass PDMA of 8000 and a low concentration of 0.2% (w/v), the incompatibility of PAM and PDMA has been alleviated. At the same time, the advantage of using polymer mixtures revealed a higher efficiency for such a polymer mixture when compared with PAM. The mixture also endowed the separation medium with a dynamic coating ability. An efficiency of over 10.10⁶ theoretical plates per meter has been achieved by using the bare capillaries without the additional chemical coating

step.

Soper, S. A., S. M. Ford, et al. (1999). "Nanoliter-scale sample preparation methods directly coupled to polymethylmethacrylate-based microchips and gel-filled capillaries for the analysis of oligonucleotides." Journal of Chromatography A **853**(1-2): 107.

<http://www.sciencedirect.com/science/article/B6TG8-3X64GMY-F/2/2af1f44dfc2c5f321186c1f17011334f>

We are currently developing miniaturized, chip-based electrophoresis devices fabricated in plastics for the high-speed separation of oligonucleotides. One of the principal advantages associated with these devices is their small sample requirements, typically in the nanoliter to sub-nanoliter range. Unfortunately, most standard sample preparation protocols, especially for oligonucleotides, are done off-chip on a microliter-scale. Our work has focused on the development of capillary nanoreactors coupled to micro-separation platforms, such as micro-electrophoresis chips, for the preparation of sequencing ladders and also polymerase chain reactions (PCRs). These nanoreactors consist of fused-silica capillary tubes (10-20 cm x 20-50 [μm] I.D.) with fluid pumping accomplished using the electroosmotic flow generated by the tubes. These reactors were situated in fast thermal cyclers to perform cycle sequencing or PCR amplification of the DNAs. The reactors could be interfaced to either a micro-electrophoresis chips via capillary connectors micromachined in polymethylmethacrylate (PMMA) using deep X-ray etching (width 50 [μm]; depth 50 [μm]) or conventional capillary gel tubes using zero-dead volume glass unions. For our chips, they also contained an injector, separation channel (length 6 cm; width 30 [μm]; depth 50 [μm]) and a dual fiber optic, near-infrared fluorescence detector. The sequencing nanoreactor used surface immobilized templates attached to the wall via a biotin-streptavidin-biotin linkage. Sequencing tracks could be directly injected into gel-filled capillary tubes with minimal degradation in the efficiency of the separation process. The nanoreactor could also be configured to perform PCR reactions by filling the capillary tube with the PCR reagents and template. After thermal cycling, the PCR cocktail could be pooled from multiple reactors and loaded onto a slab gel or injected into a capillary tube or microchip device for fractionation.

Journal of Chromatography B (7)

de Carlos, A., D. Montenegro, et al. (2003). "Purification of human alpha-fucosidase precursor expressed in Escherichia coli as a glutathione S-transferase fusion protein." Journal of Chromatography B **786**(1-2): 7.

<http://www.sciencedirect.com/science/article/B6X0P-46X8XN2-3/2/14c7cb7608dc310e9f52efab581dac97>

Alpha-fucosidase (FUC) is a glycosidase involved in the degradation of fucose-containing glycoconjugates. A cDNA representing the complete sequence of human FUC was inserted into the prokaryotic expression vector pGEX-2T. High levels of the glutathione S-transferase (GST) fusion protein were detected in Escherichia coli cells after induction with isopropyl thio-beta-galactopyranoside. The GST-FUC protein was mostly found as inclusion bodies and attempts to optimise its expression as a soluble form were unsuccessful. Nevertheless, the recombinant protein was purified by affinity chromatography on glutathione-sepharose and its fucosidase

activity was characterised. After thrombin cleavage of the GST tag, the FUC precursor protein was purified by electro-elution.

Havlis, J. and M. Trbusek (2002). "5-Methylcytosine as a marker for the monitoring of DNA methylation." Journal of Chromatography B **781**(1-2): 373.

<http://www.sciencedirect.com/science/article/B6X0P-46FMKN4-2/2/05a178a664df754b1fca3f12fe848ee8>

The extent of the DNA methylation of genomic DNA as well as the methylation pattern of many gene-regulatory areas are important aspects with regard to the state of genetic information, especially their expression. There is growing evidence that aberrant methylation is associated with many serious pathological consequences. As genetic research advances, many different approaches have been employed to determine the overall level of DNA methylation in a genome or to reveal the methylation state of particular nucleotide residues, starting from semiquantitative methods up to new and powerful techniques. In this paper, the currently employed techniques are reviewed both from the point of view of their relevance in genomic research and of their analytical application. The methods discussed include approaches based on chromatographic separation (thin-layer chromatography, high-performance liquid chromatography, affinity chromatography), separation in an electric field (capillary electrophoresis, gel electrophoresis in combination with methylation-sensitive restriction enzymes and/or specific sequencing protocols), and some other methodological procedures (mass spectrometry, methyl accepting capacity assay and immunoassays).

Liu, Z.-J., W.-C. Peng, et al. (2003). "Relative mRNA expression of the lactate dehydrogenase A and B subunits as determined by simultaneous amplification and single strand conformation polymorphism: Relation with subunit enzyme activity." Journal of Chromatography B **793**(2): 405.

<http://www.sciencedirect.com/science/article/B6X0P-49679S6-3/2/1cbbf7a1b8714ecbf56602e73163cb9d>

To explore if it is correlated in human tumor cells that the expression of LDH homologous gene and LDH isoenzymes, we used RT-PCR-SSCP technique to measure the relative expression of genes with homologous sequences. The combination of PCR using common primers designed in the highly conserved regions and single-strand conformation polymorphism analysis of the products is used for quantitative determination of the proportions of LDH-A mRNA in human cancer cell lines. The proportion is compared with that of the activities of isoenzymes. The results indicated that the enzyme activity of LDH-A was consistent with mRNA levels in the human tumor cell. The present procedure using a single pair of primers for two fragments can overcome disadvantages in quantitative analysis using multiplex PCR. Template concentrations and PCR cycles did not affect the proportions of LDH-A and LDH-B in the product.

Muhr, D., T. Wagner, et al. (2002). "Polymerase chain reaction fidelity and denaturing high-performance liquid chromatography." Journal of Chromatography B **782**(1-2): 105.

<http://www.sciencedirect.com/science/article/B6X0P-46W1CRX-C/2/a6fba080eda63a1a5c9ddbf705aa6c2>

Incorporation of non-complementary nucleotides during polymerase chain reaction can result in

ambiguous denaturing high-performance liquid chromatography profiles that reduce both sensitivity and specificity of mutation analysis. The use of proofreading DNA polymerases increases the fidelity of polymerase chain reaction and, consequently, reduces background noise in the chromatograms. This is demonstrated for several BRCA1 and BRCA2 mutations that had yielded previously chromatograms of poor quality using non-proofreading enzyme for amplification. Interestingly, despite the reduced level of background heteroduplexes, the ability of denaturing high-performance liquid chromatography to detect mutant alleles at a frequency <10% in pools of chromosomes did not improve significantly.

Oefner, P. J. and C. G. Huber (2002). "A decade of high-resolution liquid chromatography of nucleic acids on styrene-divinylbenzene copolymers." Journal of Chromatography B **782**(1-2): 27.

<http://www.sciencedirect.com/science/article/B6X0P-470M7NT-5/2/8eaa2ae4fc0066683bbf40f474bd9c8d>

The introduction of alkylated, nonporous poly-(styrene-divinylbenzene) microparticles in 1992 enabled the subsequent development of denaturing HPLC that has emerged as the most sensitive screening method for mutations to date. Denaturing HPLC has provided unprecedented insight into human origins and prehistoric migrations, accelerated the cloning of genes involved in mono- and polygenic traits, and facilitated the mutational analysis of more than a hundred candidate genes of human disease. A significant step toward increased sample-throughput and information content was accomplished by the recent introduction of monolithic poly(styrene-divinylbenzene) capillary columns. They have enabled the construction of capillary arrays amenable to multiplex analysis of fluorescent dye-labeled nucleic acids by laser-induced fluorescence detection. Hyphenation of denaturing HPLC with electrospray ionization mass spectrometry, on the other hand, has allowed the direct elucidation of the chemical nature of DNA variation and determination of phase of multiple alleles on a chromosome.

Vazquez, M., G. McKinley, et al. (2002). "Electrophoresis using ultra-high voltages." Journal of Chromatography B **779**(2): 163.

<http://www.sciencedirect.com/science/article/B6X0P-46DKYNB-2/2/c311c42e604e705e8bd70bdeb9dda2ed>

Optimization of electrophoretic techniques is becoming an increasingly important area of research as microdevices are now routinely adapted for numerous biology and engineering applications. The present work seeks to optimize electrophoresis within microdevices by utilizing ultra-high voltages to increase sample concentration prior to separation. By imaging fluorescently-tagged DNA samples, the effects of both conventional and atypical voltage protocols on DNA migration and separation are readily observed. Experiments illustrate that short periods of high voltage during electrophoretic injection do not destroy the quality of DNA separations, and in fact can enhance sample concentration five-fold. This study presents data that illustrate increases in average resolution, and resolution of longer fragments, obtained from electrophoretic injections utilizing voltages between 85 and 850 V/cm.

Zhao, C., G. Xu, et al. (2003). "Simultaneous genotyping of multiplex single nucleotide polymorphisms of the K-ras gene with a home-made kit." Journal of Chromatography B **795**(1): 55.

<http://www.sciencedirect.com/science/article/B6X0P-49D6GG6->

5/2/2e98b99a743539907a30c719c990ffec

Accurate and fast genotyping of single nucleotide polymorphisms (SNPs) is important in the human genome project. Here an automated fluorescent method that can rapidly and accurately genotype multiplex known SNPs was developed by using a homemade kit, which has lower cost but higher resolution than commercial kit. With this method, oncogene K-ras was investigated, four known SNPs of K-ras gene exon 1 in 31 colorectal cancer patients were detected. Results indicate that mutations were present in 8(26%) of 31 patients, and most mutations were localized in codon 12. The presence of these mutations is thought to be a critical step and plays an important role in human colorectal carcinogenesis.

Journal of Chromatography B: Biomedical Sciences and Applications (9)

Arakawa, H., S. Nakashiro, et al. (1998). "Analysis of polymerase chain reaction products by high-performance liquid chromatography with fluorimetric detection and its application to DNA diagnosis." Journal of Chromatography B: Biomedical Sciences and Applications **716**(1-2): 119.

<http://www.sciencedirect.com/science/article/B6TG9-3TWXX3J-F/2/4bcd3a2bd25c34364c42e63e3d324b76>

We describe the development of a sensitive high-performance liquid chromatographic (HPLC) method for polymerase chain reaction (PCR) products using bisbenzimidazole (Hoechst 33258 dye) based fluorimetric detection. The detection limit and specificity for double-strand DNA detection are improved in comparison with HPLC with UV absorbance detection. This HPLC, using a column packed with diethylaminoethyl-bonded non-porous resin particles, was applied to the detection of allele-specific PCR and restriction fragment length polymorphism analysis. We also developed a hybridization method analyzed by HPLC. DNA fragments (149 bp) containing the mutation site (C→A,G,T) in the N-ras gene were amplified by PCR. Fluorescein isothiocyanate (FITC)-labeled DNA probes were also prepared by PCR using FITC-labeled 5' primer. Analysis of mutation was performed by the separation of a hybrid and non-reactive DNA probe with HPLC with fluorimetric detection after the hybridization of target DNA (149 bp) and a FITC DNA probe. The effects of various factors on hybridization were examined to establish optimal assay conditions. Under the conditions determined, a point mutation in PCR products obtained from the N-ras gene could be detected specifically by this method. The analysis of PCR products by HPLC may potentially be useful for DNA diagnosis.

Belgrader, P., J. M. Devaney, et al. (1996). "Automated polymerase chain reaction product sample preparation for capillary electrophoresis analysis." Journal of Chromatography B: Biomedical Sciences and Applications **683**(1): 109.

<http://www.sciencedirect.com/science/article/B6TG9-3V9DKTF-D/2/7bef7f875bfae2ff4ad1577af1e715e8>

The analysis of crude polymerase chain reaction (PCR) products by capillary electrophoresis (CE) is often compromised due to the presence of a high concentration of salt. Salt interferes with the electrokinetic injection and induces localized heating within the column; hence, PCR products must be desalted or cleaned-up prior to CE analysis. A variety of commercial clean-up systems

are available that have been traditionally used to prepare PCR products for cloning, sequencing and digestion with restriction enzymes. These systems were tested for their effectiveness in preparing PCR products for CE analysis and were evaluated based on CE resolution, salt removal, DNA recovery, processing time and cost. One particularly effective clean-up system, membrane dialysis, was automated using a robotic workstation.

Butler, J. M., B. R. McCord, et al. (1994). "Quantitation of polymerase chain reaction products by capillary electrophoresis using laser fluorescence." Journal of Chromatography B: Biomedical Sciences and Applications **658**(2): 271.

<http://www.sciencedirect.com/science/article/B6TG9-457CV9F-NK/2/f89b7ab0576841826e0a63b0f0aed563>

In samples where the amount of DNA is limited, the polymerase chain reaction (PCR) can amplify specific regions of the DNA. A quantitative analysis of the PCR product would be desirable to ensure sufficient DNA is available for analysis. In this study, we examine the use of capillary electrophoresis (CE) with laser fluorescence detection for quantitation of PCR products. A coated open tubular capillary was used with a non-gel sieving buffer and a fluorescent intercalating dye to obtain results within 20 minutes. Using an internal standard, peak migration time was below 0.1% relative standard deviation (R.S.D.) with a peak area precision of 3% R.S.D. In comparison to quantitation by hybridization, (i.e., slot blot) and spectrophotometric analysis, capillary electrophoresis shows distinct advantages due to its ability to separate unincorporated primers and PCR byproducts from the targeted PCR product. The results demonstrate that CE can be used to monitor the quality and quantity of the PCR product.

Gong, X. and E. S. Yeung (2000). "Genetic typing and HIV-1 diagnosis by using 96 capillary array electrophoresis and ultraviolet absorption detection." Journal of Chromatography B: Biomedical Sciences and Applications **741**(1): 15.

<http://www.sciencedirect.com/science/article/B6TG9-4037929-2/2/a6327c53460a46ed95d6199ac7a1123d>

Current high-throughput approaches to the analysis of PCR products are based primarily on electrophoretic separation and laser-excited fluorescence detection. We show that capillary array electrophoresis can be applied to HIV-1 diagnosis and D1S80 VNTR genetic typing based simply on UV absorption detection. The additive contribution of each base pair to the total absorption signal provides adequate detection sensitivity for analyzing most PCR products. Not only is the use of specialized and potentially toxic fluorescent labels eliminated, but also the complexity and cost of the instrumentation are greatly reduced.

Liang, Q., P. A. Davis, et al. (2001). "High-performance liquid chromatography multiplex detection of two single nucleotide mutations associated with hereditary hemochromatosis." Journal of Chromatography B: Biomedical Sciences and Applications **754**(1): 265.

<http://www.sciencedirect.com/science/article/B6TG9-42MFDJP-13/2/db2c17567a9a8d1668d6deda412f5c53>

High-performance liquid chromatography (HPLC) has been applied to the multiplex detection of the two single nucleotide mutations commonly found in hereditary hemochromatosis (HH). HH is

associated with a major G to A transition at position 845 (mutation Cys282Tyr) and a minor C to G transition at position 187 (mutation His63Asp) in the cDNA of the HFE gene. Two detection assays were developed based on HPLC analysis of restriction fragment length polymorphism (RFLP) or single nucleotide extension (SNE) products following multiplex PCR amplification. RFLP genotypes the two sites as dsDNA fragments of different lengths generated by restriction enzymes Rsa I/Bcl I. SNE extends primers 5'-adjacent to the sites of interest with a dideoxynucleotide triphosphate (ddNTP) to generate extended ssDNA. The identity of the added ddNTP reveals the identity of the original possible mutation site(s). Application of these methods with HPLC analysis provides simple and reliable genotyping for HH and can be applied to other single nucleotide polymorphism studies.

Mischiati, C., G. Feriotto, et al. (1995). "Chromatography in DNA radiolabeling: Hands-off automation using a robotic workstation." Journal of Chromatography B: Biomedical Sciences and Applications **664**(2): 303.

<http://www.sciencedirect.com/science/article/B6TG9-4002HC1-8F2/2effe9bccf4578aa7f110e5556c35504>

The experiments described in the present paper were performed in order to determine whether the Biomek-1000 (Beckman Instruments, Fullerton, CA, USA) automated laboratory workstation can be used in a fully automated DNA labeling method followed by automated gravity-driven size exclusion purification of molecular probes. To this aim, we performed random oligodeoxyribonucleotide priming of a HIV-1 LTR probe that was used for molecular hybridization to Southern blotted polymerase chain reaction products. The results obtained demonstrate that the automatically labeled probe can be efficiently purified by automated and gravity-driven Sephadex G-50 chromatography, without any major changes in hybridization property. This robotic methodology can be used in several procedures employing radioisotope labeling.

Tomita, M., T. Okuyama, et al. (1996). "Application of capillary gel electrophoresis to the diagnosis of the aldehyde dehydrogenase 2 genotype." Journal of Chromatography B: Biomedical Sciences and Applications **685**(1): 185.

<http://www.sciencedirect.com/science/article/B6TG9-3VVMN7T-W/2/cc670e2e65309fe0a91a0d293229f76e>

This study dealt with the application of capillary gel electrophoresis (CGE) to diagnosis of the aldehyde dehydrogenase 2 (ALDH-2) genotype. Electrophoresis was performed on a low cross-linked polyacrylamide gel {3% T [g acrylamide + g Bis (N,N'-methylenebisacrylamide)], 0.5% C (g Bis/%T)} in 100 mM Tris-borate buffer (pH 8.3) at -10 KV with on-column UV detection (260 nm). During the PCR reaction, DNA from the wild-type allele generated a MboII restriction site, which is an amplification created restriction site. This did not occur, however, with DNA fragments from the mutant allele. Therefore, determination of the heterozygous genotype, the coexistence of wild-type and mutant alleles, was easily possible. Analysis of the MboII restriction digests of the PCR products was completed in less than 20 min, showing two peaks corresponding to fragments of 125 (cleaved) and 135 (uncleaved) base pairs (bp), respectively. On the other hand, determination of the homozygous genotype, wild-type or mutant, was difficult in one electrophoresis run. The CGE of the MboII restriction digests gave a single peak and the identification, cleaved or uncleaved, was difficult under our experimental conditions. However, the addition of aliquots of the PCR reaction mixture to the restriction digests, followed by re-electrophoresis, allowed successful diagnosis, yielding two peaks (cleaved and uncleaved) for the wild-type and one peak (uncleaved) for the mutant allele. This study demonstrated that CGE offers a high-speed, high-resolution analytical tool for determining genetic types, as compared

with the conventional slab gel methodologies.

Verheyden, G., G. Volckaert, et al. (2000). "Expression of chymotrypsin(ogen) in the thioredoxin reductase deficient mutant strain of Escherichia coli AD494(DE3) and purification via a fusion product with a hexahistidine-tail." Journal of Chromatography B: Biomedical Sciences and Applications **737**(1-2): 213.

<http://www.sciencedirect.com/science/article/B6TG9-3Y9G71W-VI2/c4c9d747dfd4255b783a1b02d9a9e77b>

A reliable protocol was designed for fast expression and purification of recombinant chymotrypsin(ogen). The zymogen was overexpressed in soluble form as a (His)6-fusion construct in the cytoplasm of the thioredoxin reductase deficient Escherichia coli strain AD494(DE3). This allowed purification of chymotrypsinogen in a highly selective affinity chromatography capture step using a Ni-NTA column. After activation with enterokinase, the enzymatically active chymotrypsin was purified in a polishing step using a modified soybean trypsin inhibitor agarose column. This expression system and the use of affinity chromatography for capture and polishing, offers an easier and faster route to recombinant chymotrypsin(ogen) than the previously described use of Saccharomyces cerevisiae.

Wei, W. and E. S. Yeung (2000). "Improvements in DNA sequencing by capillary electrophoresis at elevated temperature using poly(ethylene oxide) as a sieving matrix." Journal of Chromatography B: Biomedical Sciences and Applications **745**(1): 221.

<http://www.sciencedirect.com/science/article/B6TG9-40WDPWN-M/2/b69c080b31109ce5a637857961160f0d>

DNA sequencing in poly(ethylene oxide) (PEO) matrix by capillary electrophoresis was demonstrated at high temperature. The optimal separation temperature is around 40[deg]C. The effects of polymer concentration and types of buffers on the separation performance were investigated. A new buffer system consisting of Tris-Taps-His-EDTA works well with PEO. High-speed separation and good resolution can be fulfilled by using a single-MW PEO polymer. It offers similar separation performance as before for the small DNA fragments, but better performance for large DNA fragments.

Journal of Clinical Forensic Medicine (1)

Chattopadhyay, P. and S. Guha "RAPD & FINS (mitochondrial 16s rRNA gene sequence) in analysis of food contamination: detection of food poisoning by Lizard." Journal of Clinical Forensic Medicine In Press, Corrected Proof <http://www.sciencedirect.com/science/article/B6WHN-4DJ49HS-1/2/ac01b2bcf45336052a58c9df386af167>

In poisoning, detection of the nature of causative agent is important for management of trauma and forensic investigation. Most of the methods in clinical toxicology are developed for detection of toxins and poisons. A random amplified polymorphic DNA (RAPD)-based method has been

described for detection of species of animal from its morphologically unrecognizable fragments, recovered from food substances, consumption of which caused even death. Pre-mixed RAPD reaction beads and six RAPD primers were used in polymerase chain reaction analysis. Among six RAPD primers used, any one of them was sufficient in resolving this practical forensic situation. To enhance the probability values for matching in the present study of fixing identity of an animal, six set of market available RAPD primers were used. This is the first report of a forensic application of RAPD DNA typing in identification of charred skeleton remnants of Lizard species in food material. Furthermore unique amplicons were generated for different reptilian species, which can be used as species specific markers for species identification in forensic situation, however, no variations among individuals of same species were observed.

Journal of Clinical Microbiology (2)

Al-Soud, W. A. and P. Radstrom (2001). "Purification and Characterization of PCR-Inhibitory Components in Blood Cells." Journal of Clinical Microbiology **39**(2): 485.

<http://jcm.asm.org/cgi/content/abstract/39/2/485>

In a recent study, immunoglobulin G in human plasma was identified as a major inhibitor of diagnostic PCR (W. Abu Al-Soud, L. J. Jonsson, and P. Radstrom. J. Clin. Microbiol. 38:345-350, 2000). In this study, two major PCR inhibitors in human blood cells were purified using size exclusion and anion-exchange chromatographic procedures. Based on N-terminal amino acid sequencing and electrophoretic analysis of the purified polypeptides, hemoglobin and lactoferrin were identified as PCR-inhibitor components in erythrocytes and leukocytes, respectively. When different concentrations of hemoglobin or lactoferrin were added to PCR mixtures of 25 {micro}l containing 10 different thermostable DNA polymerases and 1 ng of *Listeria monocytogenes* DNA as template DNA, AmpliTaq Gold, Pwo, and Ulta were inhibited in the presence of [≤]1.3 {micro}g of hemoglobin and [≤]25 ng of lactoferrin, while rTth and Tli were found to resist inhibition of at least 100 {micro}g of hemoglobin. In addition, the quantitative effects of seven low-molecular-mass inhibitors, present in blood samples or degradation products of hemoglobin, on real-time DNA synthesis of rTth using the LightCycler Instrument were investigated. A reaction system based on a single-stranded poly(dA) template with an oligo(dT) primer annealed to the 3' end was used. It was found that the addition of 0.25 to 0.1 mg of bile per ml, 2.5 mM CaCl₂, 0.25 mM EDTA, 5 {micro}M FeCl₃, and 0.01 IU of heparin per ml reduced the fluorescence to approximately 76, 70, 46, 17, and 51%, respectively. Finally, the effects of nine amplification facilitators were studied in the presence of hemoglobin and lactoferrin. Bovine serum albumin (BSA) was the most efficient amplification facilitator, so that the addition of 0.4% (wt/vol) BSA allowed AmpliTaq Gold to amplify DNA in the presence of 20 instead of 1 {micro}g of hemoglobin and 500 instead of 5 ng of lactoferrin. Including 0.02% (wt/vol) gp32, a single-stranded-DNA binding protein, in the reaction mixture of AmpliTaq Gold was also found to reduce the inhibitory effects of hemoglobin and lactoferrin.

Hendolin, P. H., L. Paulin, et al. (2000). "Clinically Applicable Multiplex PCR for Four Middle Ear Pathogens." Journal of Clinical Microbiology **38**(1): 125.

<http://jcm.asm.org/cgi/content/abstract/38/1/125>

The multiplex PCR method for the detection of *Alloiococcus otitidis*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumoniae* (P. H. Hendolin, A. Markkanen, J. Ylikoski, and J. J. Wahlfors, *J. Clin. Microbiol.* 35:2854-2858, 1997) in middle ear effusions (MEEs) was modified to be better suited for clinical use. To detect false-negative results, an internal amplification was added to the reaction, and to prevent carryover contamination, the dUTP-uracil-N-glycosidase system was incorporated into the procedure. Labor was minimized by using the heat-activatable AmpliTaq Gold polymerase in order to circumvent manual hot start and by detecting the amplification products on an automated sequencer. The performance of the improved protocol was verified with MEEs from patients with otitis media with effusion. In addition, a ligase detection reaction (LDR) was developed for confirmation of the PCR products. The modifications increased the reliability of the protocol and the hands-off time significantly. However, when two DNA extraction protocols were compared, gram-negative bacteria were detected more often in phenol-treated MEEs (94 versus 46%; $P < 0.001$), and gram-positive bacteria were detected more often in MEEs dissolved in sodium dodecyl sulfate-NaOH-chaotropic salt (83 versus 27%; $P < 0.001$). The LDR was found to be 100% specific. In all, the results demonstrate the feasibility of the rapid (7-h) multiplex PCR method for routine laboratory use.

Journal of Clinical Virology (52)

Aalto, S. M., E. Juvonen, et al. (2003). "Lymphoproliferative disease after allogeneic stem cell transplantation--pre-emptive diagnosis by quantification of Epstein-Barr virus DNA in serum." *Journal of Clinical Virology* **28**(3): 275.

<http://www.sciencedirect.com/science/article/B6VJV-481N3TM-1/2/548944775c9856ebe2d62b6d2f001967>

Background: Lymphoproliferative disease (PTLD) is a life-threatening complication of organ transplantation. In matched, allogeneic, non-T-cell-depleted stem-cell transplantations (SCT) the disease develops early but has been thought to be rare. **Objectives:** We determined by strict histopathological criteria the incidence of fatal Epstein-Barr-virus (EBV)--related PTLD in a large number of SCT, and assessed the diagnostic value of a real-time quantitative polymerase chain reaction (qPCR) for EBV-DNA in serum specimens. **Study design:** Of the 257 SCT performed in Helsinki during 1994-1999, 132 (51%) recipients were alive and 125 (49%) had succumbed by June 2001. The necropsies were analyzed for EBV-related PTLD as evidenced by disseminated lymphocytic infiltrates labeled histochemically for antigens and RNA (EBER 1 and 2) detectable by in situ technology. From a subset of the PTLD cases (N=12) and a series of corresponding stem-cell recipient controls (N=36), consecutive samples of serum (N=103 and 364, respectively) were studied by qPCR for EBV-DNA, and the clinical data were reviewed. **Results:** The post-mortem analysis revealed 18 cases of PTLD (14% of the deceased), all of whom had received intensive immunosuppressive treatment including anti-thymocyte globulin for treatment or prophylaxis of graft versus host disease (GVHD). By using qPCR all the PTLD patients became EBV-DNA positive, in progressively rising copy numbers. EBV-DNA was first detectable 70 (median; range 24-154) days after SCT or 23 (4-86) days before death; i.e. earlier than the symptoms which appeared 15 (2-85) days before death. Among the SCT controls, EBV-DNA occurred sporadically (in only 3.9% sera). **Conclusions:** qPCR for EBV-DNA in serum is a highly sensitive (100%) and specific (96%) diagnostic approach. Intensely immunosuppressed stem-cell recipients are at a great risk of developing PTLD, and should be carefully monitored for EBV-DNA, for pre-emptive treatment of this life-threatening disorder.

Abe, K., E. Hayakawa, et al. (2004). "Molecular epidemiology of hepatitis B, C, D and E viruses among children in Moscow, Russia." Journal of Clinical Virology **30**(1): 57.

<http://www.sciencedirect.com/science/article/B6VJV-4B0WSYR-1/2/c6b387c19e3e472a4620c9f358329d21>

Background: It is known that the prevalence of HBV and HCV infections vary according to geographical areas. However, in Russia, an adequate level of information on the molecular epidemiology of hepatitis viruses has not been available so far. Objectives: To investigate the characterization of various hepatitis viruses in Russia, we conducted molecular-based epidemiological survey of hepatitis viruses including hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV) and hepatitis E virus (HEV) among children in Moscow, Russia. Study design: The study population of 374 subjects (ranging in age from 1 to 14 years old) consisted of 195 patients with liver diseases and 179 patients without liver diseases. Viral DNA/RNA was determined by nested PCR. Genotyping of HBV and HCV were examined by PCR using type-specific primers. Anti-HEV antibody was assayed by ELISA. Results: The infection rate of each virus among patients with liver diseases including acute hepatitis, chronic hepatitis or cirrhosis was 65.6% for HBV and 15.9% for HCV. In contrast, among non-liver disease patients, the infection rates were 14.4% for HBV and 0.6% for HCV, respectively. The most common viral genotypes were type D (85%) of HBV and type 1b (79.3%) of HCV. HDV RNA was detected in 7 of 149 (4.7%) HBV DNA-positive children tested. Moreover, testing for HEV among 341 subjects resulted in the detection of anti-HEV IgG in 62 cases (18.2%). Conclusions: Our results suggest that HBV infection is widespread in Moscow and have led to a high incidence of acute and chronic liver diseases among children in this region.

Aho, J., J. R. Kornegay, et al. (2004). "Evaluation of a convenient enzyme immunoassay to assess the quality of genital specimens submitted for the detection of human papillomavirus DNA by consensus PCR." Journal of Clinical Virology **29**(2): 127.

<http://www.sciencedirect.com/science/article/B6VJV-4BH98CW-3/2/c44d338febd6971d7dc63bbcb554e269>

Background: In the PGMY-line blot assay, a human [beta]-globin fragment is co-amplified with human papillomavirus (HPV) DNA, and both analytes are detected by hybridization with probes fixed on a strip in a linear array. The [beta]-globin DIG-MWP test also detects [beta]-globin amplicons, but in a microtiter plate-based enzyme immunoassay format. Although the PGMY-line blot assay detected 50 cells per test, the [beta]-globin DIG-MWP test generated a signal above the detection cut-off with five cells per test. Objective: The performance of the [beta]-globin DIG-MWP assay to detect [beta]-globin DNA was assessed. Study design: The [beta]-globin DIG-MWP assay was compared to a standard [beta]-globin PCR and to the PGMY-line blot strips on 401 genital specimens. Overall, the three [beta]-globin assays were compared on 325 undiluted lysates, 14 diluted lysates and DNA extracted from 62 lysate samples. Results: Concordance between the PGMY-line blot and the standard [beta]-globin assay reached 99.5% (399 of 401 results), for a kappa value of 0.95. Concordant results were also obtained between the [beta]-globin DIG-MWP assay and PGMY-line blot assay for 387 (96.5%) of 401 test results, for a kappa value of 0.57. Discordant results were due to the increased sensitivity of the DIG-MWP assay. Using a cut-off for positivity at 1.500 optical density (OD) units for [beta]-globin DIG-MWP, concordance improved to 100% (401 of 401 results, kappa at 1.00). Conclusion: The [beta]-globin DIG-MWP assay was adequate to screen for sample adequacy for HPV analysis in genital specimens.

Alhababi, F., T. A. Sallam, et al. (2003). "The significance of 'anti-HBc only' in the clinical virology

laboratory." Journal of Clinical Virology **27**(2): 162.

<http://www.sciencedirect.com/science/article/B6VJV-47GHSK0-2/2/227591233d6afc4b0a10cc4e54d949ad>

Background: Isolated detection of hepatitis B core antibody (anti-HBc) in the absence of surface antigen (HBsAg) or antibody (anti-HBs) has been reported, particularly among individuals infected with human immunodeficiency virus (HIV) and hepatitis C virus (HCV). The significance of this phenomenon is unknown and it is unclear whether all individuals with such serological pattern need further molecular investigations. **Objectives:** To determine the prevalence of 'anti-HBc only' in samples referred to a clinical virology laboratory and to evaluate its significance and possible mechanisms. **Study design:** Samples identified as anti-HBc positive (389/4359, 8.9%) during an 11-month period were investigated for HBsAg, anti-HBs, anti-HCV and anti-HIV. 'Anti-HBc only' samples were tested for HBV DNA using a nested qualitative PCR. Viral loads were measured in samples with detectable HBV DNA and the DNA sequences were analysed. **Results:** Of 379 samples with detectable anti-HBc, 155 (40.9%) were 'anti-HBc only'. HBV DNA was detected in 6/151 (4%), all of which had a viral load. **Conclusions:** 'Anti-HBc only' is a common phenomenon in the clinical virology laboratory but only a small proportion of samples had detectable HBV DNA. The presence of HBsAg mutants with possible false negative HBsAg test result is of concern. Samples with 'anti-HBc only' could be used to monitor the emergence of these mutants.

Behzad-Behbahani, A., P. E. Klapper, et al. (2004). "Detection of BK virus and JC virus DNA in urine samples from immunocompromised (HIV-infected) and immunocompetent (HIV-non-infected) patients using polymerase chain reaction and microplate hybridisation." Journal of Clinical Virology **29**(4): 224.

<http://www.sciencedirect.com/science/article/B6VJV-49569PK-1/2/b636d3c894f1b48d112df279e87ccdb9>

Background: The majority of the human population is infected with two human polyomaviruses BK virus (BKV) and JC virus (JCV) during childhood. After initial infection both viruses persist within renal system. Reactivation of both viruses may be linked with immunodeficiency or immunosuppressive therapy. **Objective:** To evaluate the relationship between immunodeficiency and viruria, prevalence of BK and JC viruria over time was investigated in a cohort of HIV seropositive individuals at different stages of disease. The excretion in this group was compared with virus excretion in their HIV seronegative partners and in an unselected cohort of patients attending a Genito-Urinary Medicine (GUM) clinic. **Study design:** The excretion of BKV and JCV DNA in multiple urine samples from HIV-infected patients at different stages of disease and their HIV-negative partners, and in single samples from a cohort of patients at a GUM clinic was investigated. A microplate hybridisation method was developed to increase both the sensitivity and specificity of detection of the PCR product. The method was also applied to estimate the DNA copy numbers of BKV and JCV in urine samples. **Results:** Within the HIV group, the level of immunosuppression (CD4+ category) was not associated with JCV viruria. By contrast, there was a modest correlation between immunodeficiency as indicated by a decline in CD4+ count and BKV viruria. Shedding of both BKV and JCV DNA together in urine samples of HIV-infected patients was much higher than in control groups ($P=0.02$), indicating that HIV infection may associate with polyomavirus reactivation. The incidence of flu-like syndrome was much higher in HIV-infected asymptomatic individuals than acquired immunodeficiency syndrome (AIDS)-related complex (ARC)/AIDS patients. In general, the concentration of BKV DNA viruria (DNA copy number) was dependent to CD4+ counts ($P=0.008$) while concentration of JCV DNA was independent to CD4+ cell count ($P=0.54$). The prevalence of BKV and JCV DNA in patients who were infected with *C. trachomatis* was 9/50 (18%) and 11/50 (22%), respectively. BKV and JCV DNA was detected in 3/19 (15%) and 2/19 (10%) of patients who were infected with *N. gonorrhoea*. Results suggested that persons infected with *C. trachomatis* were more likely to

show BKV and JCV viruria. Conclusion: These results confirm that shedding of BK and JC viruses in urine is not exclusively found in immunosuppression, it may also occur in healthy individuals. The frequency of virus excretion is however, apparently increased in HIV-infected patients, although no firm statistical difference could be established. One of the interesting aspects of these findings was the relatively high incidence of BKV and JCV viruria in both control groups, i.e. HIV-negative partners of HIV-infected patients and patients attending a GUM clinic.

Bettinger, D., C. Mougin, et al. (1999). "Direct in situ reverse transcriptase-linked polymerase chain reaction with biotinylated primers for the detection of hepatitis C virus RNA in liver biopsies." Journal of Clinical Virology **12**(3): 233.

<http://www.sciencedirect.com/science/article/B6VJV-3WHKSV2-6/2/a7d035831900f1fbaf831a7f78205515>

To assess the presence and the cellular distribution of hepatitis C virus (HCV) RNA in the liver of 11 patients with confirmed HCV infection, a direct in situ reverse transcriptase-linked polymerase chain reaction (RT-PCR) method was performed on formalin-fixed and paraffin-embedded biopsies. The oligonucleotide primers used were specific to the 5' non coding region. An unlabelled downstream oligonucleotide served as a primer for reverse transcription as well as PCR. The upstream oligonucleotide serving as a primer for PCR was biotinylated, allowing a direct enzymatic detection of PCR products. HCV infected cells revealed cytoplasmic staining mainly concentrated towards the interface of the nucleus and cytoplasm. Most of the stained cells were hepatocytes and sometimes Kupffer cells. The results were compared with those obtained by RT-PCR of RNA extracted from the corresponding tissue block. Extracted HCV RNA could be detected in liver tissues of nine out of 11 (82%) infected patients. The detection rate using in situ RT-PCR was 7/11 (63%). The use of labelled primers improved specificity of direct in situ methods, by preventing non-specific incorporation of labelled dNTPs into fragmented DNA. Further studies are however required in order to increase detection sensitivity of HCV infection by in situ molecular methods.

Bogh, M., R. Machuca, et al. (2001). "Subtype-specific problems with qualitative Amplicor HIV-1 DNA PCR test." Journal of Clinical Virology **20**(3): 149.

<http://www.sciencedirect.com/science/article/B6VJV-428FK8V-B/2/4ca8226839097a126d7f83b338fa219c>

Background: commercial HIV-1 qualitative DNA PCR tests have the potential to detect virus in patients in whom antibody tests may be ineffective, such as patients with primary HIV infection and infants born to HIV seropositive mothers. However, the genetic diversity of HIV-1 raises concern about the ability of the PCR tests to detect all current subtypes. Objectives: to assess the sensitivity of the Amplicor HIV-1 test on 126 whole-blood samples representing seven different subtypes and to investigate the sensitivity when the standard assay was modified by including the primer pair SK145 and SKCC1B. Results: of the 126 HIV-1 infected persons, 113 were tested positive and 13 were DNA PCR negative. On the basis of these results, the standard Amplicor HIV-1 test had a sensitivity of 90% in our cohort. In addition, 9% of the positive samples showed a low reactivity but above the cut-off of the assay. The standard assay yielded sensitivities of 100% for subtype B (n=16), D (n=9) and G (n=1), but only 83% for subtype A (n=41), 98% for subtype C (n=43), 79% for subtype E (n=14) and 0% for subtype F (n=2). All samples with low reactivity were non-B subtype. Eight of the DNA PCR negative samples, four subtype A, one C and three E were amplified with the modified Amplicor HIV-1 test with addition of SK145/SKCC1B primers. Using this modified protocol, six samples out of eight became positive. However, two samples (one A and one C) remained DNA PCR negative. Conclusion: this study confirms that

the Amplicor HIV-1 test does not detect all subtypes with equivalent sensitivity and 10% of the samples, tested negative. Thus, it is preferable to add the SK145/SKCC1B primers to the standard test, where infection with non-B subtype is suspected.

Brinkman, J. A., M. Z. Rahmani, et al. (2004). "Optimization of PCR based detection of human papillomavirus DNA from urine specimens." *Journal of Clinical Virology* **29**(4): 230.

<http://www.sciencedirect.com/science/article/B6VJV-497RH9B-1/2/dc95c75967e7fa4e323afe877e6df9d6>

Background: Human papillomavirus (HPV) causes cervical cancer. Current screening requires a yearly pelvic exam and Pap smear. However, these procedures are impractical for screening all women at risk for disease. Urine sampling has been successfully utilized to screen for Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) infections and has been considered for HPV DNA detection by several investigators. However, no study to date has been performed to specifically optimize HPV detection in urine. Objectives: To compare handling and extraction techniques in order to optimize the HPV specific PCR system in urine specimens. Study design: Examination of 10 characteristics that may contribute to PCR inhibition in urine was performed utilizing 10SG multistix. Five different DNA extraction methods were compared in spiked specimens and in 10 clinical specimens. After the optimal extraction technique was identified, concentration of the sample with and without prior dilution was compared to the original protocol. Lastly, specimen handling was compared between immediate processing, refrigerating overnight, or freezing overnight. Results and conclusions: the presence of protein in urine enhanced amplification while nitrites decreased amplification. Of the extraction methods tested, the QIAamp DNA Mini Kit demonstrated the best amplification from urine samples spiked with HPV DNA and clinical specimens. The addition of a dilution step and a concentration step before applying the Qiagen protocol further increased amplification of [beta]-globin (from 50 to 63%) and the HPV L1 gene (from 13 to 33%). Lastly, refrigerating the specimens at 4 [deg]C overnight appears to produce better amplification (62% [beta]-globin and 17% HPV positive) than either immediate processing (46% [beta]-globin and 13% HPV+) or freezing the specimen for 24 h prior to processing (46% [beta]-globin and 10% HPV+). In these studies, amplification was low despite optimization. Additional improvements are required prior to clinical application of a urine-based HPV DNA detection system.

Castelain, S., V. Descamps, et al. (2004). "TaqMan amplification system with an internal positive control for HCV RNA quantitation." *Journal of Clinical Virology* **31**(3): 227.

<http://www.sciencedirect.com/science/article/B6VJV-4C9HS4H-1/2/f0938d77c3b23f73a154830dc6101c58>

Background: Quantitation of hepatitis C virus (HCV) RNA has become an essential tool for monitoring antiviral therapies in chronically infected patients. Different quantitative HCV RNA assays have been reported, mainly using techniques based on signal amplification with branched DNA (bDNA) technology or target sequence amplification by reverse-transcription PCR method (RT-PCR). Objectives and study design: An RT-PCR assay using TaqMan (fluorescence-based real-time PCR) and minor groove binding (MGB) probes was designed for the quantitative determination of HCV RNA in the clinical samples. Calculation of the concentration of HCV RNA was based on an external standard curve in the presence of an internal positive control (IPC). Results: The assay detected 550 international units (IU)/mL with >95% probability of a positive result, with a linear range extending up to 10,000,000 IU/mL. The test exhibited good reproducibility with intra-assay and inter-assay coefficients of variation (CV) of 1.6% and 3.2%, respectively. All the major HCV genotypes were quantified with equivalent efficiency and

accuracy. HCV genotypes 5 and 6 have also been amplified but too few samples have been tested. The performance of this new assay for quantitation of HCV viremia was evaluated with 213 anti-HCV positive sera, 120 of which corresponded to 30 patients sampled during the therapy. We used the Amplicor HCV Monitor assay (Roche Diagnostics, France) and the bDNA VERSANT HCV RNA assay (Bayer Diagnostics, France) to analyze 173 and 40 samples, respectively. The assay described here was significantly correlated with both commercial assays ($R^2=0.9535$, $PR^2=0.8508$, P -Conclusion: The present study illustrated the high reproducibility and reliability of our TaqMan HCV assay. Moreover, the monitoring of viral decline with our assay gave the same results as those obtained with the commercial assays indicating that this new technique provides an attractive approach for measuring HCV viral load.

Comanor, L., C. Elkin, et al. (2003). "Successful HCV genotyping of previously failed and low viral load specimens using an HCV RNA qualitative assay based on transcription-mediated amplification in conjunction with the line probe assay." Journal of Clinical Virology **28**(1): 14.

<http://www.sciencedirect.com/science/article/B6VJV-47CYDC0-2/2/92e5183f511803a8068fe5bd0d1415ff>

Background: Hepatitis C virus (HCV) genotyping is a critical part of the diagnostic work-up for chronic hepatitis C. The VERSANT(R) HCV line probe assay (LiPA) marketed by Bayer Corporation requires PCR-derived amplicons for genotyping usually obtained from commercial assays, including Amplicor(R) HCV 2.0 (Amplicor 2.0), Amplicor HCV Monitor(R) 2.0, or SuperQuant(R). Occasionally, PCR-based methods in conjunction with LiPA fail to give a genotyping result. Although most genotyping failures occur among low viral load specimens, some occur in specimens with relatively high viral loads. The Bayer HCV RNA Qualitative assay (HCV TMA), with a limit of detection of approximately 5-10 IU/ml, is more sensitive than other commercial assays. Objectives: An HCV genotyping protocol using HCV TMA linked with LiPA (TMA-LiPA) was developed and tested for ability to genotype samples that had previously failed genotyping by PCR-based methods in conjunction with LiPA. Study design: Clinical specimens were obtained from eight independent laboratories in Canada and the US and tested with TMA-LiPA at the Bayer Reference Testing Laboratory. Specimens included those that failed to produce a genotype result when a PCR-based assay was used in conjunction with LiPA and specimens for which genotyping was not attempted because the viral load was below the validated cut-off determined in the laboratory of origin. Results and conclusions: TMA-LiPA successfully genotyped 68 of 75 (90.7%) specimens that had failed genotyping by PCR-based methods used in conjunction with LiPA and 36 of 40 (90.0%) specimens that were rejected for genotyping due to low viral load. Moreover, TMA-LiPA assigned subtype for 79 of 107 (73.8%) specimens. Our TMA-LiPA results reflected the distribution of HCV genotypes found in North America, and were 100% concordant with those of Amplicor 2.0 in conjunction with LiPA for control specimens genotyped by both assays. TMA-LiPA may prove useful both in optimizing LiPA performance and genotyping patient specimens.

Drosten, C., T. Nippraschk, et al. (2004). "Prevalence of hepatitis B virus DNA in anti-HBc-positive/HBsAg-negative sera correlates with HCV but not HIV serostatus." Journal of Clinical Virology **29**(1): 59.

<http://www.sciencedirect.com/science/article/B6VJV-48NJ8FR-1/2/9f53c5175f130b046a8377e65674cf25>

Background: Hepatitis B virus (HBV) DNA often remains detectable in serum despite clinical recovery and loss of HBsAg. Objective: To study whether coinfection with HIV and HCV influence the chance of detecting HBV DNA in sera with markers of past hepatitis B. Study design and

results: The test panel included 160 anti-HBc-positive/HBsAg-negative sera collected in the diagnostic setting. The following parameters were determined in the sera: anti-HIV (32% positive), anti-HCV (34% positive), HCV RNA (18% positive), and anti-HBs (37% positive). A highly sensitive PCR (90%-detection limit 100 copies/ml) amplifying the terminal protein (TP) region of HBV was established and HBV DNA was detected in 12.5% of the samples. In 70% of these samples, the HBV DNA concentration was below 500 copies/ml as measured by real-time PCR in the S gene. Logistic regression analysis revealed that the chance of detecting HBV DNA was increased by a positive HCV serostatus (odds ratio 5.0, 95%-CI 1.6-15.7), whereas HIV coinfection (odds ratio 2.0, 95%-CI 0.7-5.8), anti-HBs (odds ratio 0.9, 95%-CI 0.3-2.6), and HCV RNA status (odds ratio 0.4, 95%-CI 0.1-1.7) had no statistically significant influence. In contrast, the chance of detecting HCV RNA in the subgroup of anti-HCV-positive sera was increased by HIV coinfection (odds ratio 4.5, 95%-CI 1.2-17.4). Sequencing of the TP PCR products revealed neither a specific phylogenetic origin of the circulating HBV DNA nor clustering of uncommon mutations in the TP region. Conclusions: The prevalence of HBV DNA in serum of anti-HBc-positive/HBsAg-negative subjects correlates with HCV rather than HIV serostatus.

Feucht, H.-H., B. Zollner, et al. (1999). "High rate of chronicity in HCV infection determined by antibody confirmatory assay and PCR in 4110 patients during long-term follow-up." Journal of Clinical Virology **13**(1-2): 43.

<http://www.sciencedirect.com/science/article/B6VJV-3WMK61W-6/2/3115c987578d07d017c1adb25e9dd118>

Background: It is still unclear how many patients with hepatitis C virus (HCV) antibodies have viremia and hence are infectious. Objectives: To determine the chronicity of HCV infection by correlation of HCV antibodies with presence of viremia in long-term follow-up. Study design: In a longitudinal study sera of 4110 patients were analyzed with second generation HCV-enzyme immunoassay (EIA) and polymerase chain reaction (PCR). Only those patients were included in this study in whom sequential serum samples over a period of 2 years were available. To avoid preanalytical and analytical failures, we used a transport solution to prevent RNA degradation and a four-antigen recombinant immunoblot assay, established in our laboratory, for confirmation of antibody reactivity. Results: Of 2815 patients with confirmed HCV antibodies 2784 (98.9%) were also positive in HCV-PCR assay. False reactive EIA results were detected in 177 (13.7%) individuals as shown by confirmatory assay and PCR. Only one patient (0.04%) spontaneously lost detectable HCV viremia and subsequently HCV-specific antibodies. Conclusions: Our study clearly demonstrates that presence of confirmed HCV-specific antibodies correlates significantly (98.9%; $P < 0.001$) with HCV viremia, and that spontaneous loss of viremia is a very rare event in HCV infection. We also found that elimination of HCV infection is not sufficiently predicted by the loss of detectable viremia in PCR, but can be concluded from the disappearance of virus-specific antibodies.

Gahan, M. E., F. Miller, et al. (2001). "Quantification of mitochondrial DNA in peripheral blood mononuclear cells and subcutaneous fat using real-time polymerase chain reaction." Journal of Clinical Virology **22**(3): 241.

<http://www.sciencedirect.com/science/article/B6VJV-43YR0F4-4/2/c1710dcc7bccf794d09bb5108f348499>

Background: With decreased rates of HIV mortality and disease progression attributable to treatment with nucleoside analogue reverse transcriptase inhibitors (NRTIs), attention has now become focused on the toxicities of these forms of treatment. It is believed NRTIs cause a decrease in mitochondrial DNA (mtDNA) synthesis due to their inhibition of DNA polymerase

gamma. This hypothesis is supported by in vitro data from muscle biopsies and human lymphoblastic cell lines. The resulting mitochondrial toxicity is thought to manifest itself in a variety of clinical symptoms including fatigue, fat wasting and peripheral neuropathy. A non-invasive test of mitochondrial toxicity is needed to assess toxicity and optimise HIV treatment strategies. Peripheral blood mononuclear cells (PBMC) and subcutaneous fat could be ideal and accessible sources of mtDNA for examining toxicity. Objectives: The objectives of this study were (a) to develop an assay to quantify the mtDNA copy number of PBMC and obtain reproducible results and (b) to establish the utility of subcutaneous fat as a source of mtDNA for quantification. Study Design: PBMC were isolated from blood by centrifugation over Ficoll-Paque(R) and subcutaneous fat was obtained from two 3 mm punch skin biopsies. Following DNA extraction, the mtDNA copy number in each sample was quantified by real-time polymerase chain reaction (PCR). Results: The real-time PCR assay was found to generate consistent and reproducible results with replicates of samples undertaken within the same run, and in two or more different runs, having a mean coefficient of variation of 11.3 and 17.2%, respectively. PBMC and subcutaneous fat contained 409+/-148 and 2042+/-391 copies of mtDNA per cell, respectively. Conclusions: From the work carried out it can be concluded that firstly, the real-time PCR assay generates consistent and reproducible results, and secondly that mtDNA can be extracted and quantified from PBMC and subcutaneous fat.

Gaidano, G., D. Capello, et al. (2000). "Molecular characterization of HHV-8 positive primary effusion lymphoma reveals pathogenetic and histogenetic features of the disease." Journal of Clinical Virology **16**(3): 215.

<http://www.sciencedirect.com/science/article/B6VJV-3YWXPNR-6/2/aca85e33d15e7a3314566d8bd2eac048>

Background: Primary effusion lymphoma (PEL) associates with HHV-8 infection, preferentially develops in immunodeficient patients and grows in the serous body cavities. PEL derives from post-germinal center, pre-terminally differentiated B-cells. The pathogenesis of PEL is unclear and the sole identified genetic lesions are human herpesvirus type-8 (HHV-8) infection in all cases and EBV infection in 70% of cases. Epstein-Barr virus (EBV) infection in PEL displays a latency I phenotype. Objectives: To clarify the pathogenesis and histogenesis of PEL by investigating (1) the lymphoma karyotype; (2) the expression status of the Met tyrosine kinase receptor and of its ligand hepatocyte growth factor (HGF); (3) the molecular profile of EBV, with particular focus on mutations of EBNA-1 genes, which are thought to affect viral tumorigenicity in EBV-infected neoplasms displaying the latency I phenotype. Study design: Twenty-four PEL (nine cell lines and 15 primary specimens) formed the basis of the study. Karyotypes were investigated by conventional cytogenetics and fluorescent in situ hybridization (FISH) in selected cases. The expression status of Met and HGF was defined by multiple techniques, including RT-PCR, FACS analysis, immunocytochemistry, Western blot studies and ELISA. The molecular profile of EBNA-1 genes of EBV were investigated by DNA direct sequencing. Results: Trisomy 7, trisomy 12 and breaks at 1q21-q25 are recurrently associated with PEL. PEL consistently co-express Met and HGF both at the mRNA and protein level. Among aggressive B-cell lymphomas, Met/HGF co-expression appears to be relatively specific for PEL. The EBNA-1 gene of EBV displays a high degree of genetic heterogeneity in PEL, with no preferential association with one specific variant. Conclusions: PEL associates with recurrent chromosomal alterations, suggesting that viral infection is not sufficient for tumor development and that lesions of cellular genes may be required. The expression of Met/HGF by PEL cells may bear implications for the lymphoma proliferation and growth pattern, since Met/HGF interactions influence cell mitogenesis and motogenesis. EBV infection in PEL displays a latency I phenotype and fails to associate with specific EBNA-1 variants, suggesting that the role of EBV in PEL is not mediated by the major transforming pathways currently known in EBV positive lymphomas.

Gibellini, D., P. Monari, et al. (2003). "A rapid hemi-nested PCR for HTLV-I detection." Journal of Clinical Virology **28**(3): 341.

<http://www.sciencedirect.com/science/article/B6VJV-49FGX81-1/2/92ed4730be53788e5815bf9904d64250>

A hemi-nested PCR approach was adopted to detect HTLV-1 infection in clinical samples of peripheral blood mononuclear cells (PBMCs) from subjects with positive or indeterminate serological results. Our results showed that the hemi-nested PCR quickly solved the diagnostic query, detecting the presence of proviral HTLV-1 DNA in two of the 252 patients with inconclusive serological results. The main advantage of this method are the typology of DNA extraction, allowing a consistent DNA recovery without amplification problems, the rapidity (4-5 hours), the performance of the assay and its comparable or better sensitivity than other HTLV-1 PCR formats.

Giovannelli, L., G. Capra, et al. "Atypical squamous cells of undetermined significance-favour reactive compared to atypical squamous cells of undetermined significance-favour dysplasia: association with cervical intraepithelial lesions and human papillomavirus infection." Journal of Clinical Virology **In Press, Corrected Proof** <http://www.sciencedirect.com/science/article/B6VJV-4FCG29P-1/2/09f10e693d5fefc26923f6ad4a411cd0>

Background and objectives:The current study compared the cervical cytological sub-category "atypical squamous cells of undetermined significance-favour reactive (AFR)", recently recommended to be eliminated by the Bethesda system, to the sub-category "atypical squamous cells of undetermined significance-favour dysplasia (ASC-US)", in terms of prevalence of coexistent squamous intraepithelial lesions of either low-grade (LSIL) or high-grade (HSIL) and rate of human papillomavirus (HPV) infection. **Study design:**One hundred women with AFR and 100 with ASC-US were consecutively included in the study. All patients underwent colposcopy, followed by biopsy when necessary, and were screened for HPV infection by the combined use of Hybrid Capture II (DIGENE) and PCR with MY09/11 primers, the latter followed by direct sequencing of the amplifications products for HPV genotyping. **Results:**LSIL were detected in 5.6% of AFR and 18.5% of ASC-US ($p = 0.00812$), HSIL only in 4.3% of ASC-US. HPV infection was diagnosed in 11.2% of AFR and 38.0% of ASC-US ($p = 0.00003$); high-risk HPV types (namely, HPV-16, -18, -31, -66, -67 and -70) were found in 6.7% of AFR and 22.8% of ASC-US ($p = 0.00239$). Evidence of HPV infection in absence of SIL was proven in 7.1% of AFR and in 22.5% of ASC-US ($p = 0.00622$). **Conclusion:**The association of AFR with SIL and high-risk HPV infection is low but not inexistent. Thus, to avoid the risk of leaving some high-risk AFR patients untreated or without follow-up, it could be proposed to keep AFR as a cytological category and to triage it by HPV testing, similarly to what has been already recommended for ASC-US.

Halwachs-Baumann, G., M. Wilders-Truschnig, et al. (2001). "Cytomegalovirus diagnosis in renal and bone marrow transplant recipients: the impact of molecular assays." Journal of Clinical Virology **20**(1-2): 49.

<http://www.sciencedirect.com/science/article/B6VJV-423JBCT-8/2/c3af3a6c3906aa4cbdca8d9c6cd01125>

Background: Cytomegalovirus (CMV) infections are a major threat in transplant recipients. In recent years, new assays for routine CMV diagnosis, based on molecular techniques, have become available. **Objective:** The impact of molecular assays for CMV diagnosis in transplant recipient was evaluated. **Study design:** A total of 51 transplant recipients were screened for CMV

infection. Serological (AxSYM CMV IgG and recombinant CMV IgM assays), antigenemia, CMV DNA (qualitative in house PCR and the quantitative COBAS AMPLICOR CMV MONITOR Test), and CMV mRNA (NucliSens CMV pp67 Test) tests were compared. Results: In 11/20 bone marrow transplant (BMT) recipients and 10/31 renal transplant (RTX) recipients there was no evidence of active CMV infection. Ten RTX recipients and one BMT recipient were antigenemia positive, 21 RTX and seven BMT recipients were PCR positive (qualitative CMV PCR). There were more BMT recipients CMV DNA positive in serum (7/21) than antigenemia positive (1/21). CMV mRNA was found positive in two BMT recipients (one case with no other evidence of CMV infection, the other one CMV DNA positive and antigenemia negative). The only antigenemia positive BMT recipient was found negative for CMV mRNA, but positive in all other tests. Eight RTX recipients were found positive for CMV mRNA. Six of them were also antigenemia positive and five of those were also found positive for CMV IgM. One CMV mRNA positive RTX recipient was CMV IgM positive but antigenemia negative and the other one CMV mRNA positive RTX recipient was found negative in all other tests. Two antigenemia positive RTX recipients were found negative for mRNA and CMV IgM. Conclusion: Antigenemia was found to be a good screening test for CMV infection in RTX recipients. In BMT recipients, tests based on molecular techniques appeared to be superior compared to antigenemia.

Helbig, K. J., J. George, et al. (2005). "A novel I-TAC promoter polymorphic variant is functional in the presence of replicating HCV in vitro." *Journal of Clinical Virology* **32**(2): 137.

<http://www.sciencedirect.com/science/article/B6VJV-4DS6BHW-4/2/0141689e165dbbe2b2b777732461391a>

Background: Chemokines are strong candidate genes for outcome of HCV infection. I-TAC is a chemokine known to be involved in the inflammatory process of HCV infection, and its expression is upregulated in chronic hepatitis C (CHC). Objectives: The aim of this study was to investigate genetic variability in the I-TAC promoter and to determine the correlation of these variants with HCV disease progression. Study design: I-TAC genotyping was performed in 60 chronic HCV patients and 60 controls using GeneScan analysis. Functional analysis of the I-TAC promoter was performed with the aid of luciferase reporter constructs transfected into Huh-7 cells or Huh-7 cells harbouring HCV genomic and sub-genomic replicons. Cytokine induced production of I-TAC from whole blood cultures was measured using enzyme-linked immunosorbent assay (ELISA). Results: Sequencing of approximately 1 kb upstream of the I-TAC gene start codon revealed the presence of a novel 5 bp deletion mutant (-599del5) in a number of chronic HCV patients. Analysis of the functional potential of this deletion revealed no transcriptional change in Huh-7 cells transfected with luciferase reporter constructs, and this was confirmed in cytokine stimulated whole blood cultures where similar levels of I-TAC were liberated regardless of -599del5 genotype. Conversely, the -599del5 deletion variant significantly reduced transcriptional activity of the I-TAC promoter in the presence of replicating HCV. The distribution frequency of the allele was found to be significantly increased in a chronically HCV infected population compared to healthy controls. Conclusions: The novel I-TAC -599del5 promoter polymorphism is a functional variant in the presence of replicating HCV. Furthermore, this deletion mutant is significantly increased in a chronic HCV cohort and may predispose to HCV disease susceptibility.

Hoffmann, D., B. Buchberger, et al. (2005). "In vitro synthesis of enzymatically active HIV-1 protease for rapid phenotypic resistance profiling." *Journal of Clinical Virology* **32**(4): 294.

<http://www.sciencedirect.com/science/article/B6VJV-4DJ4W65-1/2/84ba6fb2c7b5b20c71dd1c43532e39a7>

Background: Given the expanding antiretroviral therapy, inexpensive and fast HIV drug resistance assays are urgently needed. In this view, we have developed a novel phenotypic resistance test for HIV-1 protease inhibitors (PIs) based on recombinant expression of patient-derived HIV PR in *Escherichia coli* and subsequent enzymatic testing in a fluorescent readout. Objectives: To facilitate and expedite the test procedure, we have introduced coupled in vitro transcription/translation using a commercially available technology called RTS for producing enzymatically active HIV-1 protease (PR). Study design: We expressed one wild type PR and one highly resistant mutant starting from molecular clones as well as three patient-derived PRs. The amplified PR gene was either ligated into an expression vector or directly used as a template for the in vitro transcription/translation reaction. Enzymatic susceptibility data derived from in vitro expressed PRs were correlated to the respective results from *E. coli* expression and genotypic evaluation. Results: All tested enzymes were obtained in sufficient quantities for complete resistance profiling to five PIs. The PRs required no purification prior to the enzymatic assay. Inhibition constants and enzymatic resistance factors compared well to corresponding data from PRs expressed in parallel in *E. coli*. Enzymatic resistance was in good agreement with the respective PR genotype. Conclusion: The presented in vitro transcription/translation system represents a novel approach for HIV PR expression starting from molecular clones or patient samples. Coupled with the enzyme-kinetic PR assay recently developed in our group it allows to sensitively quantify resistance to PIs. The test system is significantly less laborious and faster than currently available phenotypic drug resistance assays.

Huang, L.-W., S.-L. Chao, et al. (2004). "Multiple HPV genotypes in cervical carcinomas: improved DNA detection and typing in archival tissues." *Journal of Clinical Virology* **29**(4): 271.

<http://www.sciencedirect.com/science/article/B6VJV-49FRF8P-1/2/607178c723e215454028f226ce541269>

Background: Human papillomaviruses (HPV) have been considered to be the necessary and central agents of cervical carcinoma. Objective: The aim of this study was to determine the prevalence and genotypes of HPV in archival cervical carcinomas. Study design: The study included 152 paraffin-embedded, formaldehyde-fixed cervical carcinoma specimens. To improve the detection and typing of HPV in archival tissues, we conducted a comprehensive study in which, polymerase chain reaction (PCR)-based methods using E7 type-specific (TS) and L1 modified general primers (MY11/GP6+ and GP5+/GP6+) were employed. Results: Overall HPV prevalence was 98% in the cervical carcinomas. HPV 16 was detected in 66% of the tumors, HPV 18 in 22%, HPV 31 in 13%, HPV 33 in 9%, and HPV 58 in 9%. Notably, multiple HPV types were present in 44 (28.9%) of the 152 cervical carcinomas. The most common co-infections were HPV types 16/18 (12 cases), followed by HPV types 16/31 (7 cases). Additionally, HPV 18 was more frequent in adenocarcinomas and adenosquamous carcinomas (86%) than in squamous cell carcinomas (15.8%) ($P=0.0002$). Conclusions: The combination of L1 general primers and E7 type-specific primers can be of use in detecting HPV DNA in archival tissues. The present study showed a high frequency of multiple HPV infections in cervical carcinomas. Hence, relevant HPV typing information in cervical carcinoma is very important for further HPV vaccine design and application.

Ilona, L.-F., O. Pia, et al. (2003). "Detection of herpes simplex virus DNA in dried blood spots making a retrospective diagnosis possible." *Journal of Clinical Virology* **26**(1): 39.

<http://www.sciencedirect.com/science/article/B6VJV-45TY876-2/2/9b89f8327158f45f6f1bf3fe27d4d812>

Background: Herpes simplex virus (HSV) infections in neonates are associated with life-

threatening disease. Early diagnosis and treatment with antiviral therapy has decreased the morbidity, mortality and long-term sequelae in surviving children. The aim of the study was to investigate if herpes simplex virus DNA detection in dried blood spots on filter papers (Guthrie cards) sampled for screening of metabolic diseases may contribute to early diagnosis of neonatal HSV infection and enable pre-emptive therapy. Methods: For detection of HSV-1 and -2 DNA, two different DNA extraction methods were evaluated. A minimal essential medium (MEM) extraction method was found superior and was used in combination with detection of HSV-1 and -2 DNA by PCR in dried blood spots from children with verified neonatal HSV infection. Cards from 28 children were included. The onset of illness varied from day 0 to 42 days and was the result of different types of maternal infection (27 cases) and an external source (one case). Results: HSV DNA was detected in seven of the 28 Guthrie cards, two were HSV-1 and five were HSV-2 DNA positive. Positive dried blood spot cards were sampled within the interval 5 days before, to 6 days after onset of neonatal herpes. In cases of late onset CNS disease, viremia, was not demonstrable at the age of 3-5 days, the time period when the blood spot cards are normally sampled. Conclusion: Viremia, the prerequisite for demonstrating HSV DNA in dried blood spot cards preceded the onset of illness by up to 5 days and lasted at least up to 6 days thereafter. Analysis of HSV DNA in dried blood spot cards may be of value in the diagnostic arsenal for early onset of neonatal herpes and also have a role in the follow up of a child exposed at delivery. As the majority of the later onset neonatal herpes encephalitis cases are missed, a large-scale neonatal screening does not seem appropriate.

Kamar, N., K. Sandres-Saune, et al. (2004). "Effects of long-term lamivudine therapy in renal-transplant patients." Journal of Clinical Virology **31**(4): 298.

<http://www.sciencedirect.com/science/article/B6VJV-4D34PKK-1/2/9b1aa899c423f4cff33af368f4585fee>

Background: Following renal transplantation (RT), chronic immunosuppression is associated in hepatitis B virus (HBV) (+) patients with a flare-up of the disease, which might be harmful in the long term. Objectives: We report on the effect of long-term lamivudine therapy given at an initial daily dose of 100 mg in 18 HBV (+) RT patients. Results: When lamivudine therapy was commenced, 14 patients (77%) had an increase in their aspartate (AST) and alanine (ALT) aminotransferase levels. During a mean follow-up, under treatment, of 36.5 [plus-or-minus sign] 3.5 months (up to 66 months), 10 patients (55%) had a sustained partial (HBV DNA 5 copies/ml) (n = 4) or complete (HBV DNA n = 6) virological response. Overall, 12 virological breakthroughs were observed. Of those who were HBe Ag(+) prior to lamivudine therapy (n = 4), one seroconverted to HBe Ab during therapy. At the last follow-up, AST and ALT levels were normal in 13 patients. When liver biopsy was repeated during treatment (n = 15), the virological responders showed a significant decrease in total Knodell score from 10 [plus-or-minus sign] 0.6 to 7 [plus-or-minus sign] 1 (P = 0.04), but no significant change in the stage of fibrosis. Conversely, in those patients with high HBV DNA titers, there were no significant changes in the total Knodell score or in the grade of fibrosis. Conclusion: In conclusion, lamivudine therapy is safe in HBV(+)ve renal-transplant patients. However, even if the full and partial virological response rates are still high (55%) in the long term, relapse or primary non-responses occur. The implementation of alternative efficient strategies is warranted.

Koch, N., C. Tamalet, et al. (2001). "Comparison of two commercial assays for the detection of insertion mutations of HIV-1 reverse transcriptase." Journal of Clinical Virology **21**(2): 153.

<http://www.sciencedirect.com/science/article/B6VJV-433PC0B-6/2/f87e44f18d558832caf7358a5664b254>

Insertions in the [beta]3-[beta]4 fingers subdomain of HIV-1 reverse transcriptase (RT) confer cross-resistance to various nucleoside analogs. The detection of these rearrangements in the region of codons 67-70 of RT is of primary importance for adapting and optimizing combination treatment regimen. Recent reports suggest that some genotyping techniques based on the hybridization of oligonucleotide probes may fail to detect insertion mutants of HIV-1 RT. In the present study, we have evaluated the efficiency of two commercial kits TruGene (based on Dye Primer sequencing) and Viroseq (Big Dye Terminator technique) for the detection of insertion mutations. The data were compared with an in-house dRhodamine sequencing method. Overall, all these cycle sequencing techniques were operative in the detection of insertion mutants. The best peak homogeneity in the electrophoregrams was observed with the Dye primer technique. However, specific compression artifacts were frequently encountered with this technique, rendering ambiguous the interpretation of the electrophoregrams in several regions of the sequence. This shortcoming did not occur with dRhodamine Dye terminator or Bigdye terminator cycle sequencing. In any case, a manual inspection of the electrophoregrams is highly recommended, for all types of cycle sequencing techniques, especially for detecting new mutational patterns of the RT and protease genes. Finally, some specific problems were encountered with the softwares provided with both Trugene and Viroseq kits.

Lau, W., R. Onizuka, et al. (1998). "Polymerase chain reaction based assessment of leukoreduction efficacy using a cytomegalovirus DNA transfected human T-cell line." Journal of Clinical Virology **11**(2): 109.

<http://www.sciencedirect.com/science/article/B6VJV-3VXY4S9-G/2/0aad66c6f29d1420d5cbec74c04e8bb4>

Background and Objectives: Detection of CMV DNA by PCR in seropositive blood donors is affected by nucleic acid extraction, amplification conditions and PCR product detection sensitivity. Clinical studies have shown that leukoreduced blood products are as effective as CMV-seronegative blood products in minimizing transfusion-associated CMV infection. We developed a PCR-based test model to assess the efficacy of leukoreduction on the removal of CMV DNA from blood donations. **Materials and Methods:** Whole blood units were spiked with a human Jurkat T-lymphocyte cell line which had been stably transfected with a CMV DNA immediate early sequence. All blood units were either CMV seronegative or shown to be CMV PCR negative. The amount of CMV DNA by PCR before, during and after filtration of blood units with third-generation leukocyte reduction filters were determined using a semi-quantitative adaptation of the Digene SHARP Signal(TM) System Assay (DSSSA). **Results:** Whole blood units spiked with $1-2 \times 10^6$ CMV transfected Jurkat cells contained no detectable CMV DNA after leukoreduction. With a higher inoculum of 2.9×10^8 transfected Jurkat cells per unit, CMV DNA was detected after leukoreduction; however, there was an approximate 3 log decrease in the amount of CMV DNA detected. **Conclusion:** This CMV DNA transfected human Jurkat cell line in conjunction with semi-quantitative CMV PCR may be used to model leukoreduction efficacy and provides evidence that leukoreduction of blood products can decrease the amount of cellular CMV DNA by approximately 3 logs.

Lee, T.-H., D. M. Chafets, et al. (2004). "Quantitation of HTLV-I and II proviral load using real-time quantitative PCR with SYBR Green chemistry." Journal of Clinical Virology **31**(4): 275.

<http://www.sciencedirect.com/science/article/B6VJV-4D2N7V9-1/2/b7cf2fa286d4facb578a432147f3ecf0>

Background: Human T-lymphotropic virus type I (HTLV-I) is linked etiologically with adult T cell leukemia/lymphoma and HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP).

Human T-lymphotropic virus type II (HTLV-II) is associated with HAM/TSP and, in HIV coinfecting patients only, rare cases of cutaneous T cell lymphoma. Proviral load may be important in the pathogenesis of HTLV-associated disease. Materials and Methods: A real time quantitative PCR assay using SYBR Green intercalation was established. Primers targeting the tax region were standardized against MT2 and MOT cell line DNA for HTLV-I and HTLV-II, respectively. HTLV-I/II copy number was normalized to the amount of cellular DNA by quantitation of the HLA-DQ alpha gene. We measured proviral load in peripheral blood mononuclear cells (PBMCs) in a large cohort of 120 HTLV-I and 335 HTLV-II seropositive former blood donors. We also assessed the intra- and inter-assay reproducibility of the assay. Results: Proviral load for HTLV-I infected patients ranged from 3.1×10^0 to 1.8×10^5 copies/106 PBMCs with a mean of 1.6×10^4 and a median of 3.0×10^3 . HTLV-I was undetectable in 7 of 120 cases (5.8%). Proviral load for HTLV-II infected patients ranged from 1.1×10^0 to 1.0×10^6 copies/106 PBMCs with a mean of 2.8×10^4 and a median of 5.0×10^2 . HTLV-II was undetectable in 31 out of 335 cases (9.3%). Conclusion: The assay has excellent dynamic range from 106 to 100 copies/reaction, good intra- and inter-assay reproducibility, and a lower limit of detection of a single copy per reaction. The sensitivity and high dynamic range allow determination of a broad range of HTLV-I/II proviral load in clinical subjects. This assay will facilitate the study of the relationship between proviral load and pathogenesis.

Legler, T. J., M. Kohler, et al. (1999). "High-throughput extraction, amplification, and detection (HEAD) of HCV-RNA in individual blood donations." *Journal of Clinical Virology* **13**(1-2): 95.

<http://www.sciencedirect.com/science/article/B6VJV-3WMK61W-C/2/579c944e3fe95b7540dcf2f61ee9664c>

Background: High-throughput nucleic acid amplification techniques (NATs) are required for the detection of viral genomes in individual blood donations and might be helpful in any virological laboratory. Objective: To develop and automate a method for the detection of hepatitis C virus RNA in individual blood donations, compatible with the time schedule of routine blood bank screening and product release. Study design: The viral RNA was isolated with the use of target specific capture oligonucleotides and magnetic beads. This extraction method was combined with reverse transcription/amplification (RT/PCR) and fluorescence detection. We adapted our method on a pipetting robot and pipetted all steps in a single room. When the pipetting was completed, microtiter plates were heat-sealed with foils and placed into a thermocycler. Positive reactions were detected with a fluorescent dye in a second room. Aerosols were avoided with programmed slow pipetting steps and with a special device constructed for the removal of the used disposable tips. During a 7 month period, we used this method in routine testing of individual donations prior to the release of all blood components. Results: The total number of 11700 individual donations including platelet concentrates were analysed. We tested up to 192 specimens in one run within 7 h. The frequency of cross-contamination using the automated procedure was 0.1%. Five specimens have been found repeatedly reactive for HCV-RNA, four of these were anti-HCV positive, one sample from a repeat donor was negative in anti-HCV assays. A seroconversion was detectable at his next presentation, 6 months later. Conclusion: In this pilot study, we demonstrate that automated HCV-RT-PCR testing is practicable for individual donations in high-throughput. Additionally, the described PCR approach could easily be adapted to the detection of other viral genomes by the use of specific primers.

Maudru, T. and K. W. C. Peden (1998). "Analysis of a coded panel of licensed vaccines by polymerase chain reaction-based reverse transcriptase assays: A collaborative study." *Journal of Clinical Virology* **11**(1): 19.

<http://www.sciencedirect.com/science/article/B6VJV-3VXY4NF->

Background: A recent publication reporting the presence of low levels of reverse transcriptase (RT) activity in certain vaccines for human use necessitated that regulatory agencies address the issue of whether this RT activity presented a risk to humans. Detection of low levels of RT activity corresponding to fewer than ten virions became possible with the development of highly-sensitive polymerase chain reaction (PCR)-based RT (PBRT) assays. Variations of the PBRT assay were developed in three laboratories. These assays were reported as being at least one million-fold more sensitive than conventional RT assays. **Objective:** To ascertain the sensitivity and reliability of PBRT assays in different laboratories and to determine which vaccine samples possessed RT activity. **Study design:** Coded panels of licensed vaccines together with positive and negative controls was assembled at the Center for Biologics Evaluation and Research (CBER) of the Food and Drug Administration (FDA) and distributed to five cooperating laboratories as well as to our laboratory at CBER. Each laboratory carried out their version of the PBRT assay and submitted the results to the coordinator at CBER. **Results:** Results of the PBRT analyses carried out in the six laboratories are presented. Five of the six laboratories reported results that were highly consistent. RT activity was detected in live attenuated vaccines that were prepared in chick embryo cells (mumps, measles and yellow fever), but very low or undetectable RT activity was found in vaccines produced in mammalian cells (rabies and rubella). Influenza vaccines from several manufacturers included in the panel displayed the most variability, with different products of this inactivated vaccine having differing amounts of RT activity. **Conclusion:** Only vaccines produced in chick embryo cells had significant RT activity. Because RT activity was present in the allantoic fluid of uninfected chick embryos and culture medium from chick embryo fibroblasts, the RT activity arises from the cell substrate used for vaccine production. The PBRT assays were reliably able to detect the low levels of RT activity in chicken-derived vaccines.

Pas, S. D., R. A. de Man, et al. (2002). "The dynamics of mutations in the YMDD motif of the hepatitis B virus polymerase gene during and after lamivudine treatment as determined by reverse hybridisation." *Journal of Clinical Virology* **25**(1): 63.

<http://www.sciencedirect.com/science/article/B6VJV-44PVT3G-2/2/3172db28eabd35c3b69c4b10600c4493>

We have analysed the dynamics of HBV variants related to Lamivudine resistance in 22 chronically infected patients during and after the end of Lamivudine therapy. Thirteen patients had a confirmed methionine mutation in the YMDD region of the reverse transcriptase domain determined by sequence analysis. They responded to therapy having a mean reduction of HBV DNA of 4.55 log (range 2.93-8.91). Nine patients partly responded to therapy, with a small decline of HBV DNA (mean reduction of 3.39 log, range 1.72-5.12) and no indication for an YMDD variant. Samples were re-analysed with a HBV Drug Resistance Line Probe assay (InnoLipa HBV-DR), which detected as low as 10% of a variant HBV population. With this assay, changes in the YMDD region were detected with a mean of 2 weeks (range -8 to 10) earlier than by an increase of HBV DNA levels. Increase of ALT was observed with a mean of 31 weeks (range 29-51) later than the methionine changes in the YMDD motif. Indications for a methionine into valine change could be determined in only one of the partial responders. An unexpected observation was the predominant presence of variant virus populations after end of therapy. In ten patients, we detected the wild type virus with a mean of 14 weeks after end of therapy (range 1-42 weeks). In three patients, no variant virus population could be detected at 25, 36 and 37 weeks, respectively, after cessation of treatment. This observation is important for the inclusion of the so-called naive patients in clinical trials.

Pas, S. D. and H. G. M. Niesters (2002). "My Favourite Reagent: Detection of HBV DNA using real time

analysis." Journal of Clinical Virology **25**(1): 93.

<http://www.sciencedirect.com/science/article/B6VJV-45WGJP2-7/2/d05850f4e49ce245f3270db233fad8a7>

Pas, S. D., S. Noppornpanth, et al. (2005). "Quantification of the newly detected lamivudine resistant YSDD variants of Hepatitis B virus using molecular beacons." Journal of Clinical Virology **32**(2): 166.

<http://www.sciencedirect.com/science/article/B6VJV-4DW3FTG-1/2/7e7114bce50095c613ef1464e90b307b>

A real-time based amplification assay with molecular beacons was used to detect and quantify PCR amplicons to discriminate between the newly described Lamivudine-resistant YSDD variant, a known YIDD variant and wild-type Hepatitis B virus (HBV) DNA in the YMDD region of the polymerase gene. Using this assay, we retrospectively analysed samples from two HBV chronically infected Asian twin sisters, starting 9 weeks before therapy, during and between two periods of treatment with Lamivudine. In order to analyse more accurately the dynamics of variant DNA loads during and after therapy, this real time assay was compared to three other mutation analysis techniques, restriction fragment length polymorphism (RFLP), InnoLipa HBV-DR assay and direct sequence analysis. With this technique, new information on the dynamics of variants during and after therapy was obtained.

Perrons, C., R. Jelley, et al. (2005). "Detection of persistent high risk human papillomavirus infections with hybrid capture II and SPF10/LiPA." Journal of Clinical Virology **32**(4): 278.

<http://www.sciencedirect.com/science/article/B6VJV-4DHX9B0-1/2/d9a41df63ccc8a3bbe049fb6c0adbdbc>

Background: HPV infection in young women is common. However only a certain number of HPV genotypes are oncogenic. It is necessary for high risk HPV infection to persist at the cervix for a considerable time before oncogenesis occurs. Objectives: To look for persistence of high risk HPV in women attending a colposcopy clinic. Two DNA detection methods were used and the results compared to determine the rates of persistent, resolved and acquired infections over a 6-month period. HPV genotyping was used to determine type specific persistence. Study design: One hundred and thirty-eight women were tested for HPV infection when attending the colposcopy clinic at UCLH and then tested again at a subsequent visit approximately 6 months later. HPV DNA was detected by the Digene HC II assay using the high risk probes only and by PCR with the SPF10 primer set. All SPF10 PCR-positive samples were then specifically genotyped by a Line Probe Assay (LiPA) [Kleter et al. 1999. J. Clin. Microbiol. 1999;37:2508]. Results: At entry of the study high risk HPV was detected in 43% of the samples by Digene HC II and in 60% of the samples by SPF10/LiPA. Thirty-eight (28%) of the women had a true persistent infection with the same high risk HPV genotype over a median period of 6.3 months. Nine (7%) women resolved one HR HPV infection after their first colposcopy visit, but obtained a different high risk HPV infection by the time they were tested at their second visit as identified by LiPA. Thirty-seven (27%) of the 138 women had mixed HPV infections, representing 45% of all those infected. Conclusions: The SPF10/LiPA assay detected more high risk infections than the Digene HC II assay. The Digene HC II assay was unable to distinguish between persistent infections with the same high risk genotype and those where the genotype had changed between visits.

Picone, O., J.-M. Costa, et al. (2005). "Human cytomegalovirus (HCMV) short tandem repeats analysis in congenital infection." Journal of Clinical Virology **32**(3): 254.

<http://www.sciencedirect.com/science/article/B6VJV-4F3NY6G-1/2/73e4ee34295d2bf8a08ac06b2757cd28>

Pignatelli, S., P. Dal Monte, et al. (2003). "Intrauterine cytomegalovirus infection and glycoprotein N (gN) genotypes." Journal of Clinical Virology **28**(1): 38.

<http://www.sciencedirect.com/science/article/B6VJV-47CYDC0-1/2/cf3ff0819df9531cfef2c668828bd799>

Background: Human cytomegalovirus (HCMV) clinical isolates display genetic polymorphisms, supposed to be related with strain-specific tissue-tropism and HCMV-induced immunopathogenesis. One recently discovered polymorphic gene is ORF UL73, encoding for the envelope glycoprotein gN. Among HCMV clinical strains, it shows four distinct genomic variants denoted as gN-1, gN-2, gN-3 and gN-4. Objectives: Aims of this study were to assess the prevalence of the different gN types in the populations examined and to investigate the possible relationship between genotypes and severity of congenital CMV disease. Study design: The gN genotyping was carried out by sequencing analysis of the HCMV ORF UL73. Comparisons were made by chi-square test and contingency tables. Results: All the four gN genotypes can cause congenital infections and the overall distribution was as follows: gN-1, 23.6%; gN-2, 1.1%; gN-3, 12.9%; gN-4, 62.4%. None of them seems to be preferentially associated with vertical transmission or with acute outcome of congenital infection. However, considering the chronic outcome and long-term sequelae, there was a statistically significant (PConclusions: HCMV congenital infections, which displayed a prevalence of the gN-1 variants, seem to be associated with favorable chronic outcome.

Piiparinen, H., K. Hockerstedt, et al. (2004). "Comparison of two quantitative CMV PCR tests, Cobas Amplicor CMV Monitor and TaqMan assay, and pp65-antigenemia assay in the determination of viral loads from peripheral blood of organ transplant patients." Journal of Clinical Virology **30**(3): 258.

<http://www.sciencedirect.com/science/article/B6VJV-4BRPC38-1/2/fca40238ecf77b77fe81a9fe288e56bc>

Background: Quantitative PCR assays have become the most common methods in the determination of viral load during cytomegalovirus (CMV) infection of transplant patients. However, usually these tests are still quite time-consuming and labor-intensive which diminishes their utility of these tests in routine diagnostic laboratories. Objectives: The objective of this study was to develop a quantitative CMV PCR test which is time-saving and easy to perform for the detection and monitoring of CMV infection of transplant patients. Study design: The quantitative real time CMV PCR assay using TaqMan chemistry and an automated sample preparation system, MagNA Pure LC, was developed. The designed quantitative CMV test was compared to commercial quantitative PCR test, Cobas Amplicor Monitor, in the determination of CMV DNA loads in plasma samples of liver and kidney transplant patients. The results were also correlated with the CMV pp65-antigenemia test. The clinical material of 270 blood specimens of transplant patients were tested using these two PCR methods and pp65-antigenemia test in parallel. Plasma samples were used for PCR assays and leucocytes for the antigenemia test. Results: The TaqMan assay described was easy to perform, it was rapid (3-4 h) and hands-on time needed for performing the test was short. The detection limit of the assay was 250 copies/ml

(cps/ml) plasma and the linear range up to 25,000,000 cps/ml. TaqMan assay was the most sensitive test detecting 92% of the CMV positive findings. Cobas Monitor detected 80% and pp65 test 88% of the positive findings. The correlations between TaqMan and antigenemia assays, and between Cobas Amplicor and antigenemia were statistically significant and high, $R=0.84$ ($PR=0.80$) ($PR=0.64$, P Conclusions: The developed real time TaqMan assay was rapid and easily performed and could be the best alternative for the diagnosis of CMV infection and monitoring of liver and kidney transplant patients.

Poon, L. L. M., K. H. Chan, et al. (2003). "Early diagnosis of SARS Coronavirus infection by real time RT-PCR." Journal of Clinical Virology **28**(3): 233.

<http://www.sciencedirect.com/science/article/B6VJV-49KYTJ5-3/2/96dc5a1dab0e5a8d02df7d06c4fe876c>

Background: A novel coronavirus was recently identified as the aetiological agent of Severe Acute Respiratory Syndrome (SARS). Molecular assays currently available for detection of SARS-coronavirus (SARS-Cov) have low sensitivity during the early stage of the illness. Objective: To develop and evaluate a sensitive diagnostic test for SARS by optimizing the viral RNA extraction methods and by applying real-time quantitative RT-PCR technology. Study design: 50 nasopharyngeal aspirate (NPA) samples collected from days 1-3 of disease onset from SARS patients in whom SARS CoV infections was subsequently serologically confirmed and 30 negative control samples were studied. Samples were tested by: (1) our first generation conventional RT-PCR assay with a routine RNA extraction method (Lancet 361 (2003) 1319), (2) our first generation conventional RT-PCR assay with a modified RNA extraction method, (3) a real-time quantitative RT-PCR assay with a modified RNA extraction method. Results: Of 50 NPA specimens collected during the first 3 days of illness, 11 (22%) were positive in our first generation RT-PCR assay. With a modification in the RNA extraction protocol, 22 (44%) samples were positive in the conventional RT-PCR assay. By combining the modified RNA extraction method and real-time quantitative PCR technology, 40 (80%) of these samples were positive in the real-time RT-PCR assay. No positive signal was observed in the negative controls. Conclusion: By optimizing RNA extraction methods and applying quantitative real time RT-PCR technologies, the sensitivity of tests for early diagnosis of SARS can be greatly enhanced.

Preiser, W., N. S. Brink, et al. (2003). "Development and clinical application of a fully controlled quantitative PCR assay for cell-free cytomegalovirus in human plasma." Journal of Clinical Virology **26**(1): 49.

<http://www.sciencedirect.com/science/article/B6VJV-4604319-1/2/d9f3013c29d95e32448f3b7ff624eadc>

Background: Cytomegalovirus (HCMV) disease continues to be a major problem in certain patient groups, including bone marrow transplant (BMT) recipients. The quantification of HCMV genome is clinically useful for the diagnosis of HCMV disease, for the virological surveillance of high-risk patients and for monitoring antiviral therapy. Objectives: To develop a novel, robust, and fully controlled PCR (qPCR) for the quantification of HCMV DNA in plasma samples and to demonstrate its clinical usefulness in the BMT setting. Study design: The newly developed HCMV qPCR employs cell culture-derived murine CMV as an internal control for both extraction and amplification. Following amplification using common primers, detection of both internal control and patient HCMV amplicons is by specific probes and a chemiluminescence microtitre plate system. Its performance was evaluated using the routine non-quantitative nested HCMV PCR on whole blood (NQPCR) and correlated with clinical events such as disease and antiviral therapy. Results: A high level of concordance (85.1%) was found between the novel assay and the

NQPCR, with the qPCR being slightly more sensitive. The samples giving discordant results generally had levels of HCMV DNA close to the limit of detectability or had been stored for prolonged periods. Conclusions: The use of plasma as an analyte by the newly developed assay avoids the detection of cell-associated virus. On the other hand, testing a comparatively large volume of plasma ensures that sensitivity is not compromised by not detecting cell-associated HCMV. In a small preliminary evaluation in BMT recipients, changes in HCMV 'viral load' correlated with initiation and discontinuation of antiviral therapy and were biologically plausible.

Puppe, W., J. A. I. Weigl, et al. (2004). "Evaluation of a multiplex reverse transcriptase PCR ELISA for the detection of nine respiratory tract pathogens." *Journal of Clinical Virology* **30**(2): 165.

<http://www.sciencedirect.com/science/article/B6VJV-4BBKHBS-4/2/0a35233520b490dced01da781d803955>

Background: A multiplex reverse transcription (RT) polymerase chain reaction combined with a microwell hybridization assay (m-RT-PCR-ELISA) was previously developed to detect nine different microorganisms: enterovirus (EV), influenza virus type A (IVA) and type B (IVB), respiratory syncytial virus (RSV), parainfluenzavirus type 1 (PIV1) and type 3 (PIV3), adenovirus (AV), *Mycoplasma pneumoniae* (Mpn), *Chlamydia pneumoniae* (Cpn) in a single test. These organisms do not usually colonize the respiratory tract of humans, but, if present, it may be assumed they are involved in respiratory disease. Objectives and study design: The m-RT-PCR-ELISA was tested on (i) culture supernatants of unknown contents, (ii) by determining the analytical sensitivity of 10-fold serial dilutions of culture supernatants and (iii) by determining clinical sensitivity in a retrospective study on 411 clinical specimens. The specimens were re-tested in parallel by m-RT-PCR-ELISA versus the gold standard culture and immunofluorescence, and versus individual RT-PCR. Results: (i) The 9-valent m-RT-PCR-ELISA shows 83% to 100% concordant results on 103 culture supernatants containing different organisms. (ii) The analytical sensitivity was as follows: higher sensitivity of the 9-valent m-RT-PCR-ELISA in comparison to culture in the cases of PIV3, IVA and IVB (factor 10) and AV and EV (factor 100), and lower sensitivity in case of RSV and PIV1 (factor 10). (iii) The agreement with the gold standard in the kappa statistic was excellent for RSV ($\kappa=0.937$), IVA ($\kappa=0.940$), very good for PIV1 ($\kappa=0.914$), IVB ($\kappa=0.907$) and satisfactory for PIV3 ($\kappa=0.410$). For AV, EV and Mpn the m-RT-PCR-ELISA preliminary could be qualified as very good, based on the data derived on culture supernatants. Information about the validity for Cpn is limited. Conclusion: The m-RT-PCR-ELISA is a feasible, sensitive and specific method for detection of a broad spectrum of organisms. It is suitable for individual as well as epidemiological diagnosis.

Rautenberg, P., C. Lubbert, et al. (1999). "Evaluation of the AmpliSensor PCR and the SHARP signal detection system for the early prediction of symptomatic CMV infection in solid transplant recipients." *Journal of Clinical Virology* **13**(1-2): 81.

<http://www.sciencedirect.com/science/article/B6VJV-3WMK61W-B/2/d0ff9f0a42ffc3155735fff6c804ccd>

Background: Cytomegalovirus (CMV) is associated with high morbidity and mortality in transplant patients. Specific antiviral treatment at an early stage of CMV infection may effectively ameliorate, but not eliminate CMV disease in these patients. Presently, the pp65 antigenemia test on peripheral leukocytes is the method most widely used for predicting and monitoring transplant patients for active CMV infection. Nucleic acid amplification methods are less well defined since they lack standardisation. Objective: A seminested fluorometric PCR assay (AmpliSensor-CMV, BAG, Germany) and a one-step PCR with a signal-amplification step (SHARP, Abbott, Germany) specific for the fragments of the CMV UL 122 and UL 123 genes, respectively, were evaluated for

the early diagnosis of CMV infection. Design: A total of 26 recipients of heterogeneous solid organs were monitored prospectively for a median of 99 days after transplantation. By testing 371 clinical samples parallel with the pp65-antigen assay and IgM and IgG EIA assays the sensitivity, specificity, correlation and quantitation potential of both PCRs was evaluated. Results: Eight out of 26 patients developed active CMV infection. A total of 48 samples of these patients exceeded a CMV-DNA load threshold of 15 genome equivalents/10⁵ leukocytes (AmpliSensor-CMV) and 41 samples exceeded the critical cut-off for the SHARP system. The AmpliSensor PCR exceeded its threshold consistently before the clinical onset of CMV disease (median 8 days). There was very good agreement between symptomatic CMV infection in patients and AmpliSensor-PCR, SHARP PCR, and pp65-antigen results (κ -coefficient > 0.900). IgM and IgG EIA showed moderate agreement (κ -coefficient = 0.591 and 0.552, respectively). Conclusion: Both PCRs and pp65 antigen assay correlated significantly better with CMV disease than serodiagnosis. The AmpliSensor PCR allowed more precisely than the SHARP system a quantitative determination of viral load and an early and reliable prediction of active CMV infection. The use of AmpliSensor PCR may improve the diagnosis and management of active CMV infection in organ transplant recipients.

Sagir, A., O. Adams, et al. "SEN virus seroprevalence in HIV positive patients: association with immunosuppression and HIV-replication." *Journal of Clinical Virology* **In Press, Corrected Proof** <http://www.sciencedirect.com/science/article/B6VJV-4F0GC8W-1/2/00f60ac92d166f5015f6642059c6923f>

Background: Patients infected with HIV are often co-infected with other viruses. SEN virus (SENV) was isolated from a HIV positive patient with intravenous drug use and post-transfusion hepatitis. SENV strains D and H seem to be relevant for the development of post-transfusion hepatitis. We compared the prevalence of SENV strains D and H and the viral load of SENV H in HIV-infected patients with healthy blood donors. The results were correlated with clinical markers such as HIV stage, CD4 cell count, HIV-RNA positivity, HAART or the transmission mode in HIV infected individuals. Objectives: Blood samples of 143 HIV-positive patients were analysed and compared with a control group of 122 healthy blood donors. SENV D and -H was detected by PCR. Results: SENV was detectable in 15.4% (22/143) of HIV-positive patients compared to 10.4% (12/122) in the control group ($P = 0.18$). SENV H DNA-levels were significantly higher in HIV-positive patients ($P = 0.01$). The prevalence in patients with CD4 cells less than 200/mm³ was 31% (13/42), compared to 12.3% (8/65) in cases with CD4 cells between 200 and 500/mm³, and 2.8% in cases with CD4 cells above 500/mm³ ($P = 0.002$ for CD4 cells 200, $P = 0.031$ for CD4 cells 500). Prevalence of these strains was not significantly influenced by CDC stages. SENV was detected significantly more frequent in patients with detectable HIV-RNA ($P = 0.005$). Patients undergoing HAART were significantly less frequent positive for SENV D or -H ($P = 0.029$) than patients without HAART. In a multivariate analysis using a logistic regression model HIV-RNA positivity and CD4 cell count were identified as independent factors for SENV prevalence. Conclusion: SENV (D and H) prevalence is not significantly higher in HIV-positive patients in comparison to healthy blood donors. SENV prevalence depends on CD4 cell count and HIV-RNA.

Sarcinella, L., T. Mazzulli, et al. (2002). "Cytomegalovirus glycoprotein B genotype does not correlate with outcomes in liver transplant patients." *Journal of Clinical Virology* **24**(1-2): 99.

<http://www.sciencedirect.com/science/article/B6VJV-44MFSG6-H/2/38fc78f83eba978953332a7df73b77e4>

Background: Based on sequence variation in the UL55 gene that encodes glycoprotein B (gB), human cytomegalovirus (CMV) can be classified into four gB genotypes. Previous studies have

suggested there could be an association between CMV gB genotype and clinical outcome in transplant patients. Objectives: The goal of this study was to determine the distribution of gB genotypes in a cohort of liver transplant recipients with CMV infection and the effect of gB type on clinical outcomes including CMV disease and rejection. Study design: DNA was extracted directly from the blood of 58 liver transplant recipients with CMV infection. The gB genotype of CMV was determined using the polymerase chain reaction to amplify a region of UL55, followed by restriction analysis based on HinfI and RsaI digestion. Results were correlated with CMV viral load, symptomatic disease, and development of acute rejection. Results: The distribution of CMV gB genotypes was: gB1, 15/58 (25.9%); gB2, 16/58 (27.6%); gB3, 21/58 (36.2%); gB4, 2/58 (3.4%) and four patients (6.9%) had mixed infection. No correlation between CMV genotype and peak CMV viral load was observed. Symptomatic CMV disease developed in 25/58 (43.1%) patients and 26/58 (44.8%) had acute rejection. The rate of CMV disease and acute graft rejection in patients infected with the different CMV gB genotypes was not significantly different. However, all four patients with infection with a mixture of CMV gB genotypes developed progression to CMV disease ($P=0.030$). Conclusions: The gB genotype did not correlate with peak CMV viral load and with the development of CMV disease or acute rejection following liver transplantation.

Schwaiger, M. and P. Cassinotti (2003). "Development of a quantitative real-time RT-PCR assay with internal control for the laboratory detection of tick borne encephalitis virus (TBEV) RNA." Journal of Clinical Virology **27**(2): 136.

<http://www.sciencedirect.com/science/article/B6VJV-47GYM1T-7/2/0774bb9e027fc813d0abee7bce4471ef>

Background: Tick borne encephalitis virus (TBEV), is a human flavivirus causing tick borne encephalitis (TBE), a viral infection of the central nervous system endemic in Europe and Asia. Objectives: To develop a reverse transcription polymerase chain reaction (RT-PCR) assay based on quantitative real-time RT-PCR technology (TaqMan) for detection and quantification of TBEV RNA. The test includes an internal control (IC) to avoid false negative results. Study design: The system was established and validated using wild-type (WT) non-infectious synthetic RNA representing a fragment of the 3' non-coding region of the TBEV genome. In addition, synthetic RNA differing from the WT synthetic RNA by a unique probe binding region was used as IC to monitor the overall efficiency of the RT-PCR. Results: The analytical sensitivity of the assay was at least ten copies of the TBEV synthetic transcript in presence of 50 copies of the IC. Successful amplification was obtained for different strains within the TBEV complex (Hypr, Hochosterwitz, Laibach, Elsass=Alsace, ZZ9, Wladiwostok). Among 14 serum and 21 cerebrospinal fluid (CSF) samples obtained from 28 patients with clinical suspicion of TBEV 1 CSF sample tested positive for TBEV RNA. In addition, no TBEV RNA could be detected in blood samples obtained from three vaccinated people 1 and 3 days post-vaccination. Thus indicating that a positive result is unlikely to be caused by recent vaccination. Conclusions: A quantitative, highly sensitive and specific real-time RT-PCR assay has been developed for the detection of TBEV RNA. Inclusion of an IC is important to monitor the possible occurrence of false-negative results caused by the presence of inhibitory factors. This assay should be an important asset for the routine laboratory detection of TBEV RNA.

Sen, A., N. Kobayashi, et al. (2000). "Amplification of various genes of human group B rotavirus from stool specimens by RT-PCR." Journal of Clinical Virology **17**(3): 177.

<http://www.sciencedirect.com/science/article/B6VJV-416C03J-5/2/15a86bfaa1a404ee084efc080f82cddb>

Background: The detection of the human group B rotavirus (HuGBR) CAL strain from India has given us an opportunity to design suitable primers for the detection of HuGBR since CAL is the second HuGBR detected until now, the Chinese Adult Diarrhoea Rotavirus (ADRV) being the first reported human pathogen belonging to this group of viruses. The primers described here may thus be used for the detection of human group B rotaviruses by reverse transcription-PCR (RT-PCR) in a diagnostic laboratory. Objective: To establish a set of primers suitable for the detection of various genes of human group B rotaviruses using a rapid RT-PCR assay. Study design: Until recently, the Chinese ADRV strain was the only HuGBR strain that had been partially sequenced by cloning various viral genes using vector-specific primers. Consequently, there are very few reports in the literature describing primers that may be used for the detection of HuGBR viruses using RT-PCR in a clinical laboratory. The sequences of various genes from the ADRV strain that had been submitted to the nucleotide sequence database GenBank were analyzed in order to design several putative detection primer pairs for an RT-PCR assay. The rationale was to amplify the cognate genes from five isolates of the HuGBR CAL strain (CAL-1 to CAL-5) that have been detected to date from India. Primers that resulted in a specific product of the expected size from the CAL isolates were used to standardize a protocol for amplifying various genes of the CAL isolates under identical reaction conditions. Results: Out of several synthetic oligonucleotides designed, 12 were found to be satisfactory for the amplification of gene segments 4, 5, 6, 7, and 9 from the five CAL isolates and are presented here. A set of previously described primers that have been shown to be specific for human group B rotavirus gene segment 8 were also found to amplify the cognate gene from the CAL isolates. All the reactions were carried out using the same thermal cycling conditions. Conclusions: The extreme virulence potential of HuGBR has been documented in several epidemics in China. Until recently, the Chinese ADRV strain was the only known HuGBR strain. As there have not been any reports of HuGBR infections outside China, there are no consensus nucleotide sequences available for HuGBR that may be used to validate primers for the detection of HuGBR. Here we report a set of 12 primer sequences that were designed from ADRV sequences and also found to amplify various genes from the different CAL isolates and hence may represent consensus primers suitable for the detection of HuGBR.

Smith, I. L., J. A. Northill, et al. (2002). "Detection of Australian bat lyssavirus using a fluorogenic probe." Journal of Clinical Virology **25**(3): 285.

<http://www.sciencedirect.com/science/article/B6VJV-46YXTNN-1/2/50322ec858befaa15e57a4c54e7050ae>

Background: Australian bat lyssavirus (ABLV) has been transmitted to humans following a scratch or bite from an infected bat in two cases. Following a scratch or bite to a person, the bat is usually submitted for testing and diagnosis is made using a direct fluorescent antibody test on a brain smear. A nested RT-PCR assay has also been utilised to confirm diagnosis. If positive for lyssavirus, post-exposure prophylaxis is administered. Objectives: The TaqMan(TM) assay was developed to improve the diagnosis of ABLV infection, following problems encountered with the generation of spurious PCR products in the nested RT-PCR and also to reduce the high risk of contamination inherent with nested PCRs. Study design: RNA was extracted from 161 bat brains and the samples were compared using a conventional RT-PCR and the TaqMan based assay. Samples from a patient with an ABLV infection collected antemortem and postmortem were also tested. Results: The sensitivity of the new TaqMan based PCR assay compared favourably with the nested PCR previously in use in our laboratory. This assay was able to detect RNA in samples collected antemortem and postmortem for the diagnosis of a human case of ABLV. Conclusions: The major advantage of the TaqMan(TM) based assay was the speed of diagnosis with a result within minutes of completing the PCR (a result within 4 h of receiving the specimen). This test greatly reduces the chance of false positives through the elimination of second-round PCR and the requirement for agarose gels. The assay is sensitive and specific and should be invaluable for future antemortem and postmortem diagnosis of ABLV infection in humans.

Song, L. H., V. Q. Binh, et al. (2003). "Serum cytokine profiles associated with clinical presentation in Vietnamese infected with hepatitis B virus." Journal of Clinical Virology **28**(1): 93.

<http://www.sciencedirect.com/science/article/B6VJV-47RBSYB-4/2/6c08d3f7a693d6210e426733385b29d5>

Background/objectives: An ineffective cytokine response is thought to be one of the reasons for the failure to suppress hepatitis B virus (HBV) replication and to eliminate the virus. We investigated the serum levels of interleukin (IL)-6, IL-10, IL-12, and interferon (IFN)-[gamma] in HBV-infected Vietnamese patients to determine whether they were related to the outcome of HBV infection. **Study design:** Samples from a total of 154 HBV-infected patients with well-characterised clinical profiles and 56 healthy controls were assessed. **Results:** Serum IL-6 levels, which were inversely correlated with transaminase levels, were highest in patients with liver cirrhosis (LC) and hepatocellular carcinoma (HCC) and lowest in those with either asymptomatic (ASYM), acute or chronic HBV, and thus, represented the best marker of HBV-related clinical progression. Compared with the healthy control group, serum IL-12 was uniformly elevated in all HBV-infected patients apart from those with ASYM infections, implying no impairment of production of this cytokine in HBV-infected individuals. Serum IL-10 and IFN-[gamma] levels, however, were uniformly low and showed no association with clinical presentation. Cytokine profiles were not influenced by the presence of hepatitis B e antigen (HBeAg). **Conclusions:** Serum IL-6 and IL-12 but not IL-10 and IFN-[gamma] are associated with the clinical presentation in HBV-infected Vietnamese patients.

Stocher, M., V. Leb, et al. (2002). "A simple approach to the generation of heterologous competitive internal controls for real-time PCR assays on the LightCycler." Journal of Clinical Virology **25**(Supplement 3): 47.

<http://www.sciencedirect.com/science/article/B6VJV-47C3NXK-7/2/c7e743df374d09e92db3ff575e657e31>

Background: The real-time PCR technology allows convenient detection and quantification of virus derived DNA. This approach is used in many PCR based assays in clinical laboratories. Detection and quantification of virus derived DNA is usually performed against external controls or external standards. Thus, adequacy within a clinical sample is not monitored for. This can be achieved using internal controls that are co-amplified with the specific target within the same reaction vessel. **Objectives:** We describe a convenient way to prepare heterologous internal controls as competitors for real-time PCR based assays. **Study design:** The internal controls were devised as competitors in real-time PCR, e.g. LightCycler-PCR. The bacterial neomycin phosphotransferase gene (neo) was used as source for heterologous DNA. Within the neo gene a box was chosen containing sequences for four differently spaced forward primers, one reverse primer, and a pair of neo specific hybridization probes. Pairs of primers were constructed to compose of virus-specific primer sequences and neo box specific primer sequences. Using those composite primers in conventional preparative PCR four types of internal controls were amplified from the neo box and subsequently cloned. **Results:** A panel of the four differently sized internal controls was generated and tested by LightCycler PCR using their virus-specific primers. All four different PCR products were detected with the single pair of neo specific FRET-hybridization probes. **Conclusion:** The presented approach to generate competitive internal controls for use in LightCycler PCR assays proved convenient and rapid. The obtained internal controls match most PCR product sizes used in clinical routine molecular assays and will assist to discriminate true from false negative results.

Tonon, S. A., M. A. Picconi, et al. (2001). "Physical status of the E2 human papilloma virus 16 viral gene in cervical preneoplastic and neoplastic lesions." Journal of Clinical Virology **21**(2): 129.

<http://www.sciencedirect.com/science/article/B6VJV-433PC0B-3/2/e0af9fc63749666b9a43ae511db58ca9>

Background: Integration of human papilloma virus (HPV) 16 DNA is considered an important genetic change in cervical lesion progression towards ICC. The viral E2 gene is often disrupted by this process, releasing suppression of viral E6/E7 oncogenes, a key factor for oncogenic progression. Objectives: To evaluate the physical status of HPV 16 E2 gene in cervical preneoplastic and neoplastic lesions and its relation with lesion severity. Study design: A sensitive PCR approach for the detection of an intact E2 HPV 16 gene in infected epithelial cells from the cervix with low grade squamous intraepithelial lesion (LGSIL), high grade squamous intraepithelial lesion (HGSIL) and invasive cervical carcinoma (ICC) diagnosis was applied. The correlation between gene disruption and lesion stage was examined. Results: Sixty-two LGSIL, 39 HGSIL and 24 ICC samples were analyzed. Fifty-seven LGSIL [92%], 13 HGSIL [33%] and 4 ICC [17%] showed results compatible with an intact E2 gene, while 5 LGSIL [8%], 26 HGSIL [67%] and 20 ICC [83%] samples gave no signal. Conclusions: HPV 16 E2 gene disruption showed a positive correlation with cervical lesion progression, particularly from LGSIL to HGSIL. Although additional genetic events are very likely to be needed for HGSIL to ICC progression, the E2 gene disruption is a putative early marker to consider in the prognostic analysis of HPV 16 chronically infected women.

Torfason, E. G., Gueth, et al. (2002). "Polymerase chain reaction for laboratory diagnosis of orf virus infections." Journal of Clinical Virology **24**(1-2): 79.

<http://www.sciencedirect.com/science/article/B6VJV-44MFSG6-D/2/a29a0db4914205b5fa880a097795af9c>

Background: The orf virus of sheep and goats is one of several zoonotic parapoxviruses. In the ovine/caprine host it causes contagious ecthyma (contagious pustular dermatitis, scabby mouth), but in humans it normally causes solitary or clustered orf lesions, typically on hands, arms or face. In addition to disease in the animals, the virus can be quite a nuisance as an occupational hazard in farmers and butchers. Clinical diagnosis is often possible, but laboratory diagnosis is sometimes necessary. For virus isolation, primary ovine or bovine cells, not routinely present, are needed. Serological methods exist, but electron microscopy is the most commonly used method. Objectives: To develop a reliable method for the laboratory diagnosis of orf zoonoses, without virus culture and without access to an electron microscope. Study design: A suitable primer pair was designed for orf polymerase chain reaction (PCR), using the Oligo software and sequence information from GenBank. Orf positive controls and specimens were kindly provided by several public health centers. Molluscum contagiosum specimens were provided by a dermatologist. HSV-1, HSV-2 and VZV positive swab specimens came from our routine diagnostic service. Asymptomatic skin specimens were obtained from sheep heads from the abattoir, and swab specimens from the heads of asymptomatic sheep. Selected amplified orf PCR positive specimens were sequenced to ensure the authenticity of the PCR products. Orf positive specimens were sent to another laboratory for electron microscopy. Results and conclusions: A robust PCR was developed, with very small inter-run variation. All specificity demands were met, and the sensitivity seems to be good or excellent. All negative specificity controls from cell cultures and non-orf viruses were negative. Twenty-two (95.7%) of 23 scab or swab specimens with suspected orf etiology were orf PCR positive. Five of eight skin specimens from sheep heads from the abattoir were positive, and all 11 swab specimens from asymptomatic sheep were negative. Electron microscopy demonstrated orf-like particles in orf-PCR positive specimens. This PCR seems to be suitable as a diagnostic test for orf in humans, but asymptomatic virus shedding in sheep or goats may complicate veterinary applications of the assay.

Vennema, H., E. de Bruin, et al. (2002). "Rational optimization of generic primers used for Norwalk-like virus detection by reverse transcriptase polymerase chain reaction." Journal of Clinical Virology **25**(2): 233.

<http://www.sciencedirect.com/science/article/B6VJV-46W05HR-4/2/572d7fb1e5547f759bc60083122ae04f>

Verstrepen, W. A., P. Bruynseels, et al. (2002). "Evaluation of a rapid real-time RT-PCR assay for detection of enterovirus RNA in cerebrospinal fluid specimens." Journal of Clinical Virology **25**(Supplement 1): 39.

<http://www.sciencedirect.com/science/article/B6VJV-45TY876-1/2/e90b30d9c98812640c2c978f5c901cc3>

Background: We previously described the characteristics of a single-tube real-time enterovirus reverse transcriptase polymerase chain reaction (RT-PCR) assay based on a fluorogenic probe and primers directed to highly conserved sequences in the 5'-untranslated region (UTR) of the enterovirus genome. Objectives: To evaluate the performance of the assay on a larger number of cerebrospinal fluid (CSF) specimens from patients suspected of having viral meningitis. Study design: Real-time enterovirus RT-PCR and viral culture were performed on CSF specimens received from March 2000 to November 2001. Patient records were retrospectively reviewed for final clinical diagnosis. Results: From the 186 CSF specimens tested, culture was positive for enterovirus in 31 cases, whereas real-time RT-PCR detected enterovirus RNA in 45 CSF specimens. The sensitivity of real-time RT-PCR in relation to the clinical diagnosis of viral meningitis was 72.6%, whereas the sensitivity of viral culture reached only 57.4%. Enterovirus RNA was also found in a number of specimens with low leukocyte counts. Conclusions: We confirm that the real-time enterovirus RT-PCR assay for CSF specimens is significantly more sensitive than viral culture.

Ward, C. L., M. H. Dempsey, et al. (2004). "Design and performance testing of quantitative real time PCR assays for influenza A and B viral load measurement." Journal of Clinical Virology **29**(3): 179.

<http://www.sciencedirect.com/science/article/B6VJV-492W0P8-1/2/7575686b4ad97fc4190e40940c372112>

Background: The antiviral effect of anti-influenza drugs such as zanamivir may be demonstrated in patients as an increased rate of decline in viral load over a time course of treatment as compared with placebo. Historically this was measured using plaque assays, or Culture Enhanced Enzyme Linked Immunosorbent Assay (CE-ELISA). Objectives: to develop and characterise real time quantitative PCR (qPCR) assays to measure influenza A and B viral load in clinical samples, that offer improvements over existing methods, in particular virus infectivity assays. Study design: The dynamic range and robustness were established for the real time qPCR assays along with stability of the assay components. Cross validation of the real time PCR assays with CE-ELISA was performed by parallel testing of both serial dilutions of three different subtypes of cultured virus and a panel of influenza positive throat swab specimens. Results: the assays were specific for influenza A and B and the dynamic ranges were at least seven logs. The assay variability was within acceptable limits but increased towards the lower limit of quantification, which was 3.33 log₁₀ viral cDNA copies/ml of virus transport medium (ten viral RNA copies/PCR). The components of the assay were robust enough to withstand extended

storage and several freeze-thaw cycles. For the real time PCR assays the limit of quantification was equivalent to the virus infectivity cut off, which equates to a 93-fold increase in sensitivity. Conclusion: Well characterised real time PCR assays offer significant improvements over the existing methods for measuring the viral load of strains of influenza A and B in clinical specimens.

Yancoski, J., S. Danielian, et al. (2004). "Quantification of Epstein-Barr virus load in Argentinean transplant recipients using real-time PCR." Journal of Clinical Virology **31**(1): 58.

<http://www.sciencedirect.com/science/article/B6VJV-4CDJNN4-1/2/551efab17df8d5383a4ae57bf62f4bd5>

Background: Post-transplant lymphoproliferative disease (PTLD) is a frequent and severe Epstein-Barr virus (EBV)-associated complication in transplant recipients that is caused by suppression of T-cell function. Objective: Evaluation of the diagnostic value of EBV DNA load in non-fractionated whole blood samples (n=297) from 110 pediatric transplant patients by real-time PCR. Results and conclusions: Patients with PTLD had a median viral load of 1.08×10^5 copies/ml blood (n=24), which was significantly higher compared with patients without PTLD (median: 50 copies/ml blood, n=273, PPP=0.0391). These patients who had a high viral load may benefit from a close follow-up of the viral burden.

Zhou, L., T. C. Harder, et al. (1999). "Rapid detection by reverse hybridization of mutations in the UL97 gene of human cytomegalovirus conferring resistance to ganciclovir." Journal of Clinical Virology **13**(1-2): 53.

<http://www.sciencedirect.com/science/article/B6VJV-3WMK61W-7/2/44c76d63117c8bfd8e6bc132c86bde35>

Background of study: Diseases due to human cytomegalovirus (HCMV) infection constitute a major threat in marrow and solid organ transplant recipients. Ganciclovir (GCV) is widely used in prophylaxis and pre-emptive therapy of active HCMV infection. Resistance to ganciclovir (GCV) may arise at variable frequency under GCV therapy and is conferred by mutations (i) in the UL97 gene (codons 460, 520, and 591-607) encoding a phosphotransferase which is essential for monophosphorylation of GCV and, to a lesser extent, (ii) in the UL54 gene coding for the DNA polymerase of HCMV. Objective: The purpose was to develop a rapid assay to screen for emerging GCV resistance mutations in the UL97 gene of HCMV whereby avoiding virus isolation and nucleotide sequencing procedures. Study design: A nested PCR (nPCR) amplifying UL97 codons 450-672 was developed. Nested amplicons were subsequently sequenced directly. Oligonucleotides for use in a reverse hybridization assay were designed to detect relevant non-synonymous mutations at codons UL97 460, 520, 603 and 607. Strain AD169 served as a wild-type control. Results: UL97-specific nPCR amplicons were obtained from 18 EDTA blood samples of ten transplant recipients receiving GCV for more than 30 days. In three consecutive samples from a single patient a GCV resistance mutation at codon 603 (C->W) was detected. In addition, two out of four cell culture-adapted HCMV isolates known to exhibit GCV resistance in vitro revealed mutations at codons 460 (M->V) and 607 (C->Y), respectively. By reverse hybridization a discrimination of single nucleotide changes at codons 460, 520, 603 and 607 was possible whereby matching exactly the results of the nucleotide sequence analysis for all 23 amplicons examined. Conclusions: Reverse hybridization appeared to be a rapid and convenient alternative to nucleotide sequencing when screening the UL97 gene of HCMV for selected markers of GCV resistance.

Narama, I., Y. Kobayashi, et al. (2005). "Pigmented Cutaneous Papillomatosis (Pigmented Epidermal Nevus) in Three Pug Dogs; Histopathology, Electron Microscopy and Analysis of Viral DNA by the Polymerase Chain Reaction." Journal of Comparative Pathology **132**(2-3): 132.

<http://www.sciencedirect.com/science/article/B6WHW-4F6CRBN-3/2/1f5a8a0efb70018e602710da697325c7>

Summary Canine pigmented epidermal nevus (CPEN) is a skin disorder of some breeds of dog characterized by multiple black plaques of the haired and non-haired skin. Three cases of pigmented cutaneous papillomatosis (previously described also as CPEN) in pug dogs were investigated histopathologically, immunohistochemically and electron microscopically. Additionally, DNA analyses with the polymerase chain reaction (PCR) were performed in two cases. Many nuclei of the stratum granulosum were diffusely immunolabelled for specific structural antigens of bovine papillomavirus (subgroup A), but nuclear inclusion bodies were not detected by retrospective examination of haematoxylin and eosin-stained sections of the affected skin. Aggregates of small numbers of viral particles (ranging from 37 to 43 nm in diameter) with a hexagonal structure were sparsely scattered throughout the nuclei of some of the superficial keratinocytes. PCR amplification targeted for the L1 gene of papillomavirus cloned from a case of CPEN yielded an expected fragment of 194-bp in the two CPEN cases examined but not in a case of canine oral papilloma.

Schmidbauer, S. M., M. Venner, et al. (2004). "Compensated Overexpression of Procollagens [alpha]1(I) and [alpha]1(III) Following Perilla Mint Ketone-induced Acute Pulmonary Damage in Horses." Journal of Comparative Pathology **131**(2-3): 186.

<http://www.sciencedirect.com/science/article/B6WHW-4CPM121-1/2/b38125619b296ea4fc709c8aab578a7b>

Interstitial lung disease with chronic fibrosis is a frequent cause of reduced performance in horses. The aim of this study was to establish a model of acute alveolar damage and interstitial lung disease in horses that could be used to monitor the histopathological lesions and changes in expression levels of genes relevant to pulmonary fibrosis. Six adult horses were given a single intravenous injection (6 mg per kg body weight) of perilla mint ketone (PMK). Transthoracic lung biopsy samples (1 x 0.2 x 0.2 cm) were collected before and after (days 1, 4, 8, 11, 15, 18, 22, 25 and 29) the administration of PMK. Light and electron microscopy revealed severe acute alveolar damage (days 1 to 4), proliferation of type II pneumocytes (days 4 to 11) and finally complete healing at about day 18. However, unexpectedly severe clinical signs necessitated euthanasia in two horses on days 9 and 11. The expression levels of the collagen genes COL1A1 and COL3A1 as well as transforming growth factor (TGF)-[beta] were examined in the biopsy samples by reverse transcription-real time quantitative polymerase chain reaction. COL1A1 and COL3A1 gene expressions were upregulated (3- and 17-fold, respectively) between days 1 and 29 in all six horses, whereas TGF-[beta] was upregulated in two horses (2- and 4-fold, respectively), between days 4 and 18. Although the gene expression analyses indicated a strong activation of the pro-fibrotic pathway, no interstitial fibrosis was seen in any horse. A complete necropsy performed on day 60 revealed complete recovery of the lungs of the four surviving horses, with no evidence of fibrosis. Unidentified compensatory mechanisms may have prevented pulmonary fibrosis, despite strong upregulation of pro-fibrotic genes.

Journal of Cystic Fibrosis (1)

Ramalho, A. S., S. Beck, et al. (2004). "Methods for RNA extraction, cDNA preparation and analysis of CFTR transcripts." Journal of Cystic Fibrosis **3**(Supplement 2): 11.

<http://www.sciencedirect.com/science/article/B6X2D-4CSG1X7-1/2/f47450de20d468e83c5bf97211802381>

The scope of this article is to outline some of the basic methods for good quality RNA preparation from mammalian tissues and cells (including epithelial cells). Additionally, we give an outline of common techniques of measuring CFTR gene expression such as quantitative and semi-quantitative reverse transcription (RT) PCR and ribonuclease protection assay (RPA). These methods are designed to detect low abundance transcripts, which apply to CFTR mRNA in most cell types and tissues.

Journal of Dermatological Science (13)

Abiko, Y., M. Nishimura, et al. (2003). "Upregulated expression of human [beta] defensin-1 and -3 mRNA during differentiation of keratinocyte immortalized cell lines, HaCaT and PHK16-0b." Journal of Dermatological Science **31**(3): 225.

<http://www.sciencedirect.com/science/article/B6T87-4817HWB-1/2/8b2c2750dfb01bede7d627b022d47187>

Background: Human [beta]-defensins (hBDs) belong to a group of antimicrobial peptide that are expressed in the epithelial cells. Objective: The present study investigated mRNA expression levels of the [beta]-defensins, hBD-1, -2 and -3, in human keratinocytes during differentiation in vitro. Methods: Immortalized keratinocyte cell lines, HaCaT and PHK16-0b, were used in this study; in order to stimulate differentiation, the Ca²⁺ concentration in the growth media was increased from 0.3 to 1.8 mM. Results: Four days after the increase, the expression levels of hBD-1 and -3 were increased in both cell lines, followed by an increase in the mRNA levels of the differentiation markers, involucrin and keratin 10. No increased expression of hBD-2 was observed. Conclusion: The results indicate that keratinocyte differentiation may stimulate hBD-1 and -3 expression in stratified squamous epithelia.

Asahina, A., S. Kuwata, et al. (1996). "Study of aspartate at residue 9 of HLA-C molecules in Japanese patients with psoriasis vulgaris." Journal of Dermatological Science **13**(2): 125.

<http://www.sciencedirect.com/science/article/B6T87-3RHM5V1-11/2/27f5e711dab28ab9cb8919cf6e5f5fe0>

The frequency of aspartate at residue 9 (Asp-9) of HLA-C molecules was investigated among 75 Japanese patients with psoriasis vulgaris and 50 healthy controls. We developed a technique of polymerase chain reaction sequence-specific primer (PCR-SSP) amplification of genomic DNA for HLA-C alleles with a codon for Asp-9. The specificity of amplification was confirmed by direct sequencing of the amplified products and amplification from total RNA (RT-PCR). Asp-9 was positive in all individuals with Cw6 and/or Cw7, but negative in the others, indicating that Asp-9 was specific to Cw6 and Cw7 antigens in our subjects. The frequency of Asp-9 was significantly increased in the patient group (48% vs. 20%; $P < 0.0001$). Asp-9 is located on a [beta] sheet of [alpha] 1 domain of HLA-C molecule and influences the peptide binding of the C pocket of the groove together with Ala-73. Both Asp-9 and Ala-73 could contribute to the disease susceptibility to psoriasis vulgaris in the immune responses.

Goto, M., K. C. Sato-Matsumura, et al. (2004). "Tyrosinase gene analysis in Japanese patients with oculocutaneous albinism." Journal of Dermatological Science **35**(3): 215.

<http://www.sciencedirect.com/science/article/B6T87-4DBCK7Y-1/2/f8d147dbc3c750de14c79a7a9b773f30>

SummaryBackground:Oculocutaneous albinism (OCA) is a heterogeneous congenital disorder. Tyrosinase is a key enzyme in melanin biosynthesis, and tyrosinase gene mutations cause the OCA1 subtype.Objective:This study was intended evaluate the frequency and details of tyrosinase gene mutations in Japanese OCA patients.Patients and methods:We examined nine non-consanguineous OCA families, sequenced the tyrosinase gene of the patients and also confirmed a splicing site mutation using exon trapping system.Results:Tyrosinase gene mutations were identified in five out of nine OCA families (55%). IVS2-10delTT-7t-a was present in 3 out of 18 alleles in three families (16%), P310insC was present in three alleles in three families (16%) and R278X was found in three alleles (16%), including those in one heterozygous and one compound homozygous patient. G97V (290 G-T) was found in 1 out of 18 alleles, and we could not find G97V in the mutation database. We have added this mutation as 9th mutation of Japanese OCA1 patients. In 8 of 18 alleles, four families, no tyrosinase mutations were identified. They were presumed not to be OCA1, but other subtypes of OCA. Exon trapping system demonstrated IVS2-10delTT-7t-a mutation generated the abnormal splicing site, and inserted the codon 4 bases in mRNA level resulting in premature termination codon downstream.Conclusion:This study provided new information about OCA1 mutations, and highlights the requirement of broader detailed search to make precise diagnosis of OCA.

Ha, S.-J., J.-S. Kim, et al. (2003). "Analysis of genetic polymorphisms of steroid 5[alpha]-reductase type 1 and 2 genes in Korean men with androgenetic alopecia." Journal of Dermatological Science **31**(2): 135.

<http://www.sciencedirect.com/science/article/B6T87-47T8NWX-1/2/e0b16a253659aec2a16f240e719065a7>

Background: Genetic polymorphisms of steroid 5[alpha]-reductase have been studied in androgenetic alopecia in Caucasians, but the genes encoding the two isoenzymes were not associated with male pattern baldness. Genetic polymorphisms and ethnic variations have not been studied for Asians, although it is suggested that racial difference could exist and influence clinical phenotypes. **Objective:** The purpose of our study is to investigate the genetic polymorphisms of steroid 5[alpha]-reductase type 1 and 2 (SRD5A1 and SRD5A2) genes in Korean population, and to study the association of these polymorphisms with the development, clinical types (female or male pattern) and therapeutic response of androgenetic alopecia. **Methods:** Sixty-six patients with androgenetic alopecia and controls consisted of 92 healthy men

were included. Twenty-four patients were treated with finasteride for at least 6 months, and clinical responses were assessed by a simple classification. For type 1 isoenzyme, HinfI and NspI restriction fragment length polymorphisms (RFLPs) were detected using polymerase chain reaction method. For type 2 isoenzyme, RsaI RFLPs detected valine/leucine polymorphisms at codon 89, and MspI RFLPs detected alanine/threonine polymorphisms at codon 49. Results: We could not find any significant associations of the genetic polymorphisms of these two isoenzyme genes with androgenetic alopecia in Koreans ($P>0.05$). These polymorphisms were not associated with the clinical types of baldness or the response to finasteride ($P>0.05$). Conclusion: These results suggest that polymorphisms of SRD5A1 and SRD5A2 genes may not be directly associated with the development of baldness or generation of different clinical phenotypes.

Ichihashi, N. and Y. Kitajima (2000). "Loss of heterozygosity of adenomatous polyposis coli gene in cutaneous tumors as determined by using polymerase chain reaction and paraffin section preparations." Journal of Dermatological Science **22**(2): 102.

<http://www.sciencedirect.com/science/article/B6T87-3Y2N7S8-5/2/9e4efd49a842542dea6b20de55ca4249>

It has been suggested that an alteration in the adenomatous polyposis coli (APC) gene, which is a tumor suppressor gene, is one of the earlier events in carcinogenesis of some adenocarcinomas. We undertook this study to determine the prevalence of loss of heterozygosity (LOH) of the APC gene in several kinds of cutaneous tumors. Fifty-seven unrelated Japanese patients were examined for analysis of the APC gene. The 57 cases consisted of extramammary Paget's disease, squamous cell carcinoma (SCC), eccrine poroma and porocarcinoma, metastatic tumor of rectal adenocarcinoma and malignant melanoma. DNA was extracted from the tumor and normal portions dissected from the formalin-fixed paraffin-embedding sections and amplified with the use of the PCR. The amplified DNA was examined for LOH in the APC gene. Seven samples of 32 heterozygous persons of APC gene (three out of seven eccrine poromas, two eccrine porocarcinomas and two metastatic tumors of rectal adenocarcinoma) showed for LOH in the APC gene. None of the heterozygous samples from the extramammary Paget's disease (11), SCC (five) and melanoma (five) showed LOH. These results suggest that tumor or tumor suppressor genes, other than the APC gene, may be responsible for extramammary Paget's disease and SCC and that LOH involving APC may have some relevance to the formation and progression of eccrine tumors as in rectal tumors.

Kanekura, T., T. Kanzaki, et al. (1995). "p53 gene mutations in skin cancers with underlying disorders." Journal of Dermatological Science **9**(3): 209.

<http://www.sciencedirect.com/science/article/B6T87-4037RDS-8/2/70bfae5b1537ad734767b981a2619626>

Mutations in p53, a tumor suppressor gene, are one of the most common genetic lesions of human cancers. The relationship between p53 gene mutation and ultraviolet (UV) light has been demonstrated in skin cancers of sun-exposed sites. In this study, genomic DNA from 12 skin cancers was screened for mutations in exons 5 to 9 of this gene using the polymerase chain reaction -- single strand conformation polymorphism (PCR-SSCP) analysis followed by DNA sequencing. DNA samples were obtained from 8 basal cell carcinomas (BCCs): 1 from an organoid nevus, 1 from a patient with basal cell nevus syndrome, 1 from a patient with xeroderma pigmentosum, and 1 from a recurrent and 4 from primary sporadic lesions on actinic damaged skin, and from 4 squamous cell carcinomas (SCCs): 1 from a burn scar, 1 from a patient with epidermodysplasia verruciformis, and 2 from actinic keratosis. Mutation of the p53 gene was detected in only 1 case of SCC which had arisen from actinic keratosis. The mutation occurred at

codon 159 in exon S with a GCC to CCC base-pair substitution resulting in an amino acid change of alanine to proline. This mutation does not correspond to results of UV mutagenesis studies reported in the literature. Our findings imply that, although p53 gene mutation and UV exposure play an important role in the carcinogenesis of some skin cancers, they are not crucial, especially in skin cancers that develop from underlying skin disorders.

Kapur, S., M. A. O. H. Menke, et al. (2001). "Early mycosis fungoides: molecular analysis for its diagnosis and the absence of p53 gene mutations in cases with progression." Journal of Dermatological Science **26**(1): 36.

<http://www.sciencedirect.com/science/article/B6T87-42VM82Y-5/2/137767c4955ccc5f4dea19a2f4bc4c96>

The histological diagnosis of initial mycosis fungoides (MF) and the molecular mechanisms that are responsible for its progression and transformation to the more highly malignant variants of MF remain largely unknown. Because of the rare occurrence of these tumours, the need for snap frozen skin biopsy specimens and the difficulty to obtain suitable material for karyotypic and genotypic analysis, specific cytogenetic and molecular lesions have not yet been identified. In particular the role of known oncogenes and tumour suppressor genes, including the p53 gene, in the pathogenesis and clinical progression of MF has not been extensively investigated. The present study was carried out using the polymerase chain reaction (PCR) technique combined with temperature gradient gel electrophoresis (TGGE) to detect mutations of the p53 gene in 58 patients with MF. TGGE analysis was also used in combination with clonality analysis by means of T-cell receptor [gamma] (TCRG) gene rearrangement studies to distinguish parapsoriasis en plaque and initial MF from patch/plaque stage MF. More than 83% of the diagnoses of initial MF could be confirmed using PCR-TGGE analysis. However, although the sensitive TGGE analysis was used for all exons, p53 gene polymorphisms were found in 4 and p53 gene mutation in only 1 of 58 biopsy specimens. It appears unlikely that p53 gene mutations play a role in either the pathogenesis of parapsoriasis and initial MF or their progression to advanced stages of MF. However, TCRG gene rearrangement studies by means of TCR-TGGE analysis may be useful for distinguishing histologically discordant cases of initial MF.

Kubota, T., M. Dakeishi, et al. (2000). "Probable involvement of a germ-line mutation of an unknown mismatch repair gene in a Japanese Muir-Torre syndrome phenotype." Journal of Dermatological Science **23**(2): 117.

<http://www.sciencedirect.com/science/article/B6T87-408KCH0-6/2/2944f2cdedd732fdd8c630cd8b8006a9>

A combination of haplotype analysis and direct sequencing were conducted on Japanese Muir-Torre syndrome kindred. In the kindred, two females revealed a hereditary non-polyposis colon cancer (HNPCC) phenotype and one male had a sebaceous tumor in addition to a HNPCC phenotype. Haplotype analysis and direct sequencing failed to show involvement of the known mismatch repair genes, with the exception of MSH5, in this kindred. Analysis of large fragments (from 3.9 to 6.2 kb) covering the entire 25 kb MSH5 gene in the proband revealed the absence of gross changes in the promoter region and exons. The direct sequencing of the promoter region and all 25 exons failed to demonstrate any mutations in the coding regions except for a CA repeat polymorphism in intron 3 and a C/A polymorphism in intron 15. Taken together present results indicate that a novel and yet unknown mismatch repair gene is likely involved in the HNPCC in this kindred.

Matsuba, S., Y. Suga, et al. (2002). "Sulfhydryl oxidase (SOx) from mouse epidermis: molecular cloning, nucleotide sequence, and expression of recombinant protein in the cultured cells." Journal of Dermatological Science **30**(1): 50.

<http://www.sciencedirect.com/science/article/B6T87-46TG2X9-3/2/53e544f708b5cd01166e43e3502f4367>

Skin sulfhydryl oxidase (SOx) is an enzyme that catalyzes disulfide (S-S) cross-linking through the oxidation of sulfhydryl compounds in the skin. In this study, using the enzyme purified from rat seminal vesicle, we obtained peptide sequences for SOx by mass spectrometry. We then searched for SOx nucleotides corresponding highly to the rat peptide sequences by assembling murine-expressed sequence tags (ESTs) from the GeneBank database. The assembled mouse SOx cDNA has an open reading frame of 1704-bp nucleotides, translating into a size of 568 amino acids. The calculated molecular mass of the mouse SOx protein is 65 kDa. This mouse sequence can be amplified from total RNAs of various mouse tissue samples by reverse transcription polymerase chain reaction, especially highly amplified from those of the seminal vesicles and epidermis. The cDNA fragment was subsequently cloned into the mammalian expression vector (pTARGET-MSSOx), allowing us to express mouse recombinant SOx protein in cultured cells. When pTARGET-MSSOx was transfected, Western blot analysis using anti-SOx antiserum could detect a 65 kDa-band of recombinant SOx in both samples from the whole cell extract and the medium after the harvest of the HEK cells. In immunohistochemical analysis, the Pt-K2 cells, following the introduction of pTARGET-MSSOx, seemed to generate a SOx protein reactive to anti-SOx antiserum in the cells. Moreover, the indirect staining of the S-S bonds using N-(7-dimethylamino-4-methyl coumarinyl) maleimide (DACM), following the addition of N-ethylmaleimide and dithiothreitol, showed that the formation of S-S bridges almost matched the localization of SOx expression in the Pt-K2 cells after the transfection. In essence, we cloned skin SOx cDNA and characterized it as one of the S-S cross-linking enzymes. The SOx clone from mouse epidermis seems to be useful for investigating the potential function of the enzyme in the epidermis, especially for understanding the physiological role of SOx in the differentiation of keratinocytes.

Shimizu, M., A. Adachi, et al. (2004). "Detection of various types of human papillomavirus DNA, mainly belonging to the cutaneous-group, more frequently in normal tissue than in squamous cell carcinomas of the lip." Journal of Dermatological Science **36**(1): 33.

<http://www.sciencedirect.com/science/article/B6T87-4DBKGX9-3/2/f88341acbe6ede5eb41785ffc994682e>

SummaryBackground:Mucosal high-risk human papillomaviruses (HPVs), such as type 16, are detectable in oral cancers, especially of the oropharynx and tonsils, and there is evidence that they play a pathogenetic role in some cases. However, information is limited about their significance for cancers of the vermilion of the lip.**Objective:**To determine the detection rate, types and localization of HPVs in squamous cell carcinomas (SCCs) of the lip.**Methods:**Nested PCR for cutaneous HPVs, including epidermodysplasia verruciformis-related HPV (EV-HPV), and single PCR for mucosal HPVs, were conducted for a total of 27 SCCs and normal samples from 30 individuals. Tyramide-based in situ hybridization (ISH) was also applied.**Results:**Various types of HPVs were detected, particularly from normal individuals. Among the kinds of the HPV types detected in this study, half were found by PCR using a primer pair, which we newly designed. The prevalence of HPV was 5 out of 27 SCCs (ca. 18%) and 10 out of 30 normal individuals (ca. 33%). They were the entire cutaneous-group except for two, from one SCC and one normal individual.**Conclusion:**On the surface of the normal lip various types of mainly cutaneous-group HPVs may be present, but there does not appear to be any obvious association with SCCs

developing in this site.

Suga, T., M. Setoyama, et al. (1998). "Morphological, biochemical and molecular biological characteristics of a granulocyte colony-stimulating factor-producing human eccrine carcinoma cell line." Journal of Dermatological Science **16**(3): 200.

<http://www.sciencedirect.com/science/article/B6T87-3SBW2FT-4/2/407b7f9c7607a10c7b9fd2a084d4dfc8>

We describe here a newly established cell line from an eccrine carcinoma which produced an abundant amount of granulocyte colony-stimulating factor (G-CSF). An eccrine carcinoma of the scalp of a 69 year-old-Japanese female had metastasized to the pleura. Clinically, she had marked neutrophilia (up to 60000/mm³), and a high level of G-CSF (38.7 x 10³ pg/ml) was detected in the pleural effusion, as determined by enzyme-linked immunosorbent assay (ELISA). We established a cell line in vitro and maintained the cells in culture for 30 months in 90 subcultures. We investigated whether these tumor cells were able to produce G-CSF in culture and found that they were. We also found that the amount of G-CSF produced paralleled the rise in cell number (26.5 x 10³ pg/ml at confluency). When culture media were administered to rabbits (25 ml/rabbit), the amount of circulating neutrophils increased until the number was equal to or greater than that resulting from injection of recombinant human G-CSF (rhG-CSF)(75 [mu]g). This effect persisted for 7 days. When tumors were induced in SCID and nude mice by injecting cultured cells (1 x 10⁷ cells/mouse), the number of circulating neutrophils also correlated well with tumor size in these mice (200000/ mm³, 3 cm tumor). After tumor removal, the neutrophil number returned to normal within 30 days. G-CSF mRNA in cultured cells was detected by RT-PCR. Based on these results, it was confirmed that the marked neutrophilia observed in the patient was caused by the tumor-generated G-CSF. This is the first G-CSF-producing cell line developed from a cancer of the skin.

Uchida, T., M. Inaoki, et al. (2005). "Identification of a recurrent mutation in keratin 17 in a Japanese family with pachyonychia congenita type 2." Journal of Dermatological Science **38**(1): 60.

<http://www.sciencedirect.com/science/article/B6T87-4FDJRVR-1/2/35af1d6b8250e4fa772494780a81d427>

Yang, J.-M., K. Nam, et al. (1999). "Arginine in the beginning of the 1A rod domain of the keratin 10 gene is the hot spot for the mutation in epidermolytic hyperkeratosis." Journal of Dermatological Science **19**(2): 126.

<http://www.sciencedirect.com/science/article/B6T87-3VTHPY3-7/2/a8dabdb6f57b85657a7f6d6388bccbc2>

Keratin intermediate filaments are expressed in specific type I/type II pairs in the stage of differentiation of keratinocytes. The mutations in the keratin genes expressed in the epidermis are etiologically responsible for several epidermal genetic skin diseases, such as epidermolysis bullosa simplex, epidermolytic hyperkeratosis (EHK), ichthyosis bullosa of Siemens, palmoplantar keratoderma, pachyonychia congenita and white sponge nevus. The mutations of keratins 1/10 which are expressed in spinous and granular layers are confirmed to cause EHK. There are several trials to correlate between the clinical phenotypes and sites of mutations of the keratin genes. One of these is that EHK is divided into two groups: the palms and soles involvement (PS)

group and the non-palms and soles (NPS) group. So far the PS group had the mutations in the keratin 1 and the NPS group in keratin 10. Most of the mutations of the NPS group were reported in the beginning of the 1A rod domain and over 2/3 of the mutations in the 1A rod domain were the base pair substitution of arginine. Here we find two different mutations in two unrelated Korean kindreds classified as NPS group--R156C and R156H--in the 1A rod domain of keratin 10. Our results are compatible with the above classification and suggest that the arginine in the beginning of the 1A rod domain is the hot spot for the mutation of the keratin 10 gene.

Journal of Diabetes and its Complications (3)

Lindholm, E., M. Klannemark, et al. (2004). "Putative role of polymorphisms in UCP1-3 genes for diabetic nephropathy." Journal of Diabetes and its Complications **18**(2): 103.

<http://www.sciencedirect.com/science/article/B6T88-4CB7N0F-4/2/7b93e8fdd3904b190108cd0e67ce536e>

Increased production of reactive oxygen species (ROS) has been suggested as a cause of diabetic complications. Uncoupling proteins (UCPs) have been ascribed a role in reducing the formation of ROS, and genetic variation in genes encoding for UCPs could thus be putative candidate genes for diabetic nephropathy. To test this hypothesis we searched for association between the A->G (-3862) variant in UCP1, the insertion/deletion (I/D) polymorphism in exon 8 in UCP2, and the C->T (-55) polymorphism in UCP3 and diabetic nephropathy in 218 diabetic patients with normal urinary albumin excretion rate (AER), 216 with micro- or macroalbuminuria, and in 106 control subjects without a family history of diabetes. We did not find any association between the different polymorphisms and diabetic nephropathy, nor did we observe any difference in AER among carriers of different UCP1-3 genotypes. We could, however, confirm the reported association between BMI and the UCP3 -55 C->T polymorphism; patients carrying the T allele had higher BMI than patients homozygous for the C allele (26.4+/-4.2 vs. 25.3+/-4.3 kg/m²; P=.01). We conclude that studied polymorphisms in the UCP1-3 genes do not play a major role in the development of micro- or macroalbuminuria in Scandinavian diabetic patients.

Miura, J., Y. Uchigata, et al. (2004). "AGE down-regulation of monocyte RAGE expression and its association with diabetic complications in type 1 diabetes." Journal of Diabetes and its Complications **18**(1): 53.

<http://www.sciencedirect.com/science/article/B6T88-4C6JJDH-9/2/9a3e7cf4533e1f6fda8a68f31d79c425>

Advanced glycation end product (AGE) engagement of a cell surface receptor for AGE (RAGE) has been implicated in the development of diabetic complications. In this study, we determined the RAGE mRNA levels in monocytes from type 1 diabetic patients and analyzed their relationship with diabetic vascular complications. Quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis revealed that the monocyte expression of RAGE mRNA was significantly lower in patients with retinopathy than in those without retinopathy and was also significantly down-regulated in patients with nephropathy in comparison with those without nephropathy. Experiments with monocyte-enriched cultures revealed that RAGE mRNA and protein levels were down-regulated by the exposure to glyceraldehyde-derived AGE--the recently

identified high-affinity RAGE ligand. Accordingly, we then assayed for the serum levels of glyceraldehyde-derived AGE as well as those of carboxymethyllysine (CML)--the known RAGE ligand and related them to the monocyte levels of RAGE mRNA. This screen revealed a negative correlation between the two parameters. The results thus suggest that the decrease in monocyte RAGE expression can be at least partly accounted for by the ligand engagement and may be a factor contributing to the development of diabetic vascular complications.

Murata, M., N. Ohta, et al. (2002). "Selective pericyte degeneration in the retinal capillaries of galactose-fed dogs results from apoptosis linked to aldose reductase-catalyzed galactitol accumulation." Journal of Diabetes and its Complications **16**(5): 363.

<http://www.sciencedirect.com/science/article/B6T88-46K20KJ-B/2/6d117bb4eb487153fee7b8c99f5bbf40>

Galactose-fed dogs develop retinal capillary changes similar to diabetic retinopathy with pericyte degeneration as the initial lesion. This is followed by the formation of microaneurysms, hemorrhages, and some areas of acellularity. To investigate the mechanisms for selective pericyte degeneration, retinal capillary pericytes and endothelial cells isolated from beagle dog retina were cultured for 2 weeks in Dulbecco's modified Eagle's medium (DMEM) containing 50 mM -galactose. Apoptosis was detected in pericytes but not endothelial cells by in situ terminal deoxynucleotidyl transferase (TdT)-mediated biotin-dUTP nick end labelling (TUNEL) staining and the DNA fragmentation assay on agarose gel electrophoresis. This apoptosis was prevented by the addition of the aldose reductase inhibitor AL 1576 to the culture medium containing galactose. Apoptosis was not observed when pericytes were similarly cultured in control DMEM medium. These data support the premise that the selective degeneration of retinal capillary pericytes observed in galactose-fed dogs is linked to increased aldose reductase activity in these cells.

Journal of Gastrointestinal Surgery (8)

Brabender, J., R. V. Lord, et al. (2001). "Upregulation of ornithine decarboxylase mRNA expression in barrett's esophagus and barrett's-associated adenocarcinoma." Journal of Gastrointestinal Surgery **5**(2): 174.

<http://www.sciencedirect.com/science/article/B6W8T-44423W2-8C/2/21da48e0129a76674995a5ed0c207631>

The Barrett's multistage process is characterized histopathologically by progression from Barrett's intestinal metaplasia to Barrett's esophagus with dysplasia and ultimately adenocarcinoma. Understanding the cellular and molecular events in this multistage process may contribute to improved diagnosis and treatment. Ornithine decarboxylase (ODC) is the first enzyme in the biosynthesis of polyamines. Elevated ODC activity has been found to be associated with progression during Barrett's esophagus, but the regulation of ODC gene expression in the development of Barrett's-associated adenocarcinoma has not been reported. The aim of this study was to assess the prevalence and timing of ODC mRNA expression in the Barrett's metaplasia-dysplasia-adenocarcinoma sequence. ODC mRNA expression levels, relative to the stably expressed internal reference gene [beta]-actin, were measured using a quantitative

reverse transcription-polymerase chain reaction (RT-PCR) method (ABI 7700 Sequence Detector System) in 104 specimens from 19 patients with Barrett's esophagus without carcinoma and 22 patients with Barrett's-associated adenocarcinoma. The median ODC mRNA expression levels were significantly increased in Barrett's esophagus tissues compared to matched normal tissues in patients without adenocarcinoma of the esophagus ($P = 0.002$; Wilcoxon test). A significant progressive increase in ODC mRNA expression was detectable through the stages of the metaplasia-dysplasia-carcinoma sequence in patients with Barrett's-associated adenocarcinoma ($r = 0.719$; $P \leq 0.001$; Spearman's rho test). These findings show that upregulation of ODC mRNA expression is an early event in the development and progression of Barrett's-associated adenocarcinoma of the esophagus, and they suggest that high ODC mRNA expression levels may be a clinically useful biomarker for the detection of occult adenocarcinoma.

Brabender, J., R. V. Lord, et al. (2004). "Role of retinoid X receptor mRNA expression in Barrett's esophagus." Journal of Gastrointestinal Surgery 8(4): 413.

<http://www.sciencedirect.com/science/article/B6W8T-4C88SFD-G/2/81dc8b2ff35a56b7903f0e5755be1621>

The Barrett's multistage process is characterized histopathologically by progression from Barrett's intestinal metaplasia to Barrett's esophagus with dysplasia and ultimately adenocarcinoma. Understanding of the molecular alterations in this multistage process may contribute to improved diagnosis and treatment. Retinoid X receptors (RXR) play an important role in regulating the morphogenesis, development, growth, and differentiation of cells. Alterations in RXR expression have been observed in a variety of solid tumors; however, the role in Barrett's esophagus disease has yet to be determined. The aim of this study was to assess the prevalence and timing of RXR messenger RNA expression in the Barrett's metaplasia-dysplasia-adenocarcinoma sequence and to investigate its role in the development and progression of this disease. We analyzed the mRNA expression of all three RXR subtypes (RXR- α , RXR- β , and RXR- γ) by using a quantitative real-time reverse transcription-polymerase chain reaction method in 108 specimens from 19 patients with Barrett's esophagus without carcinoma (BE group), 20 patients with Barrett's-associated adenocarcinoma (EA group), and a control group of 10 patients without evidence of gastroesophageal reflux disease (CG). RXR- α mRNA expression was significantly decreased ($P = 0.01$; Wilcoxon test) and adenocarcinoma tissues ($P = 0.018$, Mann-Whitney test). RXR- α and RXR- β mRNA expression was significantly associated in normal squamous esophagus tissues ($r^2 = 0.49$; $P = 0.63$; $r^2 = 0.68$; $P = 0.001$). There were significant differences in RXR- α ($P = 0.011$) and RXR- β ($P = 0.005$) mRNA expression in histopathologically normal squamous esophagus tissues in patients with cancer and the control group without evidence of gastroesophageal reflux disease. These findings suggest that alterations in the mRNA expression of all three RXR subtypes are frequent events in the development and progression of Barrett's esophagus and associated adenocarcinoma, that RXR mRNA expression levels may be useful biomarkers for this disease, and that a widespread "field-effect" is present in the normal esophagus of patients with esophageal adenocarcinoma.

Brabender, J., R. V. Lord, et al. (2002). "Glutathione S-Transferase-Pi Expression Is Downregulated in Patients With Barrett's Esophagus and Esophageal Adenocarcinoma." Journal of Gastrointestinal Surgery 6(3): 359.

<http://www.sciencedirect.com/science/article/B6W8T-45TY7F9-M/2/e92c1f7f89afa373e80860a4bf872a28>

The glutathione S-transferases (GSTs) are a family of enzymes that play an important role in the prevention of cancer by detoxifying numerous potentially carcinogenic compounds. GSTs

conjugate reduced glutathione to a variety of electrophilic and hydrophobic compounds, converting them into more soluble, more easily excretable compounds. Decreased glutathione S-transferase-pi (GSTPI) enzyme activity has been reported in Barrett's esophagus, and an inverse correlation was demonstrated between GST enzyme activity and tumor incidence in the gastrointestinal tract, but the role of GSTPI messengerRNA (mRNA) expression in Barrett's esophagus and associated adenocarcinomas is uncertain. The purpose of this study was to investigate the role of GSTPI mRNA and protein expression in the development and progression of the Barrett's metaplasia-dysplasia-adenocarcinoma sequence, and to investigate the potential of GSTPI quantitation as a biomarker in the clinical management of this disease. GSTPI mRNA expression levels, in relation to the housekeeping gene [beta]-actin, were analyzed using a quantitative real-time reverse transcription-polymerase chain reaction method (TaqMan) in 111 specimens from 19 patients with Barrett's esophagus without carcinoma (BE group), 21 patients with Barrett's-associated adenocarcinoma (EA group), and a control group of 10 patients without evidence of Barrett's esophagus or chronic gastroesophageal reflux disease. GSTPI mRNA expression was detectable in all 111 samples investigated. Analyzed according to histopathologic group, the median GSTPI mRNA expression was highest in normal squamous esophagus epithelium, intermediate in Barrett's esophagus, and lowest in adenocarcinoma tissues ($P = 0.001$) or the EA group ($P = 0.023$). GSTPI expression levels in adenocarcinoma tissues were decreased compared to matching normal esophagus tissues from the patients with adenocarcinoma ($P = 0.011$). Furthermore, GSTPI mRNA expression values were significantly different between metaplastic, dysplastic, and adenocarcinoma tissues ($P = 0.026$). GSTPI expression levels were also significantly lower in histologically normal squamous esophagus tissues from patients with cancer (EA group) compared to both normal esophagus tissues from patients without cancer (BE group; $P = 0.007$) and normal esophagus tissues from the control group with no esophageal abnormality ($P = 0.002$). GSTPI protein expression was generally highest in the basal layer of normal squamous esophagus epithelium and lowest in adenocarcinoma cells, with Barrett's cells showing intermediate staining intensity. Our results show that downregulation of GSTPI expression is an early event in the development of Barrett's esophagus and esophageal adenocarcinoma. Loss of GSTPI expression may have an important role in the development and progression of this disease. (2002;6:359-367.)

Glatzle, J., C. M. Leutenegger, et al. (2004). "Mesenteric lymph collected during peritonitis or sepsis potentially inhibits gastric motility in rats." *Journal of Gastrointestinal Surgery* 8(6): 645.

<http://www.sciencedirect.com/science/article/B6W8T-4D9VBRY-2/2/bf3c80631fd02e3b6908430376508d8d>

Gastrointestinal motility is strongly inhibited during peritonitis or sepsis and proinflammatory cytokines released into mesenteric lymph during an acute gastrointestinal insult mediate systemic responses. We investigated whether mesenteric lymph collected during peritonitis or sepsis inhibits gastric motility and gastric emptying. Mesenteric lymph was collected for 12 hours from three experimental groups: vehicle (saline, 1 ml, intraperitoneally [ip], control lymph), peritonitis (0.5% acetic acid, 1ml, ip, peritonitis lymph), and sepsis (lipopolysaccharide [LPS], 5 mg/kg, 1 ml, ip, sepsis lymph). Gastric motility and gastric emptying were measured in recipient rats in response to lymph injections into the jugular vein. Quantitative polymerase chain reaction (PCR) for tumor necrosis factor [alpha] (TNF[alpha]) gene expression in the jejunum and in lymph cells were measured during sepsis. Mesenteric lymph flow significantly increased during peritonitis or sepsis (lymph flow [ml] per 60 minutes; control 2.45 [plus-or-minus sign] 0.04; peritonitis 2.67 [plus-or-minus sign] 0.07; sepsis 3.25 [plus-or-minus sign] 0.1, $p < 0.05$, 1.89 [plus-or-minus sign] 1.31 minutes; peritonitis lymph: -0.56 [plus-or-minus sign] 0.06 cm H₂O, 9.9 [plus-or-minus sign] 0.9 minutes; sepsis lymph: -0.51 [plus-or-minus sign] 0.05 cm H₂O, 6.9 [plus-or-minus sign] 0.6 minutes; $p < 0.001$ vs. basal; 12 hours: 24.7 [plus-or-minus sign] 16.8, not significant [NS]; 24 hours: 7.0 [plus-or-minus sign] 3.4, NS). In conclusion, mediators in mesenteric lymph, possibly cytokines, may be responsible for the inhibition of gastric motility during peritonitis or sepsis.

Because the composition of mesenteric lymph probably reflects the interstitial fluid of the gut wall, monitoring visceral lymph might be an extremely beneficial tool to determine mediators released during impaired gut wall function.

Kuramochi, H., D. Vallbohmer, et al. (2004). "Quantitative, tissue-specific analysis of cyclooxygenase gene expression in the pathogenesis of Barrett's adenocarcinoma." Journal of Gastrointestinal Surgery 8(8): 1007.

<http://www.sciencedirect.com/science/article/B6W8T-4DYV0NT-J/2/6168ee832eaf88fdad25a84924abe1a>

Cyclooxygenase (Cox-2) is implicated in the pathogenesis of many cancers including esophageal adenocarcinoma (EAC), whereas the role of the isoform Cox-1 in carcinogenesis is not well understood. To further elucidate the role of these factors in the development of EAC, we measured the gene expressions (mRNA levels) of Cox-2 and Cox-1 by real-time quantitative polymerase chain reaction (QRT-PCR) in tissues from normal esophagus with and without erosive gastroesophageal reflux disease (GERD), Barrett's esophagus (BE), dysplasia, adenocarcinoma, and in healthy gastric antrum. All tissues were purified by laser capture microdissection from endoscopic or surgical resection specimens. Median Cox-2 gene expression did not differ significantly among the esophageal control groups but was elevated 5-fold in BE, 8-fold in dysplasia and 16-fold in EAC compared to normal esophageal controls with no erosive GERD. Erosive GERD tissue had slightly higher median Cox-2 expression but Cox-2 expression in normal antrum was much higher than that in a normal esophagus, close to that of dysplasia. In contrast to that of Cox-2, Cox-1 expression was significantly decreased in all neoplastic tissues compared to normal controls. Cox-1 and Cox-2 expression varied over a wide range in the neoplastic tissues but over a relatively narrow range in the esophageal normal tissues. The occurrence of substantial alterations in Cox-1 and Cox-2 expression at the BE stage indicates that these are early events in the development of EAC. These results confirm the important role of Cox-2 amplification in the pathogenesis of esophageal adenocarcinoma, but the unexpected down-regulation of Cox-1 raises questions about its role in carcinogenesis.

Lord, R. V. N., D. Salonga, et al. (2000). "Telomerase reverse transcriptase expression is increased early in the Barrett's metaplasia, dysplasia, adenocarcinoma sequence." Journal of Gastrointestinal Surgery 4(2): 135.

<http://www.sciencedirect.com/science/article/B6W8T-4442JP3-FS/2/c796073fd269f8a183e99a8cb5aeb0c8>

Barrett's esophagus is a multistage polyclonal disease that is associated with the development of adenocarcinoma of the esophagus and esophagogastric junction. Telomerase activation is associated with cellular immortality and carcinogenesis, and increased expression of the telomerase reverse transcriptase catalytic subunit (hTERT) has been used for the early detection of malignant diseases. To identify biomarkers associated with each stage of the Barrett's process, relative mRNA expression levels of hTERT were measured using a quantitative reverse transcription--polymerase chain reaction method (ABI 7700 Sequence Detector (TaqMan system)) in Barrett's intestinal metaplasia (n = 14), Barrett's dysplasia (n = 10), Barrett's adenocarcinoma (n = 14), and matching normal squamous esophagus tissues (n = 32). hTERT expression was significantly increased at all stages of Barrett's esophagus, including the intestinal metaplasia stage, compared to normal tissues from patients without cancer (intestinal metaplasia vs. normal esophagus, P = 0.001; adenocarcinoma, P = 0.007; all Mann-Whitney U test). hTERT expression levels were significantly higher in adenocarcinoma tissues than in intestinal metaplasia tissues (P = 0.003), and were higher in dysplasia compared with intestinal metaplasia

tissues ($P = 0.056$). hTERT levels were also significantly higher in histologically normal squamous esophagus tissues from cancer patients than in normal esophagus tissues from patients with no cancer ($P = 0.013$). Very high expression levels ([hTERT x 100: [beta]-actin] > 20) were found only in patients with cancer. These findings suggest that telomerase activation is an important early event in the development of Barrett's esophagus and esophageal adenocarcinoma, that very high telomerase levels may be a clinically useful biomarker for the detection of occult adenocarcinoma, and that a widespread cancer "field" effect is present in the esophagus of patients with Barrett's cancer.

Okami, J., K. Dono, et al. (2003). "Patterns of regional lymph node involvement in intrahepatic cholangiocarcinoma of the left lobe." Journal of Gastrointestinal Surgery 7(7): 850.

<http://www.sciencedirect.com/science/article/B6W8T-49V9HDY-6/2/ae0c6806ff7a2ec5163b8778e94ee103>

Lymph node involvement is an important prognostic factor in intrahepatic cholangiocarcinoma. Besides the nodes in the hepatoduodenal ligament, recent studies have suggested that the nodes around the cardiac portion of the stomach or along the gastric lesser curvature can be affected when the primary tumor is located in the left hepatic lobe. However, the distribution of metastatic nodes has not been well described in this disease. Thirteen patients with intrahepatic cholangiocarcinoma in the left hepatic lobe were enrolled in this study. Lymphatic mapping was performed by means of both histologic examination and reverse transcriptase-polymerase chain reaction assays. Nodal involvement around the cardiac portion of the stomach or along the lesser gastric curvature (left pathway) was found in 7 (54%) of 13 patients by histologic examination or reverse transcriptase-polymerase chain reaction, whereas positive nodes in the hepatoduodenal ligament (right pathway) were found in 6 (46%) of 13 patients. Two patients (15%) had positive nodes only in the left pathway. Therefore, for a more accurate clinical staging of intrahepatic cholangiocarcinoma in the hepatic left lobe, lymph nodes around the cardiac portion of the stomach and along the lesser gastric curvature should be examined in addition to nodes in the hepatoduodenal ligament.

Otto, C., J. Kauczok, et al. (2002). "Mechanisms of Tolerance Induction After Rat Liver Transplantation: Intrahepatic CD4+ T Cells Produce Different Cytokines During Rejection and Tolerance in Response to Stimulation." Journal of Gastrointestinal Surgery 6(3): 455.

<http://www.sciencedirect.com/science/article/B6W8T-45TY7F9-12/2/ba96303a02810beedb2dc9f4ccd44fbb>

Increasing evidence supports the existence of regulatory T cells that may inhibit the allogeneic immune response after transplantation by secreting regulatory cytokines. To determine whether rat liver tolerance is associated with intrahepatic regulatory T cells secreting a characteristic cytokine profile, we analyzed the cytokine production of freshly isolated intrahepatic CD4+ T cells at different times postoperatively by semiquantitative reverse transcription-polymerase chain reaction and by enzyme-linked immunosorbent assay before and after in vitro stimulation. Orthotopic arterialized liver transplantation was performed in two allogeneic rat strain combinations, one with fatal acute rejection (DA-to-LEW) and one with long-lasting tolerance (LEW-to-DA) without immunosuppression despite a complete major histocompatibility complex mismatch (spontaneous liver tolerance). Liver allografts of both groups showed continuously increasing cellular infiltration between day 3 and day 7 after transplantation. In this inflammatory situation, very low levels of interleukin-13 were detectable directly after cell isolation, as well as after in vitro stimulation. However, after 30 days, intrahepatic CD4+T cells in the tolerance group were then able to express elevated messenger RNA levels of the anti-inflammatory cytokine

interleukin-13 in response to stimulation. This result indicates the presence of an intra-graft Th2-like CD4⁺ T cell population, which may have a regulatory function in the induction of liver tolerance. (2002;6:455-463.)

Journal of Hepatology (38)

Baba, S., H. Fujii, et al. (2004). "Commitment of bone marrow cells to hepatic stellate cells in mouse." Journal of Hepatology **40**(2): 255.

<http://www.sciencedirect.com/science/article/B6W7C-4B26K5D-1/2/1f992d1a1bcb26a7412077e4cd8c3e8d>

Background/Aims: Recently, several cells found within the liver have been reported to derive from bone marrow (BM). This study sought to examine the commitment of BM cells to hepatic stellate cell (HSC) lineage in mouse liver. **Methods:** We transplanted BM cells from green fluorescent protein (GFP) transgenic mice into age-matched C57BL/J mice. Hepatic nonparenchymal cells were isolated from the livers of BM-transplanted mice using density gradient centrifugation with Nycodenz. The expression of lineage markers by the isolated cells was evaluated by RT-PCR and immunostaining. We then examined the histology of liver tissues obtained from BM-transplanted mice with and without carbon tetrachloride-induced injury. **Results:** GFP-expressing cells with intracytoplasmic lipid droplets comprised 33.4 $\pm$ 2.3% of the cells isolated by density gradient centrifugation. These cells expressed the HSC lineage markers, such as desmin and glial fibrillary acidic protein (GFAP), by both RT-PCR and immunostaining. During a 7-day culture, GFP-positive cells began to express α -smooth muscle actin, a marker of activated HSC. In the liver of BM-transplanted mice, GFP-positive nonparenchymal cells expressed GFAP and extended their process around hepatocytes. Upon liver injury, these cells also co-expressed desmin and α -smooth muscle actin. **Conclusions:** Nonparenchymal cells, derived from transplanted BM, acquired HSC characteristics in both quiescent and activated states.

Bedoucha, M., E. Atzpodien, et al. (2001). "Diabetic KKAY mice exhibit increased hepatic PPAR[gamma]1 gene expression and develop hepatic steatosis upon chronic treatment with antidiabetic thiazolidinediones." Journal of Hepatology **35**(1): 17.

<http://www.sciencedirect.com/science/article/B6W7C-43BXNDF-4/2/29fa0987458b34931cfb68aad084f0d0>

Background/Aims: Peroxisome proliferator-activated receptor-[gamma], which is involved in the regulation of lipid homeostasis, is upregulated in the liver of obese and diabetic mice, but the biological consequences of this induction are largely unknown. This study was aimed at further characterizing this upregulation and exploring the downstream biological effects of specific activators on hepatic lipid metabolism. **Methods:** Hepatic expression of peroxisome proliferator-activated receptor-[gamma]1 and [gamma]2 mRNA and protein was analyzed by real-time polymerase chain reaction and Western immunoblotting in KKAY mice and ob/ob mice. KKAY mice were treated with thiazolidinediones, and hepatic triglyceride content and lipid distribution were analyzed biochemically and by histopathology. **Results:** KKAY mice exhibited a marked increase in hepatic peroxisome proliferator-activated receptor-[gamma]1 mRNA and protein levels, whereas the [gamma]2 isoform was upregulated in ob/ob mice. Treatment of KKAY mice

with troglitazone or rosiglitazone resulted in severe microvesicular periportal steatosis, whereas lean control mice did not develop any pathological liver changes. Hepatic triglyceride levels, however, were not altered by the treatment. Conclusions: In mice with obesity-associated upregulated hepatic peroxisome proliferator-activated receptor- γ expression, thiazolidinediones may produce hepatic steatosis. Under pathophysiological conditions, such as non-insulin-dependent diabetes, the liver may thus become sensitized towards peroxisome proliferator-activated receptor- γ -activating drugs.

Bernal, W., P. Donaldson, et al. (1998). "Tumor necrosis factor genomic polymorphism and outcome of acetaminophen (paracetamol)-induced acute liver failure." *Journal of Hepatology* **29**(1): 53.

<http://www.sciencedirect.com/science/article/B6W7C-44FMMDV-9G/2/8613a0b1aa82c9edc9a087017d703fc9>

Background/Aims: High levels of tumor necrosis factor- α are associated with an increased risk of severe encephalopathy in acute liver failure, and experimental studies suggest that tumor necrosis factor- α plays a role in the development of acetaminophen (paracetamol)-induced liver injury and associated multiple organ failure. Inter-individual variations in the production of tumor necrosis factor- α have been linked to genomic polymorphisms within the tumor necrosis factor- α locus. This study examined whether specific tumor necrosis factor polymorphisms are associated with variations in the severity of clinical features in acetaminophen-induced acute liver failure. **Methods:** Genotypes at the -308 tumor necrosis factor A and tumor necrosis factor B Nco1 polymorphic sites were determined in 97 patients with severe acetaminophen-induced hepatotoxicity and 109 controls, using polymerase chain reaction and restriction fragment length polymorphism. The relationship between liver injury, multiple organ failure and encephalopathy, determined retrospectively from clinical notes and genotype, was examined. **Results:** No significant association was found between either tumor necrosis factor A or B genotype and parameters for multiple organ failure or liver injury. The tumor necrosis factor B1B1 genotype was significantly under-represented in those patients developing severe encephalopathy ($p = 0.03$) and a multivariate logistic regression analysis confirmed the influence of tumor necrosis factor B genotype (p_{DRB1^*03} , which is closely linked to the TNFB locus). **Conclusions:** The development of acute liver failure is unlikely to be primarily sepsis driven. However, the apparent protective effect of the tumor necrosis factor B1B1 genotype on the development of severe encephalopathy may be related to the effects of this genotype on tumor necrosis factor- α production in sepsis.

Bernal, W., M. Moloney, et al. (1999). "Association of tumor necrosis factor polymorphism with primary sclerosing cholangitis." *Journal of Hepatology* **30**(2): 237.

<http://www.sciencedirect.com/science/article/B6W7C-4406K4G-18/2/67fad28433468d9302953dcf3a736db4>

Background/Aims: Primary sclerosing cholangitis is associated with the HLA haplotypes A1-B8-DRB3*0101-DRB1*0301-DQA1*0501-DQB1*0201 and DRB3*0101-DRB1*1301-DQA1*0103-DQB1*0603. However, the interpretation of these genetic associations is controversial. One explanation may be that HLA-encoded susceptibility is due to other genes carried on these haplotypes such as the HLA class III tumor necrosis factor genes. The aim of the study was to investigate tumor necrosis factor genetics in a large series of well-defined patients. **Methods:** One hundred and ten HLA genotyped patients and 126 control subjects were studied by polymerase chain reaction genotyping for 3 different tumor necrosis factor gene polymorphisms: -308, -238 and an Nco1 restriction fragment length polymorphism in the lymphotoxin α gene. **Results:** Overall, 58% of patients had the TNF2 allele, compared with 29% of controls, $p = 0.0001$. No

association was found with either of the other tumor necrosis factor polymorphisms examined. TNF2 was significantly increased in the presence of B8 and DRB3*0101 only, and was independent of DRB1*0301 (pcB8 and TNF2 were stronger than the associations with any of the HLA class II alleles examined. Conclusion: HLA-encoded genetic susceptibility to primary sclerosing cholangitis may be determined by polymorphism within the HLA class III region, in particular with the TNF2 allele.

Brind, A., J. Jiang, et al. (1997). "Evidence for selection of hepatitis B mutants after liver transplantation through peripheral blood mononuclear cell infection." Journal of Hepatology **26**(2): 228.

<http://www.sciencedirect.com/science/article/B6W7C-44FMP1S-WB/2/4901d2d9a29e243b1464970b6ca06c33>

Background/Aims: Despite anti-HBs immunoglobulin therapy, hepatitis B virus (HBV) infection recurs in a high proportion of patients transplanted for HBsAg positive and serum HBV DNA negative chronic liver disease. The contribution of HBV genetic variability to disease recurrence has not been yet thoroughly addressed. We have therefore undertaken a detailed comparison of preS/S and preC/C sequences in two selected patients with recurrence of HBsAg and HBV DNA after transplantation. Methods: PreS/S and preC/C regions were amplified by PCR from the serum, peripheral blood mononuclear cell (PBMC) and liver tissues of two patients transplanted for end stage HBV-related cirrhosis. Samples were taken both pre- and post-transplantation. HBV-sequences from four to nine clones were determined and compared. Results: A mixing of different HBV DNA molecules was observed within and between serum, liver and PBMC samples. Sequences from both patients showed mutations in the preC region which abolished HBeAg secretion, and in the preS2 initiation codon which prevented preS2 envelope protein production. In addition, for both patients, deletions in the preS2 domains (3 and 21 base pairs) led to the expression of modified preS1 envelope protein. For one patient, the predominant HBs protein sequence found in the PBMC before transplantation showed four specific mutations. One of these mutations was in the "a" determinant (codon 144, asparagine to glycine change) of the major envelope protein. These mutations were not detected, as predominant mutations, in the liver and serum pre-orthotopic liver transplant samples. In contrast, after liver transplantation, this was the major form identified in serum, liver and PBMC. Conclusions: Our results have shown the selection of different HBV DNA molecules in liver and mononuclear cells. In addition, they provide direct evidence for the role of PBMC in the infection of liver grafts and support the hypothesis that infection of PBMC might lead to selection of HBV variants which would escape immune therapy. Finally, we provide in vivo evidence for reinfection of the liver by HBV particles lacking preS2 envelope protein expression.

Cassanelli, S., E. Pignatti, et al. (2001). "Frequency and biochemical expression of C282Y/H63D hemochromatosis (HFE) gene mutations in the healthy adult population in Italy." Journal of Hepatology **34**(4): 523.

<http://www.sciencedirect.com/science/article/B6W7C-42NH34J-6/2/6bb712051a2bd979d431b9c6297f1848>

Background/Aims: The actual prevalence of the main hemochromatosis (HFE) mutations in the Italian adult population and their phenotypic expression have not yet been established. This information is key to advocate a mass-screening program. Methods: Two thousand one hundred adults were tested for the C282Y/H63D HFE gene mutations by an automated genotyping assay as well as transferrin saturation (TS) and serum ferritin levels. Results: No homozygotes for the C282Y mutation were found. Heterozygosity for the C282Y mutation was 3.1%, while heterozygosity and homozygosity for the H63D mutation were 21.5% and 2.5%, respectively. TS

was significantly higher in C282Y heterozygotes and H63D homozygotes as compared to wild-type individuals (P). Interestingly, of the HFE wild-type subjects 5.9% had a TS value above the 45% threshold. Conclusions: This study shows that (i) the predicted prevalence for C282Y homozygosity in Italy is 1:3900; (ii) the C282Y/H63D wild-type population has an increased baseline of iron parameters possibly due to genetic factors not linked to the C282Y/H63D mutations; (iii) since in the latter population the actual tissue iron burden cannot be assessed, phenotypic (TS) screening in Italy is not recommended until the true prevalence of all mutations in the HFE gene and in other hemochromatosis genes will be established.

Colombatto, P., M. R. Brunetto, et al. (1999). "High prevalence of G1 and G2 TT-virus infection in subjects with high and low blood exposure risk: identification of G4 isolates in Italy." Journal of Hepatology **31**(6): 990.

<http://www.sciencedirect.com/science/article/B6W7C-437YWM4-8N2/5237ca463b74116f9d0e36fd305088cb>

Background/Aims: A non-enveloped single-stranded DNA virus (TTV) was detected in Japanese patients with fulminant hepatitis (47%) and chronic liver disease of unknown etiology (46%) more frequently than in blood donors (12%). Subsequent studies, however, questioned the association of TTV with liver disease. We further investigated the role of this novel virus in liver diseases. Methods: We tested 106 patients and 102 blood donors for TTV by polymerase chain reaction using conserved region primers. Results: TTV DNA was found in 19 of 102 volunteer blood donors (18.6%) and in 27 of 106 patients with liver disease (25.5%): 10 of 28 chronic hepatitis B (35.7%), 9 of 28 chronic hepatitis C (32.1%) and 8 of 50 (16%) cryptogenic liver disease patients. Previous interferon treatment was not associated with a significantly lower prevalence of TTV infection. TTV prevalence was higher in patients with blood exposure (42.8%, 6/14) than in patients without risk factors (21.4%, 18/84). Four of five patients (80%) with HBV familial infection and without blood exposure were also TTV positive. Partial nucleotide sequences from 3 Italian isolates diverged more than 30% from the 2 prototype genotypes G1 and G2 and were 88% homologous to the recently described genotype G4. Conclusions: G1 and G2 TTV are common in Italy and in the USA in liver disease patients and in blood donors. The prevalence is high in patients with blood exposure but also in subjects without risk factors; other routes of transmission should therefore be considered.

Doughty, A. L., D. M. Painter, et al. (2000). "Post-transplant quasispecies pattern remains stable over time in patients with recurrent cholestatic hepatitis due to hepatitis C virus." Journal of Hepatology **32**(1): 126.

<http://www.sciencedirect.com/science/article/B6W7C-436FVDT-53/2/76b838311dc7a942c8b80dc9581f18eb>

Background/Aims: Several studies have shown that cholestatic recurrent hepatitis is associated with very high HCV RNA loads in liver transplant recipients. The aim of this study was to investigate whether a correlation exists between cholestatic hepatitis posttransplant and the population of viral quasispecies. Methods: One hundred and nine serial sera samples were tested from 15 recurrent HCV patients. Four of these patients showed severe cholestatic recurrent hepatitis, 11 patients demonstrated non-cholestatic recurrent hepatitis post-transplant. Quasispecies were detected by RT-PCR amplification of the HVR1 followed by single-stranded conformational polymorphism analysis. Results: Forty-one samples from four cholestatic patients were tested. All four patients showed very stable quasispecies patterns post-transplant. One cholestatic patient also showed a stable quasispecies band pattern following retransplantation, again associated with severe cholestatic hepatitis. Sixty-eight samples were tested from the 11

non-cholestatic patients. In contrast, these patients showed significantly more quasispecies bands than the cholestatic patients. The noncholestatic patients also displayed fluctuating band patterns post-transplant. Serial samples were tested after retransplantation in one non-cholestatic patient, with a fluctuating pattern again seen. There was a negative correlation between the HCV RNA load in serum and the number of quasispecies bands. Conclusions: Stable hepatitis C viral quasispecies associated with persistently high viral load in post-transplant cholestatic hepatitis suggest that viral escape from immune pressures may play a role in the pathogenesis of this condition.

Dumoulin, F. L., C. Reichel, et al. (1997). "Semiquantitation of intrahepatic MDR3 mRNA levels by reverse transcription/competitive polymerase chain reaction." *Journal of Hepatology* **26**(4): 852.

<http://www.sciencedirect.com/science/article/B6W7C-437YSV7-1S/2/96c313c3c8eb02430d9f41c00eefc105>

Background/Aims: Knockout mice lacking *mdr2*, the murine analogue of human MDR3 P-glycoprotein, develop chronic non-suppurative cholangitis. Recently, a deficiency in MDR3 messenger RNA (mRNA) has been reported in a subtype of progressive familial intrahepatic cholestasis. Thus, reduced MDR3 gene expression could be involved in human cholestatic liver diseases. Methods: We developed a sensitive and specific reverse transcription/competitive polymerase chain reaction for the semiquantitation of intrahepatic MDR3 mRNA levels. Using this method we determined the MDR3 mRNA levels in 52 liver specimens from primary biliary cirrhosis (n=11), chronic hepatitis B (n=5), chronic hepatitis C (n=14), non-cholestatic cirrhosis (n=9) and controls (n=13). Results: MDR3 mRNA was detected in all specimens with some variation in mRNA levels. No significant differences in the mean MDR3 mRNA levels were present between the groups studied, including normal controls. Conclusions: We found no evidence for deficient or severely reduced intrahepatic MDR3 mRNA in primary biliary cirrhosis, nor were mRNA levels altered significantly by virus-induced inflammation or by cirrhosis. The reverse transcription/competitive polymerase chain reaction assay described here should be a useful tool in the semiquantitative study of MDR3 mRNA levels in small tissue specimens.

Fanning, P. A., J. R. Jonsson, et al. (2000). "Detection of male DNA in the liver of female patients with primary biliary cirrhosis." *Journal of Hepatology* **33**(5): 690.

<http://www.sciencedirect.com/science/article/B6W7C-436FX5D-GW/2/f987e66f0a0522691dd090bc3a6b0d71>

Background/Aims: Primary biliary cirrhosis is a chronic cholestatic liver disease characterized by progressive inflammatory destruction of bile ducts, with eventual hepatic fibrosis and cirrhosis. Since primary biliary cirrhosis affects predominantly middle-aged women and has pathological similarities to hepatic graft-versus-host-disease, we investigated whether fetal cell microchimerism might be involved in the development of this disease. Methods: The presence of Y-chromosome-specific sequences was analyzed by polymerase chain reaction using peripheral blood mononuclear cells from women with primary biliary cirrhosis (n=18) and healthy (control) women (n=18), and by in situ hybridization of liver biopsy sections from women with primary biliary cirrhosis (n=19) and women with chronic hepatitis C or alcoholic liver disease (n=20). Results: Male cells were detected in liver biopsy specimens of 8 of 19 patients (42%) with primary biliary cirrhosis. Y-chromosome-containing cells were not seen in any of the liver biopsy specimens from women with chronic hepatitis C or alcoholic liver disease. Male cells were detected in peripheral blood mononuclear cells from one healthy control at a level of 1 male cell per 106 female cells, but were not detected in peripheral blood mononuclear cells of women with primary biliary cirrhosis. Conclusions: The presence of male cells in the liver of women with

primary biliary cirrhosis raises the possibility that fetal cell microchimerism may be involved in the pathogenesis of this chronic liver disease.

Fiume, L., G. D. Stefano, et al. (1997). "Hepatotropic conjugate of adenine arabinoside monophosphate with lactosaminated poly--lysine: Synthesis of the carrier and pharmacological properties of the conjugate." Journal of Hepatology **26**(2): 253.

<http://www.sciencedirect.com/science/article/B6W7C-44FMP1S-WF/2/101480370e9ff4aac1a0979f2865aee3>

Background/Aims: The hepatotropic conjugate of adenine arabinoside monophosphate with lactosaminated poly--lysine (-Poly(Lys)) must have a high solubility in order to be injected in a small volume compatible with the intramuscular route. In this paper the molecular weights of Poly(Lys) which allowed the synthesis of conjugates with the properties of high solubility and limited loss by the kidney were determined and a procedure for obtaining Poly(Lys) preparations with the required range of polymerization has been described. **Methods:** Conjugates were prepared using Poly(Lys) of different molecular weights obtained by the procedure described here or purchased from a commercial source. Their solubility and renal loss in mice was determined. **Results:** Poly(Lys) with molecular weights ranging from 45000 and 65000 Da guarantees high solubility and low renal elimination of the conjugates. Conjugate preparations with these properties, intramuscularly administered to woodchuck hepatitis virus-infected woodchucks for 37 days at a daily dose of 5.8 mg/kg exerted a strong antiviral activity. These preparations were devoid of acute toxicity in rat and caused no toxic effects when injected intramuscularly daily for 28 days at a dose ten times higher than that active in woodchucks. **Conclusions:** The results support the possibility of a clinical use of -Poly(Lys) to obtain liver targeting of adenine arabinoside monophosphate for the treatment of chronic hepatitis B virus infection.

Fujikawa, T., T. Hirose, et al. (2003). "Purification of adult hepatic progenitor cells using green fluorescent protein (GFP)-transgenic mice and fluorescence-activated cell sorting." Journal of Hepatology **39**(2): 162.

<http://www.sciencedirect.com/science/article/B6W7C-48W2GWT-2/2/4edf902a203cabbc9bf293dd1ba80fd7>

Background/Aims: Recent advances in stem cell research have revealed that hepatic stem/progenitor cells may play an important role in liver development and regeneration. However, a lack of detectable definitive markers in viable cells has hindered their primary culture from adult livers. **Methods:** Enzymatically dissociated liver cells from green fluorescent protein (GFP)-transgenic mice, which express GFP highly in liver endodermal cells, were sorted by GFP expression using a fluorescence-activated cell sorter. Sorted cells were characterized, and also low-density cultured for extended periods to determine their proliferation and clonal differentiation capacities. **Results:** When CD45-TER119- side-scatterlow GFP^{high} cells were sorted, [alpha]-fetoprotein-positive immature endoderm-characterized cells, having high growth potential, were present in this population. Clonal analysis and electron microscopic evaluation revealed that each single cell of this population could differentiate not only into hepatocytes, but also into biliary epithelial cells, showing their bilineage differentiation activity. When surface markers were analyzed, they were positive for Integrin-[alpha]6 and -[beta]1, but negative for c-Kit and Thy1.1. **Conclusions:** Combination of GFP-transgenic mice and fluorescence-activated cell sorting enabled purification of hepatic progenitor cells from adult mouse liver. Further analysis of this population may lead to purification of their human correspondence that would be an ideal cell-source candidate for regenerative medicine.

Ghez, D., E. Nicand, et al. (2001). "Allogenic bone marrow transplantation with a donor presenting with an acute hepatitis A." Journal of Hepatology **34**(4): 625.

<http://www.sciencedirect.com/science/article/B6W7C-42NH34J-R/2/a909ff7f1d5a3d1090c5c63d17e8854d>

We report the unique occurrence of an allogenic bone marrow transplantation performed as the donor was suffering from an acute hepatitis A. The bone marrow was contaminated at the time of collection, as demonstrated by hepatitis A virus (HAV) RNA detected by RT-PCR. Hepatitis A virus infection in such a situation could have resulted in a severe liver disease in the recipient. However, although we could demonstrate that the recipient had been infected, he did not develop a symptomatic hepatitis A but only minor disturbances of liver function tests between days 35 and 55. Both the postponement of the transplantation and the use of intravenous polyvalent immunoglobulins have probably played a key role in decreasing the viral load and allowing a rapid clearance of the virus. A possible role of the grafted immune system might also be envisaged, as suggested by the de novo synthesis of IgM in the recipient.

Grassi, G., G. Pozzato, et al. (1995). "Quantitative analysis of hepatitis C virus RNA in liver biopsies by competitive reverse transcription and polymerase chain reaction." Journal of Hepatology **23**(4): 403.

<http://www.sciencedirect.com/science/article/B6W7C-44FMM3R-5X/2/c0848b0defc2c19dd9bfb547a6714fe0>

Background/Aims: The determination of HCV-RNA concentration in liver samples is likely to provide interesting insights for the study of disease progression and for evaluation of the efficacy of anti-viral therapy. **Methods:** a procedure was developed for the precise quantification of HCV-RNA in liver biopsies, based on the competitive reverse transcription-polymerase chain reaction technology. This competitive assay consists of the co-amplification of the target RNA with known amounts of a competitor RNA molecule containing the same sequence as the target plus an insertion in the middle, allowing resolution of the two amplification products by gel electrophoresis. **Results:** The amounts of HCV-genomic-RNA and [beta]-actin mRNA (the latter being used as an internal standard to overcome the problem of reproducibility of quantitative RNA extraction) were evaluated in liver biopsies of 15 patients affected by hepatitis C virus-positive chronic liver disease at the time of diagnosis. All the patients underwent [alpha]-interferon therapy for 6 months and were subsequently followed for at least 1 further year after the end of treatment. Viral RNA concentration (which ranged from 2 to 2.7×10^5 HCV-RNA molecules per 10^6 [beta]-actin molecules) directly correlated with the efficacy of treatment, indicating that low levels of viral replication in the liver are associated with a poor response to therapy. **Conclusions:** This study suggests that the determination of viral load in the liver is an important prognostic tool for the prediction of the efficacy of [alpha]-interferon therapy.

Iavarone, M., J.-B. Trabut, et al. (2003). "Characterisation of hepatitis B virus X protein mutants in tumour and non-tumour liver cells using laser capture microdissection." Journal of Hepatology **39**(2): 253.

<http://www.sciencedirect.com/science/article/B6W7C-48TK824-6/2/cd121ba4565b1e163fd36426cd90e4e6>

Background/Aims: The analysis of hepatitis B virus (HBV) X protein genetic variability and is

correlation with liver disease severity have only been addressed, so far, on whole liver extracts. We have studied, therefore, the HBV X protein (HBx) gene sequence in morphologically well-characterised tumour and non-tumour liver cells from patients with HBV-related hepatocellular carcinoma. Methods: Using laser capture microdissection (LCM), we picked up six to eight groups of tumour and non-tumour hepatocytes in serial frozen sections from six patients. After global DNA preamplification followed by HBx-specific polymerase chain reaction, the HBx gene was sequenced in each group of microdissected cells. We also validated the quantification of HBV-DNA in microdissected hepatocytes using HBV Amplicor(R). Results: Heterogeneous mutations in HBx gene were found in distinct cirrhotic nodules and tumour areas from the same patient. Mutations at aa 127, 130 and 131 were frequently detected but there was no distinct point mutation profile between tumour and non-tumour samples. In contrast, deletions in HBx gene, which were found in five/six patients, were more frequent in tumour-derived sequences (6/18) than in non-tumour-derived sequences (1/20). Conclusions: We have shown that LCM provides a direct insight of intrahepatic HBV infection. Using this technique, we demonstrated the persistence of distinct HBx encoding sequences in clonally expanding cells, thus supporting the hypothesis that HBx deletions may be implicated in liver carcinogenesis.

Ikeda, H., M. Takasu, et al. (1999). "Infection with an unenveloped DNA virus (TTV) in patients with acute or chronic liver disease of unknown etiology and in those positive for hepatitis C virus RNA." *Journal of Hepatology* **30**(2): 205.

<http://www.sciencedirect.com/science/article/B6W7C-4406K4G-13/2/b8f963051ddc0d0324b6135ac0bc3665>

Background/Aims: An unenveloped single-stranded DNA virus (TTV) has been reported in association with elevated transaminase levels in patients with posttransfusion hepatitis and in those with acute or chronic liver disease of unknown etiology. To further evaluate the association of TTV with liver disease, TTV DNA was searched for in patients with acute or chronic liver disease of various etiologies. Methods: TTV DNA was determined by polymerase chain reaction with hemi-nested primers in 64 patients with acute or chronic liver disease of unknown etiology and in 100 with acute or chronic liver disease positive for antibody to hepatitis C virus (HCV) as well as HCV RNA. Results: TTV DNA was detected in two of the seven (29%) patients with acute hepatitis of unknown etiology, but in none of the four patients with acute HCV-associated hepatitis. It was detected in 27 of the 57 (47%) patients with chronic liver disease of unknown etiology at a frequency significantly higher (p Conclusions: Based on the obtained results, TTV has a role in the development of acute and chronic liver disease of unknown etiology.

Kang-Park, S., N. Dray-Charier, et al. (1998). "Role for PKC [alpha] and PKC [epsiv] in down-regulation of CFTR mRNA in a human epithelial liver cell line." *Journal of Hepatology* **28**(2): 250.

<http://www.sciencedirect.com/science/article/B6W7C-43S17MD-2X/2/bb055edd504415efac1e7b01728e2436>

Background/Aims: In the liver, intrahepatic biliary cells are the sole site of expression of the cystic fibrosis transmembrane conductance regulator, the product of the cystic fibrosis gene. We examined the regulation of cystic fibrosis transmembrane conductance regulator gene expression by protein kinase C in the recently characterized human liver epithelial BC1 cell line which expresses, at early confluence, both biliary (cystic fibrosis transmembrane conductance regulator, cytokeratin 19) and hepatocytic (albumin) specific markers. Methods: Expression of the cystic fibrosis transmembrane conductance regulator was examined at the mRNA level by Northern blot, reverse transcription-polymerase chain reaction and nuclear run-on assays and at the protein level by Western blotting. The functionality of this protein was tested by measurement of

chloride efflux. Protein kinase C isotype expression and cytosol-to-membrane translocation were analysed by Western blotting. Results: 1) Phorbol ester down-regulated cystic fibrosis transmembrane conductance regulator mRNA expression in a time- and dose-dependent manner through a post-transcriptional mechanism with concomitant inhibition of stimulated chloride efflux. 2) Phorbol ester also activated protein kinase C as indicated by the cytosol-to-membrane translocation of both protein kinase C [alpha] and [epsilon] the two major protein kinase C isotypes expressed by BC1 cells. 3) Further, maximal down-regulation of the cystic fibrosis transmembrane conductance regulator mRNA by the phorbol ester was inhibited by H7 and by GF 109203X, two known protein kinase C inhibitors. Conclusions: These findings provide the first evidence for phorbol ester-induced down-regulation of cystic fibrosis transmembrane conductance regulator mRNA expression in a human liver epithelial cell line and point to a role for the classical protein kinase C [alpha] and the novel protein kinase C [epsilon] in this process.

Llorente, L., Y. Richaud-Patin, et al. (1996). "Cytokine gene expression in cirrhotic and non-cirrhotic human liver." *Journal of Hepatology* **24**(5): 555.

<http://www.sciencedirect.com/science/article/B6W7C-44FMMWN-GF/2/7a42c28608d1dbdb15ea894f35385607>

Background/Aims: In order to explore the role of cytokines in the pathogenesis of liver cirrhosis, we analyzed their gene expression in hepatic biopsies from patients with alcoholic liver cirrhosis, post-hepatitis C liver cirrhosis, and with idiopathic portal hypertension without cirrhosis. Methods: We assessed the gene expression of interleukins 1[beta], 2, 6, 8, and 10, as well as of tumor necrosis factor-[alpha], transforming growth factor-[beta] and interferon-[gamma] by a quantitative polymerase chain reaction. Results: We found high levels of transforming growth factor-[beta] in post-hepatitis C liver cirrhosis, high to moderate in alcoholic liver cirrhosis and low in non-cirrhotic specimens. Expression of interleukin-10, tumor necrosis factor-[alpha], and interferon-[gamma] genes was detected in most post-hepatitis C liver cirrhosis, but not in idiopathic portal hypertension or alcoholic liver cirrhosis biopsies. The interleukin1-[beta], 6 and 8 gene expression was significantly lower in alcoholic liver cirrhosis compared to post-hepatitis C liver cirrhosis, but higher compared to idiopathic portal hypertension specimens. Thus, post-hepatitis C liver cirrhosis samples showed a high degree of cytokine gene expression, whereas in alcoholic liver cirrhosis it tended to be moderate, and restricted to some cytokines (transforming growth factor-[beta], interleukin-1, 6 and 8, but not interleukin-10, tumor necrosis factor-[alpha] or interferon-[gamma]). In contrast, most non-cirrhotic specimens showed a restricted and low cytokine gene expression. Conclusions: These data suggest that transforming growth factor-[beta] may have an important role in liver fibrosis and inflammation. Interleukin-1[beta], 6, 8, tumor necrosis factor-[alpha] and interferon-[gamma], appear to participate in the pathogenesis of the mild to severe inflammatory phenomena seen in alcoholic and post-hepatitis C liver cirrhosis, respectively. Our data suggest that tumor necrosis factor-[alpha] does not participate in the hepatocellular damage of alcoholic liver cirrhosis, and indicate that neither interferon-[gamma] nor interleukin-10, at least at the levels observed in post-hepatitis C liver cirrhosis, are able to counteract the fibrotic/inflammatory process seen in this condition.

Minami, H., K. Tada, et al. (2000). "Enhancement of retrovirus-mediated gene transfer to rat liver in vivo by infusion of hepatocyte growth factor and triiodothyronine." *Journal of Hepatology* **33**(2): 183.

<http://www.sciencedirect.com/science/article/B6W7C-436FWWH-DX/2/8eb22ea1763a0b3b0268e8e974deef28>

Background/Aims: Gene transfer using recombinant Moloney murine leukemia viruses (rMoMuLV) requires mitosis of the target cell. Previously, we and others have used partial

hepatectomy for induction of hepatocellular proliferation for gene transfer to the liver in vivo by exsanguineous perfusion with rMoMuLV. We hypothesized that induction of hepatocellular proliferation by combined administration of two hepatocellular mitogens, hepatocyte growth factor (HGF) and triiodothyronine (T3), should permit rMoMuLV-mediated gene transfer into liver without invasive approaches. **Methods:** HGF (1 mg/kg) was perfused continuously into the portal vein of Wistar male rats and T3 (2 mg/kg) was injected subcutaneously. Twenty-four hours after injecting HGF and T3, the state of proliferation of hepatocytes was estimated from the incorporation of 5'-bromo-2'-deoxy-uridine (BrdU). The amphotropic retroviral receptor (Ram-1) expression of liver was evaluated at different time points after injecting HGF and T3 by means of Northern blotting using Ram-1 cDNA probe. In order to evaluate the role of hormone treatment on gene transfer, the liver was perfused exsanguineously with rMoMuLV 24 h after injection with hormones. **Results:** Rats treated with a combination of HGF and T3 expressed BrdU and [beta]-galactosidase in 8.3% and 0.7% of hepatocytes, respectively. On the other hand, there was near absence of gene transfer in untreated rats perfused with rMoMuLV. Twenty-four hours after the initial manipulation, abundant expression of Ram-1 mRNA was observed in rat hepatocytes treated with HGF plus T3. **Conclusions:** Stimulation of hepatocellular mitosis and upregulation of Ram-1 expression by HGF and T3 augment retrovirus-mediated gene transfer into hepatocytes.

Moaven, L. D., S. A. Locarnini, et al. (1997). "Hepatitis G virus and fulminant hepatic failure: evidence for transfusion-related infection." *Journal of Hepatology* **27**(4): 613.

<http://www.sciencedirect.com/science/article/B6W7C-436FV7M-3G/2/4bd283149a68f4d8d99f609b2f3f969d>

Background/Aims: In the majority of cases of fulminant "viral" hepatitis in Australia, no known aetiological agent can be isolated. We have examined the possible role of the recently discovered hepatitis G virus (HGV) in such cases. **Methods:** An HGV specific reverse transcription polymerase chain reaction (RT-PCR) was performed on pre- and post-liver transplant serum from 14 patients who were referred for transplantation at our unit between 1989 and 1995 for unexplained fulminant hepatic failure. Eleven patients successfully underwent transplantation and three died while waiting for a suitable donor organ. Hepatitis viruses A-E were excluded by standard serological and PCR based testing. HGV RT-PCR was also performed on 21 other, randomly selected, liver transplant recipients ("controls"). **Results:** The 14 fulminant cases were HGV RT-PCR negative prior to transplantation while five of 21 controls were positive. Post-transplant, eight of the 11 fulminant patients were found to be HGV RT-PCR positive and the same five controls remained HGV RT-PCR positive. In three of the eight fulminant patients the HGV infection resolved. **Conclusions:** Our data indicate that HGV infection is unlikely to be responsible for fulminant hepatitis and that it is probably acquired from blood and/or blood products during the transplantation process. Furthermore, long-term carriage of HGV post-transplant is not associated with clinically apparent liver disease.

Morimoto, O., H. Nagano, et al. (2003). "Diagnosis of intrahepatic metastasis and multicentric carcinogenesis by microsatellite loss of heterozygosity in patients with multiple and recurrent hepatocellular carcinomas." *Journal of Hepatology* **39**(2): 215.

<http://www.sciencedirect.com/science/article/B6W7C-48TK824-1/2/9b1697554f3212b8bea9eb5dde671f3b>

Background/Aims: The prognosis of hepatocellular carcinoma (HCC) is poor because of frequent intrahepatic metastasis (IM) or multicentric carcinogenesis (MC). We compared the effectiveness of loss of heterozygosity (LOH) analysis in the diagnosis of these two forms with that of histopathological diagnosis. **Methods:** Using LOH analysis of 15 specific DNA microsatellite loci,

tumor clonality was assessed in 37 cases. Results: LOH was observed in 30% of seven solitary tumors. According to these results, the selected threshold to diagnose MC was a difference in the LOH status at more than 30% of the analyzed loci, when comparing two samples in the same liver. In nine multiple HCCs, identical genetic and histopathological diagnoses were found in four (IM: 2, MC: 2). Of 21 recurrent tumors, 19 showed LOH for at least one marker. IM and MC were genetically diagnosed in five and ten patients, respectively. Genetic and histopathological diagnoses were identical in ten of 19 patients (IM: 5, MC: 5). Five genetic MC were histopathologically diagnosed as IM (3) and 'undetermined' (2). Conclusions: Genetic diagnosis by LOH analysis may be more strict and specific than histopathological diagnosis in the differential diagnosis of IM and MC.

Muller, T., B. van de Sluis, et al. (2003). "The canine copper toxicosis gene MURR1 does not cause non-Wilsonian hepatic copper toxicosis." *Journal of Hepatology* **38**(2): 164.

<http://www.sciencedirect.com/science/article/B6W7C-4753J8J-2/2/bb4c402e4d33ce7c8d529a2180b01ee1>

Background: Non-Wilsonian hepatic copper toxicosis includes Indian childhood cirrhosis (ICC), endemic Tyrolean infantile cirrhosis (ETIC) and the non-Indian disease known as idiopathic copper toxicosis (ICT). These entities resemble the hepatic copper overload observed in livers of Bedlington terriers with respect to their clinical presentation and biochemical and histological findings. We recently cloned the gene causing copper toxicosis in Bedlington terriers, MURR1, as well as the orthologous human gene on chromosome 2p13-p16. Aim: To study the human orthologue of the canine copper toxicosis gene as a candidate gene for ICC, ETIC, and ICT. Methods: We sequenced the exons and the intron-exon boundaries of the human MURR1 gene in 12 patients with classical ICC, one patient with ETIC, and 10 patients with ICT to see whether these patients display any mutations in the human orthologue of the canine copper toxicosis gene. Results: No mutations in the MURR1 gene, including the intron-exon boundaries, were identified in a total of 23 patients with non-Wilsonian hepatic copper toxicosis. Conclusions: Our results demonstrate that copper toxicosis in Bedlington terriers is not an animal model for the non-Wilsonian hepatic copper toxicosis described in this study.

Nakano, T., Y.-M. Park, et al. (1999). "TT virus infection among blood donors and patients with non-B, non-C liver diseases in Korea." *Journal of Hepatology* **30**(3): 389.

<http://www.sciencedirect.com/science/article/B6W7C-4407456-5K/2/a12c8e12be6e7e85350ce13f246a7241>

Background/Aims: A novel virus, designated the TT virus (TTV), was isolated from the serum of a patient with posttransfusion hepatitis of unknown etiology, in Japan. Subsequently, TTV was suggested to be a causative agent in a proportion of cases with cryptogenic hepatitis in Japan. This study aimed to elucidate the significance of TTV infection in cases with cryptogenic liver disease in Korea, a neighbor of Japan. Methods: The prevalence of TTV infection was studied in 120 patients with liver diseases, including 85 patients diagnosed as having non-B, non-C liver diseases. As controls, 220 blood donors were also examined. TTV DNA was detected by polymerase chain reaction, and the sequence was analyzed by phylogenetic analysis. Results: Fourteen (14.0%) of 100 accepted blood donors, 23 (19.2%) of 120 rejected blood donors, and 15 (17.6%) of 85 patients with non-B, non-C liver diseases were positive for TTV DNA. The prevalences of TTV infection among these groups were not significantly different. Phylogenetic analysis suggested the existence of four major genotypes of TTV. The proportions of each genotype among patients with non-B, non-C liver diseases were not different from those among accepted blood donors. Conclusions: TTV exists in Korea, but the prevalence among patients with

non-B, non-C liver diseases was almost the same as that among blood donors. TTV may not be the main causative agent of cryptogenic liver disease in Korea. The relationship between non-B, non-C liver diseases and TTV genotype remains unclear, although TTV can be classified into four genotypes.

Noguchi, O., N. Enomoto, et al. (1996). "Gene expressions of c-met and hepatocyte growth factor in chronic liver disease and hepatocellular carcinoma." Journal of Hepatology 24(3): 286.

<http://www.sciencedirect.com/science/article/B6W7C-44FMMN4-CX/2/776d3466bcd67827212affedf340f0>

Background/Aims: The roles of c-met proto-oncogene and hepatocyte growth factor in human livers have not been shown. **Methods:** Gene expressions of both c-met and hepatocyte growth factor were quantified in livers with chronic active hepatitis and in cirrhotic livers with hepatocellular carcinoma as well as in normal controls, using competitive reverse transcription polymerase chain reaction. **Results:** C-met expression was significantly increased in chronic active hepatitis compared with control livers, and c-met expression in chronic active hepatitis correlated with serum alanine aminotransferase levels. Hepatocyte growth factor expression was increased in some patients with chronic active hepatitis compared with controls, and there was a significant correlation between c-met expression and hepatocyte growth factor expression. On the other hand, in hepatocellular carcinoma tissues, c-met expression was increased in some cases, while that in the surrounding non-carcinomatous tissues was similar to normal controls. Hepatocyte growth factor expression was not detected in the hepatocellular carcinoma tissues and was low in the surrounding non-carcinomatous tissues. **Conclusions:** These findings suggest that hepatocyte growth factor may be involved in the regeneration of hepatocytes via paracrine mechanism in chronic active hepatitis, while the regulation of c-met expression in hepatocellular carcinoma tissues may be independent of hepatocyte growth factor stimulation.

Peck-Radosavljevic, M., J. Pidlich, et al. (1998). "Preoperative TNM-classification is a better prognostic indicator for recurrence of hepatocellular carcinoma after liver transplantation than albumin mRNA in peripheral blood." Journal of Hepatology 28(3): 497.

<http://www.sciencedirect.com/science/article/B6W7C-44FMKNF-1M/2/66d608c117602c5e5d32f7dc48040dad>

Background/Aims: Survival after orthotopic liver transplantation for hepatocellular carcinoma is limited by a high rate of tumor recurrence. A polymerase chain reaction assay based on the detection of albumin mRNA expression in peripheral blood for detection of hematogenous micrometastasis of hepatocellular carcinoma has been described, which may help to select candidates for orthotopic liver transplantation. **Methods:** The prognostic value of a highly sensitive nested reverse transcription-polymerase chain reaction assay was evaluated in comparison with the TNM-classification of the International Union against Cancer in a population of liver transplant candidates. **Results:** Eighty patients with liver disease and 42 control patients were evaluated. Six of 21 patients with hepatocellular carcinoma and 11 of 59 patients with other diseases of the liver were positive for albumin reverse transcription-polymerase chain reaction, making this assay an indicator of ongoing liver damage without absolute specificity for hepatocellular carcinoma. Twelve patients with hepatoma were followed after liver transplantation and seven of those patients had a tumor recurrence within 12 months. Six of these patients with recurrence had International Union against Cancer stage IV A tumors preoperatively, while only one of them was positive for albumin reverse transcription-polymerase chain reaction before transplantation. Only one patient with a stage I to III tumor had a recurrence within 12 months. **Conclusions:** Detection of albumin mRNA in peripheral blood by reverse transcription-polymerase chain reaction seems

to be an unreliable marker for assessing hematogenous spread of hepatocellular carcinoma. With International Union against Cancer stage IV A being a much better predictor of tumor recurrence, the practical value of albumin mRNA reverse transcription-polymerase chain reaction for patient selection in liver transplant candidates seems to be very limited.

Pina, S., M. Buti, et al. (2000). "HEV identified in serum from humans with acute hepatitis and in sewage of animal origin in Spain." *Journal of Hepatology* **33**(5): 826.

<http://www.sciencedirect.com/science/article/B6W7C-436FX5D-HJ2/7fc8e501a128c1f11d235ac6d8add1c6>

Background/Aims: Hepatitis E virus (HEV) is an enterically transmitted pathogen that appears sporadically in non-endemic countries. We studied HEV as a causal agent of acute hepatitis cases in the Spanish population, and the role of pigs as an animal reservoir. **Methods:** The presence of HEV-RNA was analysed by nested polymerase chain reaction in 37 serum samples from patients with acute viral hepatitis, 48 porcine serum samples, 6 pig faecal samples and 12 slaughterhouse sewage samples. Presence of antibodies was also tested in porcine sera. **Results:** HEV-RNA was found in 3 human serum samples from patients presenting IgG anti-HEV antibodies. Nucleotide sequence analysis identified 2 strains with 93.4% identity, phylogenetically most closely related to the Greece1 isolate, and more closely related to North American and other European strains than to those from endemic regions. HEV-RNA was also detected in slaughterhouse sewage mainly from pigs, presenting 92-94% nucleotide similarity compared to the strains detected in the human sera. Twenty-five per cent of the pigs tested presented IgG anti-HEV antibodies. **Conclusions:** These data suggest that the HEV could be more widespread than previously thought, and present new evidence of the close relationship between HEV strains detected in pigs and those from acute hepatitis patients.

Punia, P., P. Cane, et al. (2004). "Quantitation of hepatitis B lamivudine resistant mutants by real-time amplification refractory mutation system PCR." *Journal of Hepatology* **40**(6): 986.

<http://www.sciencedirect.com/science/article/B6W7C-4BSWHF1-1/2/f8f6480b8d8adf28d4203cdc3905f017>

Background/Aims: Lamivudine is an antiviral drug that is used to treat hepatitis B virus (HBV) infection. Long-term therapy does not completely suppress viral replication, and resistant mutants emerge. Resistance is mediated by changes in the tyrosine-methionine-aspartate-aspartate (YMDD) motif in the catalytic site of the HBV polymerase gene. We describe a method to detect and quantify mutant viral populations using amplification refractory mutation system (ARMS) PCR. **Methods:** We developed a real-time ARMS-PCR to detect point mutations in the polymerase gene. Using real-time PCR (LightCycler) with a Resonance probe, PCRs were performed using clones of the HBV polymerase gene containing the different YMDD mutations. Dilution series of the templates were made and tested against each of the primer pairs. This method was applied to quantify mutant virus in patient serum samples. **Results:** As little as 0.01% mutant DNA in 10⁵-10⁹ copies wild-type DNA were detected. The method is more sensitive than amplicon sequencing, which is the current method of mutant determination in the YMDD motif. **Conclusions:** This study demonstrates a rapid, highly sensitive and reproducible method of quantifying mutant HBV virus in lamivudine treated patients. It can be used to monitor patients before and during lamivudine therapy.

Saito, T., K. Shinozaki, et al. (2004). "Lack of evidence for reovirus infection in tissues from patients with biliary atresia and congenital dilatation of the bile duct." Journal of Hepatology **40**(2): 203.

<http://www.sciencedirect.com/science/article/B6W7C-4B54X84-3/2/50b419e222b118621638cb1a07e94f7d>

Background/Aims: To clarify the association between the reovirus infection of the hepatobiliary tree and the development of infantile obstructive cholangiopathy (IOC) including biliary atresia (BA) and congenital dilatation of the bile duct (CBD). **Methods:** We designed reovirus common primers for nested RT-PCR based on the L3 gene segment. The spectrum and the sensitivity of common primers were evaluated with purified reoviral RNAs and reovirus mixed with stool samples. Then, nested RT-PCRs were performed with hepatobiliary and fecal samples obtained from patients with BA, CBD, and control diseases. Additionally, electron microscopy of stool samples was performed. **Results:** The L3 common primers could amplify cDNAs synthesized from RNAs of three prototypes of reovirus, and detect as much as 5.0×10^3 plaque forming unit of serotype 3 Dearing strain in 100 mg of fecal samples. However, no amplification product was detected in 136 hepatobiliary tissues taken from 67 patients including 26 BAs and 28 CBDs, or in 65 fecal samples obtained from 15 patients including 10 BAs and 1 CBD. Additionally, viral particles were not found in any stool specimens by the electron microscope. **Conclusions:** These data do not suggest that reoviruses play a major role in the etiology of IOC or BA.

Shimizu, Y., M. Minemura, et al. (2003). "Preferential accumulation of CD103+ T cells in human livers; its association with extrathymic T cells." Journal of Hepatology **39**(6): 918.

<http://www.sciencedirect.com/science/article/B6W7C-49KS3D7-2/2/12fab404a39726e0729693863ac028df>

Background/Aims: CD103, a mucosal integrin $[\alpha]E[\beta]7$, binds to E-cadherin expressed on hepatocytes and bile duct epithelium in the liver. Although CD103+ T cells are enriched in intestinal intraepithelial lymphocytes, the localization of those cells in the liver is unknown. **Methods:** We investigated whether CD103+ cells are present in human livers, and how they are associated with the intrahepatic development of T cells by flow cytometry and immunohistochemistry. **Results:** Human livers contain significantly ($P < 0.05$) more CD4+ and CD8+ T cells (25.7 ± 13.5 and $27.1 \pm 19.3\%$, respectively) than peripheral blood lymphocytes. Moreover, CD103+ cells in the liver, but not in peripheral blood, contained T cells with intermediate expression level of T cell receptor $[\alpha][\beta]$. Those cells consist of mostly CD4+ and CD4-CD8- cells, and expressed low level of CD56 and interleukin-2 receptor beta chain in most of the population. These characteristics are distinct from natural killer T cells, which have been thought to be extrathymic T cells in human livers. Moreover, intrahepatic CD103+ cells expressed mRNA for recombination-activating gene-1, -2 and pre T cell receptor- α detected by reverse transcription-polymerase chain reaction. **Conclusions:** CD103+ T cells are preferentially accumulated in human livers, and those T cells show characteristics of extrathymic T cells.

Tada, M., O. Yokosuka, et al. (2005). "Hypermethylation of NAD(P)H: quinone oxidoreductase 1 (NQO1) gene in human hepatocellular carcinoma." Journal of Hepatology **42**(4): 511.

<http://www.sciencedirect.com/science/article/B6W7C-4DX25H6-2/2/d3cdfa20c05f3841b4415070e239965c>

Background/Aims: NAD(P)H: quinone oxidoreductase 1 (NQO1) and glutathione S-transferase P1 (GSTP1) belong to phase II xenobiotic-metabolizing enzymes. GSTP1 inactivation via CpG island

hypermethylation in hepatocellular carcinoma (HCC) was previously reported, but the involvement of NQO1 in HCC is not well known. In this study, we assessed the transcription and status of methylation of NQO1 gene in human hepatoma cells and primary human HCC tissues. Methods NQO1 transcription and DNA hypermethylation in hepatoma cells with or without 5-aza-deoxycytidine (5-Aza-CdR) treatment were investigated by reverse-transcription PCR (RT-PCR), sodium bisulfite sequencing and methylation-specific PCR (MSP). The methylation status of NQO1 and GSTP1, and NQO1 mRNA in 44 HCC cases was also analyzed by MSP and real-time PCR, respectively. Results NQO1 transcription was down-regulated and the CpG island DNA was hypermethylated in Hep3B and HuH6 cells. After treatment with 5-Aza-CdR, NQO1 transcription was restored and CpG island DNA was demethylated in these cells. MSP analysis revealed that NQO1 hypermethylation occurred in 50.0% of HCC. All of the tumors that exhibited lesser amounts of NQO1 mRNA than corresponding non-tumorous tissues showed NQO1 hypermethylation. Conclusions NQO1 transcription might be inappropriately suppressed by promoter hypermethylation in a subset of HCC, as well as GSTP1 gene.

Tanaka, A., T. P. Prindiville, et al. (1999). "Are infectious agents involved in primary biliary cirrhosis? A PCR approach." *Journal of Hepatology* **31**(4): 664.

<http://www.sciencedirect.com/science/article/B6W7C-437YTX1-58/2/4be075b047733ff704d1380c1f9a9cdf>

Background/Aims: A variety of data suggest that microbial infections and, in particular, atypical mycobacteria infections, may either initiate and/or be associated with the pathogenesis of primary biliary cirrhosis. Methods: To address this hypothesis, use was made of polymerase chain reaction techniques and primers specific for the 16s rRNA gene of Eubacteria, Archaeabacteria, Mycobacteria and Helicobacter to determine if such sequences were detectable in liver tissue specimens from 29 patients with primary biliary cirrhosis. Similar liver tissues from patients with primary sclerosing cholangitis, chronic hepatitis, alcoholic liver disease and otherwise normal donors were analyzed in parallel. Genomic DNA was extracted from each of these liver tissue specimens using sterile techniques to avoid possible laboratory contamination. The DNA was subjected to polymerase chain reaction amplification using bacterial genus specific primers and the amplified products cloned and sequenced. Sequence data were analyzed by searching for homology to existing genes. Results: Sequences from primary biliary cirrhosis and control livers corresponded to those found in a variety of bacteria, but no consensus sequence was found in primary biliary cirrhosis specimens. Neither Archaeabacteria nor Mycobacteria products were detected in liver specimens of patients with primary biliary cirrhosis, and Helicobacter pylori DNA was detected in only one primary biliary cirrhosis patient. Conclusions: Although bacterial infection, particularly with intracellular organisms, has been suggested to play a role in the initiation of primary biliary cirrhosis, there is no evidence from this study to suggest an ongoing chronic infectious process.

Tuveri, R., F. Jaffredo, et al. (2000). "Impact of TT virus infection in acute and chronic, viral- and non viral-related liver diseases." *Journal of Hepatology* **33**(1): 121.

<http://www.sciencedirect.com/science/article/B6W7C-436FWR9-DC/2/8d13f2dd3719242a1a238650ffbfa72f>

Background/Aims: The prevalence and pathogenicity of TT virus, recently identified in patients with non A-non G post-transfusional hepatitis, are questioned. Methods: We investigated the impact of this new viral infection in a large series of patients with non A-non G, cryptogenic, non-viral and viral-related, acute and chronic liver diseases (n=577) and blood donors (n=300). TTV DNA was detected in serum by heminested polymerase chain reaction. Phylogenetic analysis

was performed in 13 isolates. Results: TTV DNA was detected in 6/25 and 15/127 patients with cryptogenic non A-non G acute and chronic liver disease, respectively. TTV DNA positive subjects with post-transfusional acute hepatitis scored negative before transfusion. TTV prevalence was increased in patients with cryptogenic non A-non G acute and chronic liver disease compared to blood donors (6/300; ppConclusions: These results support the view that TTV is a widely spread infectious agent with a weak pathogenicity. It raises the possibility, however, that TTV might be implicated in a few cases of acute and chronic non A-non G hepatitis. TTV-DNA-analysed sequences are related to genotypes 1 and 2 described in Europe.

Uyama, N., Y. Shimahara, et al. (2003). "Carbenoxolone inhibits DNA synthesis and collagen gene expression in rat hepatic stellate cells in culture." Journal of Hepatology **39**(5): 749.

<http://www.sciencedirect.com/science/article/B6W7C-49CTBJP-5/2/aab5518c2bd177a427f2fe3c9f6f4e93>

Background/Aims: This study using primary-cultured rat hepatic stellate cells (HSCs) was aimed to reveal the effect of carbenoxolone and the other gap-junction blockers on the proliferation and activation of HSCs. Methods: HSC morphology was microscopically evaluated. DNA synthesis was determined by [³H]thymidine incorporation. Expression of HSC activation markers and cell cycle-related proteins was evaluated by Western blot. Collagen [alpha]1(I) mRNA expression was evaluated by quantitative reverse transcription polymerase chain reaction. Results: Carbenoxolone triggered the morphological change of activated HSCs without inducing apoptosis. Culture-induced DNA synthesis was suppressed to 22.6 and 8.51%, respectively, by 40 and 80 [mu]M carbenoxolone. The other gap-junction blockers failed to affect the morphology and the DNA synthesis of activated HSCs. Carbenoxolone decreased the expression of cyclins D1/2 and cyclin-dependent kinases 4/6. Platelet-derived growth factor (PDGF)-BB-elicited DNA synthesis was reduced to 45.6 and 3.27%, respectively, by 40 and 80 [mu]M carbenoxolone. Phosphorylation of c-Raf, MEK and mitogen-activated protein kinase, but not PDGF receptor [beta], under PDGF-BB stimulation was attenuated by carbenoxolone. Collagen [alpha]1(I) mRNA expression was significantly reduced. In addition, carbenoxolone suppressed the activation process of quiescent HSCs. Conclusions: Carbenoxolone reduced the DNA synthesis and the expression of collagen [alpha]1(I) mRNA in activated HSCs independently of its pharmacological action as gap-junction blocker.

Vidigal, P. G., J. J. Germer, et al. (2002). "Polymorphisms in the interleukin-10, tumor necrosis factor-[alpha], and transforming growth factor-[beta]1 genes in chronic hepatitis C patients treated with interferon and ribavirin." Journal of Hepatology **36**(2): 271.

<http://www.sciencedirect.com/science/article/B6W7C-44B6RS5-2/2/3bf748c5024e47ba1cffe7bc829ee049>

Background/Aims: In hepatitis C infection, the production of inappropriate cytokine levels appears to contribute to viral persistence and to affect the response to antiviral therapy. Additionally, polymorphisms in the cytokine genes may affect the production of the cytokines. In this study, we determined the frequency of the genotypes associated with polymorphisms of the interleukin-10 and tumor necrosis factor-[alpha] gene promoters, and transforming growth factor-[beta]1 gene leader sequence, and investigated their association with clinical features and the response to interferon-[alpha] and ribavirin therapy in chronic hepatitis C infection. Methods: Genomic DNA from 80 patients and 37 racially matched healthy controls was studied by polymerase chain reaction and direct automated sequencing. Results: The interleukin-10 -1082 G/G genotype was identified more frequently in patients than in controls (P=0.048). The transforming-growth factor-[beta]1 +29 (codon 10) C/C genotype was associated with resistance to the therapy (P=0.029).

After adjusting for potential confounding variables, patients exhibiting the C/C genotype were less likely to respond to treatment than patients with the T/T or T/C genotypes. Conclusions: These results suggest that inheritance of the interleukin-10 -1082 G/G and the transforming growth factor- β 1 +29 C/C genotypes, which appear to affect the cytokine production, may be associated with susceptibility to chronic hepatitis C infection and resistance to combined antiviral therapy.

Vona, G., R. Tuveri, et al. (2004). "Intrahepatic hepatitis C virus RNA quantification in microdissected hepatocytes." Journal of Hepatology **40**(4): 682.

<http://www.sciencedirect.com/science/article/B6W7C-4BDVY20-1/2/1e7a74500676dc4c2f83924dc53e3c80>

Background/Aims: Debate continues on whether serum and intrahepatic HCV viral loads are correlated and if HCV viral load correlates with the severity of liver disease. These difficulties may at least in part be linked to liver cell heterogeneity, when total liver extracts from HCV-infected individuals are tested for HCV RNA quantification. We have therefore investigated the feasibility of quantifying HCV replication using a laser-based microdissection technique. **Methods:** We compared the results with those obtained for serum HCV RNA quantification and immunochemistry in the case of HCV antigen detection in the liver. Twenty-one HCV-positive patients with chronic active hepatitis (n=10) or cirrhosis (n=11) were analyzed. **Results:** A positive correlation (P=0.0019) was observed between HCV RNA quantifications in sera and microdissected cells. Immunohistochemistry demonstrated that HCV antigen hepatocytes were randomly distributed within liver lobules. Their percentage varied in different patients (0-40%), but did not correlate with the HCV viral load. **Conclusions:** We have designed a sensitive methodology to evaluate the intrahepatic HCV viral load by combining a standardized RNA quantification method with microdissected hepatocytes from frozen liver needle biopsies. Our results directly demonstrate a positive correlation between serum and intrahepatic viral loads, which therefore provides a reliable reflection of intrahepatic HCV replication.

Watanabe, T., M. Niioka, et al. (2000). "Gene expression of interstitial collagenase in both progressive and recovery phase of rat liver fibrosis induced by carbon tetrachloride." Journal of Hepatology **33**(2): 224.

<http://www.sciencedirect.com/science/article/B6W7C-436FWWH-F3/2/0460c8b1435c3048239abd1de7e6d51c>

Background/Aims: Liver fibrosis is a dynamic state between matrix production and degradation. Since our report in 1974, many studies have examined collagenase and liver fibrosis, but not the identification of cells responsible for collagenase production in vivo. The aim of this study was to investigate the gene expression of interstitial collagenase in the progressive and recovery phases of experimental rat liver fibrosis by in situ hybridization. **Methods:** We examined the gene expression of interstitial collagenase (MMP-13) in the progressive and recovery phase of experimental rat liver fibrosis induced by chronic CCl₄ intoxication by reverse transcription-polymerase chain reaction (RT-PCR) and in situ hybridization. In order to identify the cells expressing MMP-13 mRNA by in situ hybridization, immunohistochemistry was performed using serial sections. **Results:** In normal rat liver, a faint band for MMP-13 mRNA was observed by RT-PCR, but not by in situ hybridization. The livers of rats treated with CCl₄ for 4 weeks showed fatty metamorphosis but no definite fibrosis. Positive signals for MMP-13 mRNA were observed in scattered mesenchymal cells, within lobules which seem to be stellate cells from immunohistochemical staining. Once the fibrosis became prominent, the faint band for MMP-13 mRNA was detected only by RT-PCR and very few signals, if any, by in situ hybridization. On the

other hand, in the recovery phase of liver fibrosis, gene expression of MMP-13 was markedly enhanced. Strong positive cells by in situ hybridization were observed mainly at the interface between the resolving fibrous septa and the parenchyma. Overlapping both images of in situ hybridization and immunohistochemical staining with the help of a computer revealed that some positive cells, but not all cells, were stellate cells stained with [alpha]-smooth muscle actin antibody. Conclusions: MMP-13 participates in the degradation of newly-formed matrix in the recovery from rat liver fibrosis more than in the remodeling of extracellular matrix for the formation of fibrosis. Hepatic stellate cells play a crucial role in MMP-13 production in the recovery from fibrosis, though not all stellate cells were positive for MMP-13 mRNA. Further investigation into gene expression of MMP-13 in recovery will lead to new strategies for the treatment of liver cirrhosis.

Yano, H., K. Fukuda, et al. (1996). "Expression of Fas and anti-Fas-mediated apoptosis in human hepatocellular carcinoma cell lines." Journal of Hepatology **25**(4): 454.

<http://www.sciencedirect.com/science/article/B6W7C-44FMNJ7-PS/2/12a754cbd7d611296f2d5c35965b036f>

Background/Aims: Fas transduces apoptotic signals upon cross-linking with the Fas ligand, which is experimentally replaced by anti-Fas antibodies. Because little is known about Fas expression and function in hepatocellular carcinoma, these issues are addressed in the current article. Methods: We examined Fas expressions at protein and mRNA levels, and susceptibility to anti-Fas-mediated apoptosis, on six hepatocellular carcinoma cell lines. Results: Two cell lines constitutively expressed high levels of Fas both on their cell surface and in their cytoplasm, whereas the other four cell lines expressed Fas mainly in their cytoplasm. Fas mRNA of normal size was detected in all cell lines in reverse transcriptase-polymerase chain reaction analyses. Although a Fas mRNA variant, suggesting soluble Fas molecule, was detected in the two cell lines expressing high levels of Fas, its amount was very small compared to that of normal-sized Fas transcript. Anti-Fas dose-dependently induced apoptosis exclusively in the two cell lines which constitutively express high levels of cell surface Fas. However, after preincubation with interferon-[gamma], one cell line with low surface Fas expression became anti-Fas sensitive equivalent to the two cell lines expressing surface Fas at high levels. Studies of two clonally related cell lines showed that dedifferentiated clones had lower Fas expression and resistance to anti-Fas, suggesting deterioration of Fas system after clonal cell dedifferentiation. Conclusions: These findings suggest sensitivity to anti-Fas is virtually relevant to cell surface Fas, but not to cytoplasmic Fas expression. However, its expression level does not correlate to sensitivity to anti-Fas.

Zein, N. N., A. S. Abdulkarim, et al. (2000). "Prevalence of diabetes mellitus in patients with end-stage liver cirrhosis due to hepatitis C, alcohol, or cholestatic disease." Journal of Hepatology **32**(2): 209.

<http://www.sciencedirect.com/science/article/B6W7C-436FVXF-6X/2/18366927d1bba18c941a936ff18e4162>

Background/Aims: The aims were to study: 1) the prevalence of diabetes mellitus in patients with end-stage liver cirrhosis due to hepatitis C, alcohol, or cholestatic liver disease, 2) viral and host immunogenetic factors that may predispose to diabetes, and 3) liver transplantation outcome in patients with or without diabetes. Methods: Fasting blood glucose values of patients who underwent liver transplantation because of hepatitis C-related cirrhosis (73 patients) were compared with those of patients with cirrhosis due to cholestatic (78 patients) or alcoholic liver disease (53 patients) and to a general population. Data on diabetes prevalence in a population

without liver cirrhosis was based on the prevalence of diabetes in Olmsted County, Minnesota, residents. HLA was determined using serologic assays. Hepatitis C virus genotypes were determined with polymerase chain reaction amplification and direct sequencing. Hepatitis G RNA was detected with polymerase chain reaction. Liver transplantation outcome in patients with or without diabetes was determined with rejection, retransplantation, or death at 1 year after transplantation as end points. Results: Of 64 patients with hepatitis C alone, 16 (25%) had diabetes before transplantation compared with 1 of 78 (1.3%) with cholestatic liver disease ($p=0.0001$) and 10 of 53 (19%) with alcoholic liver disease ($p=0.36$). Nine patients had hepatitis C plus cholestatic liver disease; one of these (11%) had diabetes. The prevalence of diabetes in patients with cholestatic liver cirrhosis was not different from that of the general population. The frequency of hepatitis G virus coinfection, HLA-DR3, or HLA-DR4 in hepatitis C and diabetes was not different from that of hepatitis C alone. The distribution of hepatitis C virus genotype was similar in those with and those without diabetes. Diabetes was not associated with increased risk of rejection, retransplantation, or death at 1 year after transplantation, and had no impact on overall survival after transplantation. Conclusions: 1) The risk of diabetes is not increased in patients with liver cirrhosis due to cholestatic liver disease but is in patients with liver cirrhosis due to hepatitis C or alcoholic liver disease; 2) cofactors (age, sex, body mass index, hepatitis G virus coinfection, hepatitis C virus genotype, or HLA-DR3/DR4) did not explain the increased risk of diabetes in patients with hepatitis C; 3) diabetes before liver transplantation did not change the outcome at 1 year after transplantation or survival.

Journal of Hospital Infection (4)

Chiew, Y. F. and L. M. C. Hall (1998). "Comparison of three methods for the molecular typing of Singapore isolates of enterococci with high-level aminoglycoside resistances." Journal of Hospital Infection **38**(3): 223.

<http://www.sciencedirect.com/science/article/B6WJP-4CDJ0YX-8/2/be18b13d8fc4a465721b243c12f7b9b4>

Enterococci are frequently isolated as nosocomial pathogens and have often acquired intrinsic drug resistances. Molecular typing techniques have been developed to assist in epidemiological and infection control measures. This study investigates enterococci with high-level aminoglycoside resistance (HLAR) from the National University Hospital (NUH) of Singapore, and evaluates and compares three methods for typing: restriction enzyme analysis by conventional gel electrophoresis [restriction fragment length polymorphism (RFLP)], pulsed-field gel electrophoresis (PFGE), and polymerase chain reaction (PCR) using random amplified polymorphic DNA (RAPD). Fifty-two isolates of *Enterococcus faecalis* and 13 isolates of *Enterococcus faecium* were used for the study. The numbers of patterns obtained for *E. faecalis* and *E. faecium* were 26 and 4, respectively by the RFLP method, and very similar discrimination was obtained by PFGE. RAPD PCR results were not reliably reproducible. A single pattern type by RFLP accounted for 16 of the *E. faecalis* isolates, suggesting hospital spread.

Loudon, K. W., A. P. Coke, et al. (1996). "Kitchens as a source of *Aspergillus niger* infection." Journal of Hospital Infection **32**(3): 191.

<http://www.sciencedirect.com/science/article/B6WJP-4C7633Y->

1H/2/9fca7908a67e0478a30d86b22390fc03

In this study we investigated the epidemiology of a cluster of cutaneous infections owing to *Aspergillus niger*, which occurred in neutropenic patients in a bone marrow transplant unit. Heavy environmental contamination with the mould was found in the ward kitchen adjacent to the unit. The clinical and environmental isolates were typed by random amplification of polymorphic DNA (RAPD), which showed one of the patients was infected with the same strain as that isolated repeatedly from the kitchen area. In another case, contaminated stockinette material was implicated as the source of infection. Thorough cleaning of the ward kitchen resulted in no further cases on the unit. This highlights the fact that aspergilli may spread to patients by air, food or other vehicles, and underlines the importance of searching for a source and ensuring high levels of hospital hygiene are maintained.

Skibsted, U., D. L. Baggesen, et al. (1998). "Random amplification of polymorphic DNA (RAPD), pulsed-field gel electrophoresis (PFGE) and phage-typing in the analysis of a hospital outbreak of *Salmonella* Enteritidis." *Journal of Hospital Infection* **38**(3): 207.

<http://www.sciencedirect.com/science/article/B6WJP-4CDJ0YX-6/2/20297cd3c4e6394eb02fa38c441c3d3e>

Isolates of *Salmonella* Enteritidis from 81 patients from Herlev Hospital or from Copenhagen County were analysed by random amplification of polymorphic DNA (RAPD), pulsed-field gel electrophoresis (PFGE) and phage-typing. Fourteen polymorphic markers from five decamer primers unambiguously placed all isolates into six RAPD groups: 65 isolates of phage-type 6 (PFGE type I) were resolved into three RAPD groups constituting 86, 12, and 2%, respectively. A fourth RAPD group of 10 isolates was coincident with phage type 8 (PFGE type II) and two isolates, one phage-type 1, the other phage-type 4 (both PFGE type I) formed the fifth group. The sixth group of four isolates was not phage typeable and was PFGE type III. Forty outbreak-related isolates of phage-type 6 were resolved into three strains. No diversity of phage-type 6 was found among isolates unrelated to the outbreak. It is concluded that RAPD is useful as a tool in investigations of microbial outbreaks in its own right, or to supplement phage-typing and PFGE of *Salmonella* Enteritidis.

Xu, J., C. L. Smyth, et al. (2004). "Employment of 16 S rDNA gene sequencing techniques to identify culturable environmental eubacteria in a tertiary referral hospital." *Journal of Hospital Infection* **57**(1): 52.

<http://www.sciencedirect.com/science/article/B6WJP-4C59SHV-2/2/369500e641c6fb788f1db3e26453da6e>

Universal or 'broad-range' eubacterial polymerase chain reaction (PCR) was performed on 53 isolates from environmental water-associated sites in a haematology unit (N=22), and the outer surfaces of cleaning lotion containers sited throughout a tertiary referral hospital (N=31). 16 S rDNA PCR was performed using two sets of universal primers, including the novel reverse primer, XB4, to generate a composite amplicon of 1068 bp, which was sequenced to obtain each isolate's identity. Sequence analysis was able to identify 51 isolates. Most (75% from the haematology unit and 81% from cleaner containers) were Gram-positive. Nine different genera were identified from the haematology unit and 13 from the cleaning lotion containers. This study provides the first reports of *Terrabacter* spp. and *Brachybacterium paraconglomeratum* isolated from a hospital environment. As unusual and difficult-to-identify environmental organisms are unlikely to be clinically significant, and molecular identification is costly and labour-intensive, we

recommend that molecular methods are only used as an adjunct to first-line phenotypic identification schemes where a definitive identification is required. Where molecular identification methods are justified, partial 16 S rDNA PCR and sequencing employing the novel universal primer XB4, is a valuable and reliable technique.

Journal of Immunological Methods (52)

Bardwell, P. D., A. Martin, et al. (2002). "Mutation detection of immunoglobulin V-regions by DHPLC." Journal of Immunological Methods **266**(1-2): 165.

<http://www.sciencedirect.com/science/article/B6T2Y-45XTD29-3/2/324036213b140f52aad861eec3b44d85>

One of the key features in the affinity maturation of antibodies is somatic hypermutation of the variable regions of immunoglobulin genes. The mutations that occur in immunoglobulin genes are detected by direct sequencing of cloned polymerase chain reaction (PCR) products. The frequencies of mutations in vivo are generally high enough to provide sufficient numbers of point mutations in order to generate large databases that can be analyzed in various ways. Recently, the mechanisms of variable (V)-region hypermutation have been studied in tissue culture systems and transgenic mice where mutation occurs at frequencies that are ~10-fold lower than the estimated in vivo rate. Identifying mutations by brute force sequencing of PCR products in comparative studies is limiting when trying to determine if there are statistically significant differences. Here we describe a high throughput technique that can facilitate the identification of immunoglobulin V-regions that contain one or more mutations before sequencing. This technique, known as denaturing high-performance liquid chromatography (DHPLC), utilizes a standard HPLC apparatus with a column that binds double-stranded DNA (dsDNA). In this study, we have successfully detected ~90% of previously sequenced mutated V-regions by DHPLC. Our results show that we were able to detect mutations throughout a 321-base pair (bp) region of the Ricin 45 immunoglobulin (Ig) V-region. Also, with the use of this assay, we have been able to detect mutations in multiple clones of different immunoglobulin genes.

Becknell, B., R. Trotta, et al. (2005). "Efficient infection of human natural killer cells with an EBV/retroviral hybrid vector." Journal of Immunological Methods **296**(1-2): 115.

<http://www.sciencedirect.com/science/article/B6T2Y-4F05PSP-3/2/d67a6027b3c136fde2cc6881daeb97f4>

Molecular characterization of human natural killer (NK) cells will require targeted gene delivery to inhibit and activate specific signaling pathways, yet to our knowledge, an effective means to deliver such products for long-term gene expression without disrupting normal cellular processes has not been described. In this study, we have developed a retroviral strategy to effectively express gene products in the NK cell, whereby its effector functions of cytotoxicity and cytokine production remain intact. Using an EBV/retroviral hybrid vector, we demonstrate infection of human peripheral blood NK cells with simultaneous expression of a marker for infection-the enhanced green fluorescent protein (EGFP)-along with various genes of interest. This technique results in successful infection of the CD56dim NK population that predominates among human peripheral blood NK and is the effector of antibody-dependent cellular cytotoxicity and natural

killing. In addition, we demonstrate infection of the CD56bright NK subset as well as the NK-92 cell line. In summary, we have devised an efficient and reproducible methodology for the targeted delivery of gene products to human NK cells that should now provide opportunities to dissect the molecular processes critical to normal NK cell physiology.

Bertolotto, A., F. Gilli, et al. (2001). "Evaluation of bioavailability of three types of IFN[beta] in multiple sclerosis patients by a new quantitative-competitive-PCR method for MxA quantification." Journal of Immunological Methods **256**(1-2): 141.

<http://www.sciencedirect.com/science/article/B6T2Y-43RVDJ7-D/2/41557e57795fa8085696464abc5b87fb>

Intracellular expression of human myxovirus protein A (MxA) is exclusively induced by type I IFNs (IFN[alpha],[beta],[omega]) or by some viruses and it is strongly increased under IFN treatment. We set up an internally controlled quantitative-competitive polymerase chain reaction (qc-PCR) that quantifies MxA mRNA expressed in human peripheral blood mononuclear cells (PBMC). Our qc-PCR is accurate because the mean ratio of copy number estimated by qc-PCR to that quantified spectrophotometrically is 1.08 ± 0.03 , moreover it is repeatable with high sensitivity (1 fg MxA/pg GAPDH). MxA mRNA was tested in 47 Relapsing-Remitting Multiple Sclerosis (RR-MS) untreated patients and in 48 patients treated with one of the 3 IFN[beta] licensed for MS (24 with Rebif, 14 with Avonex and 10 with Betaferon). All the 48 treated patients were negative to IFN[beta] neutralising antibodies (NABs) as tested in our laboratory using a cytopathic assay (CPE). MxA mRNA levels were detectable in all untreated patients (mean 24 ± 18 fg MxA/pg GAPDH) and significantly higher levels were found in all the treated patients 12 h after IFN[beta] administration (mean 499 ± 325 fg MxA/pg GAPDH); furthermore, the three types of IFN[beta] showed comparable bioavailability. Our data indicate that the bioavailability of the three available types of IFN[beta] can be evaluated by MxA qc-PCR.

Bettinotti, M. P., L. Hadzikadic, et al. (2003). "New HLA-A, -B, and -C locus-specific primers for PCR amplification from cDNA: application in clinical immunology." Journal of Immunological Methods **279**(1-2): 143.

<http://www.sciencedirect.com/science/article/B6T2Y-48V7JYK-1/2/be8fc33b7e1ce4739fababb4d45dc8c1>

The individual cellular immune response to intracellular antigens is modeled by the highly polymorphic major histocompatibility complex (HLA) class I molecules. The epitopes presented and the T cell repertoire that recognizes them depend on the HLA constitution of the individual. Therefore, to monitor and to modify an individual's HLA class I-driven cellular immune response, it is necessary to know the HLA class I alleles of the person and the possible epitopes of the target antigen presented by those alleles. In particular, this is necessary in order to design peptide-based vaccines and immune therapies for the treatment of diseases caused by viruses, intracellular parasites or cancer, and to monitor the immune response during those treatments. We describe a new set of HLA-A, -B, and -C locus-specific primers for the polymerase chain reaction (PCR) amplification of the whole coding sequence of these genes from complementary DNA (cDNA). We describe their use for typing and for the production of a library of recombinant HLA class I genes. We discuss two downstream applications of this gene collection: production of soluble HLA molecules and discovery of new epitopes.

Brostjan, C., T. Bellon, et al. (2002). "Differential expression of inhibitory and activating CD94/NKG2 receptors on NK cell clones." Journal of Immunological Methods **264**(1-2): 109.

<http://www.sciencedirect.com/science/article/B6T2Y-45NGR13-1/2/ab807ca30531cbeeeeb69d7d09e334ec>

Natural killer cells are known to express a variety of surface receptors involved in HLA class I monitoring. It is thus of interest to investigate the clonal distribution and relative expression levels of activating versus inhibitory NK receptors. We have developed a quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) assay designed to determine specific and absolute mRNA levels for NKG2-A/B, -C, -E, -F, -H and NKG2-D. When analyzing NK cell clones derived from a single donor we found differential expression of inhibitory (NKG2-A/B) versus triggering (NKG2-C and potentially -E, -F, -H) NK receptor chains. The generation of the splice variants NKG2-E and -H seemed to occur at a constant ratio. We further compared NKG2 transcript levels to surface receptor expression as monitored by flow cytometric analysis and to NK cell cytotoxicity as detected by reverse ADCC: a clear correlation was observed. Thus, the data obtained reveal a substantial variability in the NKG2 repertoire among NK cell subpopulations, which is likely to affect the sensitivity and reactivity towards the ligand HLA-E.

Calaman, S. D., G. R. Carson, et al. (1993). "Characterization of monoclonal antibodies specific for the V[beta]3 family of the human T cell receptor generated using soluble TCR [beta]-chain." Journal of Immunological Methods **164**(2): 233.

<http://www.sciencedirect.com/science/article/B6T2Y-476MXMB-133/2/2bca130d9534808aca16ae9c9c239ef5>

A soluble, recombinant form of the human T cell receptor (TCR) [beta]-chain containing the V[beta]3.1 sequence has been constructed, expressed in Chinese hamster ovary cells, amplified by dihydrofolate reductase selection, and purified in quantities appropriate for the generation of monoclonal antibodies (mAb). The V[beta]3 sequence was chosen because of its reported elevated usage in the synovial T cells of rheumatoid arthritis patients but the approach described should be applicable to other known human V[beta] gene sequences. By this method, two mAb were prepared which reacted with up to 10% of normal, live peripheral blood T cells but with reactivity varying greatly among individual donors. Both mAb specifically bound to a murine T cell line transfected with a human TCR V[beta]3.1 and immunoprecipitated a protein of the expected molecular weight for the TCR [beta]-chain. Both antibodies were mitogenic for T cells and analysis of peripheral blood lymphocyte cultures stimulated with the mAb suggested that both were specific for the V[beta]3.1 subfamily and not D[beta] or J[beta]. Clones expressing V[beta]3, which were derived from mAb-stimulated peripheral blood lymphocytes of a single individual, preferentially (8/13), but not exclusively, utilized the J[beta]2.7 gene segment. The V[beta]3.1 usage showed no preference for the CD8+ or CD4+ subpopulations of normal peripheral blood T cells.

Case, M. C., A. D. Burt, et al. (1999). "Enhanced ultrasensitive detection of structurally diverse antigens using a single immuno-PCR assay protocol." Journal of Immunological Methods **223**(1): 93.

<http://www.sciencedirect.com/science/article/B6T2Y-3VM79Y0-8/2/4adaf90c67f04dd070f2953d34c9b5a6>

Our studies of DNA damage and repair in autoimmune disease, lymphomagenesis, and carcinogenesis, require identification of an immunoassay approach that is capable of

ultrasensitive detection in a routine human tissue biopsy of several physicochemically diverse antigens, some of which will be present at very low level. Immuno-polymerase chain reaction (immuno-PCR) is a recently described method for ultrasensitive antigen detection that combines the amplification power of PCR with a method similar to a standard antibody capture, enzyme-linked immunosorbent assay (ELISA). As a test of the universality of immuno-PCR, and as an assessment of the suitability of this method for our studies, we used a single immuno-PCR protocol to assay purified forms of the following physicochemically diverse antigens: oligomeric pyruvate dehydrogenase complex (PDC; Mr 8.5×10^6), the promutagenic DNA base adduct O6-methylguanosine (Mr 298) and its monomeric repair enzyme, O6-methylguanine-DNA methyltransferase (MGMT; Mr 22,000), and a peptide from the N-terminus of MGMT (Mr 2310). We found that all antigens could be ultrasensitively assayed using the single immuno-PCR protocol. Assay limits observed using antigen-specific (primary) antibodies at 1 $\mu\text{g/ml}$, were in the approximate range of 10^2 - 10^9 molecules, with O6-methylguanosine being detected most sensitively. Sensitivity of the antigen assay appeared to positively correlate with primary antibody titres determined by ELISA. Furthermore, we observed a substantial increase in detection sensitivity for all antigens by the use of primary antibodies at the higher level of 10 $\mu\text{g/ml}$. The latter approach permitted antigen assay within the approximate range of 10^2 - 10^7 molecules. The combination of higher titre primary antibodies and their use at higher input level, produced an increase of immuno-PCR assay sensitivity of up to four orders of magnitude greater than those previously reported through the use of this assay to measure other antigens. This represents up to a nine order of magnitude increase in immunoassay sensitivity compared to ELISA. Our findings provide compelling evidence that immuno-PCR is indeed a universal ultrasensitive antigen detection method. Using the indicated assay enhancements, immuno-PCR performed as detailed here can offer greatly increased sensitivity for antigen measurement compared to other methods. Thus, our findings suggest that parallel quantitation of several different antigens in very small samples of human tissue will be readily attainable using immuno-PCR.

Cohen, P., D. Laune, et al. (2001). "Interaction of the octapeptide angiotensin II with a high-affinity single-chain Fv and with peptides derived from the antibody paratope." Journal of Immunological Methods **254**(1-2): 147.

<http://www.sciencedirect.com/science/article/B6T2Y-4378WTW-D/2/df443a60effa7949fa96c45962399506>

The amino-acid sequence of the very high-affinity anti-angiotensin II monoclonal antibody 4D8 was predicted from the nucleotide sequence of the heavy and light chain variable genes. The single-chain variable fragment (scFv) was constructed and expressed in *Escherichia coli* as a soluble protein and at the surface of the filamentous M13 phage and was compared with the full-length antibody (Ab). The scFv showed the same specificity profile and affinity constant as the intact antibody (5.0×10^{10} and $8.0 \times 10^{10} \text{ M}^{-1}$, respectively, by Scatchard analysis). Several peptides from the set of overlapping dodecapeptides covering the variable domains of 4D8 mAb were found to specifically bind biotinylated angiotensin II: peptides from the L1, L2, L3 and H1 regions had the strongest capacity to bind the antigen.

Damoiseaux, J. G. M. C., R. Theunissen, et al. (1998). "Comparison of detection techniques for cytokine reverse transcriptase polymerase chain reaction; digoxigenin-labeled polymerase chain reaction permits sensitive detection of cytokine mRNA in rat heart allografts." Journal of Immunological Methods **217**(1-2): 185.

<http://www.sciencedirect.com/science/article/B6T2Y-3TXK8XS-N/2/400ecc2f67f08dbd77793629e7a3a13d>

The polymerase chain reaction (PCR) is a sensitive method for the analysis of cytokine mRNA expression. The amount of specific mRNA in tissues involved in an inflammatory immune response can be low and therefore requires highly sensitive detection of the PCR products. In our study we have compared different detection techniques in order to replace the commonly used detection by means of radiolabeled probes. Besides the detection of DNA in agarose gels by ethidium bromide (EB), we used detection by digoxigenin (DIG)-labeled probes, as well as the direct incorporation of DIG-labeled nucleotides in the PCR, in comparison to detection by means of labeled probes. In vitro activated rat lymph node cells, lymph node tissue, and acutely or chronically rejected rat heart allografts were examined for expression of mRNA of the cytokines IL-2 and IFN[gamma]. The directly DIG-labeled PCR appeared to be the best alternative for detection of PCR products by means of radiolabeled probes. While IL-2 mRNA was not detected by means of EB and IFN[gamma] mRNA was only detected at the highest PCR cycle numbers in acutely and chronically rejected rat heart allografts, both cytokine mRNA's were readily detected by directly DIG-labeled PCR.

Den, W., S. R. Sompuram, et al. (1999). "A bidirectional phage display vector for the selection and mass transfer of polyclonal antibody libraries." Journal of Immunological Methods **222**(1-2): 45.

<http://www.sciencedirect.com/science/article/B6T2Y-3VCK4R5-4/2/a8a87f51cf7a47bc1195bd026f3b06b>

An approach to the creation of antigen-specific polyclonal libraries of intact antibodies is presented. A polyclonal library of Fab antibody fragments would be expressed using a phage display vector, and selected for reactivity with an antigen or group of antigens. For conversion into a sublibrary of intact polyclonal antibodies, the selected heavy (H) and light (L) chain variable (V) region gene combinations would be transferred in mass, as linked pairs, to a eukaryotic expression vector which provides immunoglobulin (Ig) constant (C) region genes. To enable this selection and transfer, a bidirectional phage display vector was generated, in which the V region gene pairs are linked head to head in opposite transcriptional orientations. The functionality of this vector was demonstrated by the selection, transfer and expression of linked V region gene pairs derived from an A/J mouse that had been immunized with p-azophenylarsonate (Ars)-coupled keyhole limpet hemocyanin (KLH). As expected, the expressed IgG2b anti-Ars antibodies with selected V region gene pairs were shown to have V region sequences and Ars-binding characteristics similar to those of anti-Ars hybridoma antibodies. The technology presented here has potential for many diagnostic and therapeutic applications. These include the generation of polyclonal antibody libraries against multiple epitopes on infectious agents or cancer cells, and of polyclonal libraries encoding chimeric molecules composed of antibody V regions and T cell receptor C regions.

Dufour, V., C. Arnault, et al. (1999). "Quantification of porcine cytokine gene expression using RT-PCR, a homologous internal control and chemiluminescence for microplate detection." Journal of Immunological Methods **229**(1-2): 49.

<http://www.sciencedirect.com/science/article/B6T2Y-3Y8N2FX-4/2/34120909a2ae4c792389927dea461ae5>

The polymerase chain reaction (PCR) has proved to be a sensitive and versatile method for the analysis of human and murine cytokine mRNA expression. This paper describes for the first time a reverse transcription-polymerase chain reaction (RT-PCR) at end-point for the quantification of five porcine cytokines: interferon (IFN)-[gamma], interleukin (IL)-2, IL-4, IL-10 and IL-18. The main features of the methodology are: (1) a unique RT for all quantifications, (2) the addition of homologous DNA internal controls (IC) of equal length to the corresponding cytokine and

consequently co-amplification of the target cytokine and the IC with equivalent efficacy, (3) PCR and detection of amplicons for all cytokines simultaneously, (4) cytokine quantification in relation to a housekeeping gene control (glyceraldehyde-3-phosphate dehydrogenase, GAPDH), (5) detection of the amplicons by enzyme linked immunosorbent assay (ELISA) using a chemiluminescent substrate with high sensitivity and wide dynamic range, (6) automation of the detection system for analysis of a large number of samples. This highly sensitive quantitative RT-PCR assay (able to detect 100-200 cytokines mRNA copies/75 x 10³ cells) was validated on peripheral blood mononuclear cells (PBMC) from pigs infected or not with pseudorabies virus (PRV), re-stimulated in vitro by a mitogen or antigens.

Ekerfelt, C., J. Ernerudh, et al. (2002). "Detection of spontaneous and antigen-induced human interleukin-4 responses in vitro: comparison of ELISPOT, a novel ELISA and real-time RT-PCR." Journal of Immunological Methods **260**(1-2): 55.

<http://www.sciencedirect.com/science/article/B6T2Y-447DF3N-4/2/23131ff534ab32980eff593fe3ff65d1>

Interleukin-4 (IL-4) is an important T-helper cell type 2 (Th2) cytokine in man, driving Th2 polarisation and exerting the most antagonistic effects to the Th1 cytokine interferon-[gamma] (IFN-[gamma]). Nevertheless, few data on spontaneous and antigen-specific secretion of IL-4 in man are available, mainly due to difficulties in the detection of IL-4. In this study, we compared three assays that can detect antigen-induced IL-4 responses; ELISPOT, ELISA after blocking the IL-4 receptor during cell culture, and real-time reverse transcription polymerase chain reaction (RT-PCR). Spontaneous, antigen- and allergen-induced responses were analysed in peripheral blood mononuclear cells from three groups with different secretion patterns for IL-4: atopic individuals, nonatopic individuals and pregnant women. ELISPOT displayed the highest sensitivity and was the only assay that could detect spontaneous secretion of IL-4 in all analysed samples. The IL-4 receptor blocking ELISA was considered best for the detection of in vitro antigen- and allergen-induced responses, since the results obtained from the ELISPOT and real-time RT-PCR displayed lower specificity, possibly because of seemingly aberrant IL-4 responses in the group of pregnant women. The real-time RT-PCR for detection of IL-4 mRNA proved to be sensitive, but expression of IL-4 mRNA was not correlated with the secretion of IL-4.

Feuchtenberger, M., H.-P. Tony, et al. (2003). "Semiquantitative and qualitative assessment of B-lymphocyte VH repertoire by a fluorescent multiplex PCR." Journal of Immunological Methods **276**(1-2): 121.

<http://www.sciencedirect.com/science/article/B6T2Y-48622T6-2/2/c6e9be04f7cbb856bc1140f45700db95>

We established a new tool to perform semiquantitative and qualitative screening for VH gene usage frequency during IgH rearrangements in human B-lymphocytes. In two separate multiplex PCRs, the rearranged VDJ regions were amplified with VH family-specific primers labeled with different fluorescent dyes (FAM, HEX, NED, or ROX). The relative amount of each of the particular VH family products and their ratios were determined by fragment analysis on a ABI PRISM 377 sequencer. We verified that the fluorescent multiplex PCR (FMPCR) shows high specificity and sensitivity, acceptable reproducibility and reliability. Data obtained were well in agreement with results revealed by sequencing following single-cell PCR. Ten healthy volunteers showed a comparable semiquantitative VH family distribution. The FMPCR also correctly detected a monoclonal peak in a CLL patient. Thus, labeling primers with various fluorescent dyes allows for an assessment of VH family usage and an immediate determination of the involved VH gene family if any clonal peaks are present. This method provides a quick, easy, and

reliable tool for VH repertoire screening of larger populations of patients suffering from diseases with changes in the VH repertoire allowing for selection of cases worth a more detailed and cumbersome sequence analysis later on.

Flores, M. G., S. Zhang, et al. (2004). "In vitro evaluation of the effects of candidate immunosuppressive drugs: flow cytometry and quantitative real-time PCR as two independent and correlated read-outs." *Journal of Immunological Methods* **289**(1-2): 123.

<http://www.sciencedirect.com/science/article/B6T2Y-4CG7PM4-2/2/52ce1d0bfeccc136aa8ecd89ef1c6be7>

Background: Immune monitoring may use flow cytometry or molecular biology techniques. Flow cytometry assays cells that are phenotypically characterized, whereas TaqMan(R) RT-PCR starts with RNA extraction from unfractionated heterogeneous cell populations. We therefore wondered how the effects of immunosuppressive drugs on cytokine production in stimulated whole blood, as determined by flow cytometry, would correlate with those obtained with quantitative real-time PCR (TaqMan(R) RT-PCR). Methods: Blood drawn from naive cynomolgus monkeys was exposed to incremental amounts of cyclosporine (CsA; 300, 600, 900 and 1200 ng/ml) or tacrolimus (TRL; 8, 20, 40 and 80 ng/ml) before lectin stimulation in vitro. Blood was then either stained for CD3, IFN-[gamma], IL-2, IL-4, and TNF-[alpha] and analyzed on a flow cytometer with various gating strategies, or submitted to RNA extraction for analysis of the above mentioned cytokines mRNA transcripts using TaqMan(R) RT-PCR. Results: Both methods revealed a parallel dose-dependent inhibition of cytokine production in stimulated blood. The 50% inhibitory concentrations (IC50's) ranged from 511-771 ng/ml (CsA) and 15-29 ng/ml (TRL) with flow cytometry, and from 275-529 ng/ml (CsA) and 11-48 ng/ml (TRL) with TaqMan(R) RT-PCR for T-helper 1 cytokines. Both assays correlated well with a Pearson product moment correlation of 0.76. Extending gating from a CD3+ gate to a lymphocyte gate improved correlation ($r=0.85$) for all cytokines investigated (except IL-2; unchanged) whereas further extending gating resulted, to the contrary, in lower correlations. Independent of gating strategy a high correlation ($r=0.97$) was observed when drug IC50's were considered. Conclusions: Flow cytometry and TaqMan(R) RT-PCR may be used interchangeably to monitor the effects of candidate immunosuppressive drugs on cytokine mRNA production in lectin-stimulated whole blood.

Frohn, C., P. Schlenke, et al. (1998). "DNA typing for natural killer cell inhibiting HLA-Cw groups NK1 and NK2 by PCR-SSP." *Journal of Immunological Methods* **218**(1-2): 155.

<http://www.sciencedirect.com/science/article/B6T2Y-3V78C2J-H/2/b69acd59079f74c5ab0873ec0928c732>

Over the last few years, natural killer (NK) cells have been shown to express MHC molecule recognizing receptors which are thought to function primarily as negative signaling receptors. HLA-Cw seems to play a key role as the corresponding ligand. Two distinct HLA-Cw groups which differ in amino acid residues 77 and 80 inhibit separate subsets of NK cells. In order to classify target cells with respect to their expression of HLA-Cw groups we established a group specific PCR-SSP which directly amplifies the relevant epitope coding sequences. The PCR protocol was validated by retyping cell lines obtained from the International Histocompatibility Workshop and by comparing those results with those acquired from allele-specific genotyping and serotyping on 80 donor-recipient pairs from our kidney transplantation unit. In the context of inhibitory HLA-Cw receptors, our protocol which definitively discriminates the two alternative epitopes is the more direct and thus more reliable approach, and is less labor intensive compared to an allele specific PCR or serotyping. In addition serotyping does not detect at all certain alleles. Basic NK cell research and clinical transplantation immunology may benefit from this newly

established PCR SSP technique.

Furuya, D., A. Yagihashi, et al. (2000). "An immuno-polymerase chain reaction assay for human interleukin-18." Journal of Immunological Methods **238**(1-2): 173.

<http://www.sciencedirect.com/science/article/B6T2Y-4007PM6-K/2/9f2fd1588a267afae8667856670ad8e5>

Conventional enzyme-linked immunosorbent assays (ELISA) are sufficient to measure normal and elevated serum interleukin (IL)-18 concentrations, but have limited sensitivity when measuring low concentrations of IL-18 such as in patients with the acquired immunodeficiency syndrome. We have developed a highly sensitive method for detecting human (h) IL-18 using an immuno-polymerase chain reaction (PCR). A mouse monoclonal anti-hIL-18 antibody and rabbit polyclonal anti-hIL-18 antibody was used for an indirect sandwich ELISA with a detection limit of 40 ng/l and a very low background. For immuno-PCR, biotinylated DNA was produced from the plasmid Bluescript by PCR amplification with biotinylated M13-20 primer and nonbiotinylated M13 reverse primer. Immuno-PCR for hIL-18 was performed for 40 cycles using 1 ng/l of biotinylated DNA. This immuno-PCR has a detection limit of 2.5 pg/l, 1.6×10^4 times lower than that of the ELISA. In addition, our system avoids sampling error caused by heat transfer from the ELISA plate to the PCR tube because all procedures from immobilization of the antibody to PCR amplification can be performed in the same tube. This immuno-PCR for hIL-18 is the most sensitive method for detecting hIL-18 reported to date.

Gallard, A., G. Foucras, et al. (2002). "Tracking T cell clonotypes in complex T lymphocyte populations by real-time quantitative PCR using fluorogenic complementarity-determining region-3-specific probes." Journal of Immunological Methods **270**(2): 269.

<http://www.sciencedirect.com/science/article/B6T2Y-46RCV6P-1/2/602f8f84b75d98b4fd25a3c18c3156a6>

The T cell receptor (TCR) [alpha] and [beta] chains are encoded by a series of stochastic rearrangements between variable (V), diversity (D) for TCR [beta] chain only, and joining (J) gene segments, creating hypervariable complementarity-determining region 3 (CDR3) regions that contact the peptide/MHC complex and confer specificity. In the present paper, we applied the recently developed real-time quantitative RT-PCR technique to the detection of rearranged TCR [beta] chain mRNA transcripts. We designed BV- and BJ-specific primers together with TaqMan(TM) probes specific for the CDR3 regions of the clones of interest. As an external reference, we used plasmids containing the entire TCR [beta] chains, making it possible to normalize the number of specific rearranged BV-J mRNA copies among the total number of TCR [beta] chains. Here, we present data validating this fluorogenic PCR-based method for the quantification of several TCR clonotypes characteristic of the CD4 T cell response to hen egg white lysozyme (HEL) in mice of the H-2d haplotype. This accurate and sensitive procedure permits the precise determination of T cell clone frequencies ranging from 10^{-2} to less than 10^{-5} in normal biological samples; it may provide an alternative approach when frequencies are too low to be assessed by flow cytometry.

Garcia-Pacheco, J. M., P. Mantilla, et al. (1995). "Routine HLA DRB/DQB oligonucleotide typing by a non-radioactive dot-blot micromethod." Journal of Immunological Methods **180**(1): 35.

<http://www.sciencedirect.com/science/article/B6T2Y-3Y6PRBM-34/2/ed5619582b64313be7ba345a3fc50a4e>

In the last few years the clinical need for HLA genotyping has become evident. However, the routine use of PCR-based DNA typing techniques has been hampered by economical and/or technical considerations. The classical PCR-SSO (product-dot) method has been widely tested and proven to be useful for large-scale HLA DNA typing. However, it is not a suitable method for routine typing of single samples because it takes several days. Using primers and probes for sequences identical to those compiled by the Eleventh International Histocompatibility Workshop, we designed a non-radioactive dot-blot technique in which each hybridization reaction is performed in a microtiter plate well containing PCR-amplified DNA that has been previously dotted on a small nylon membrane, so that a large number of oligonucleotide probes tailed with biotin-14-dATP can be simultaneously tested against the same sample. We studied 23 B-lymphoblastoid cell lines of known HLA genotype to test the method and, so far, it has been validated on more than 100 patients and healthy relatives typed prospectively. This simple, rapid, inexpensive PCR-SSO dot-blot micromethod makes DRB/DQB DNA typing of single samples possible in a short period of time, and is therefore an attractive alternative to serological typing in routine medical practice.

Govorko, D., G. Cohen, et al. (2001). "Single-chain antibody against the common epitope of mutant p53: isolation and intracytosolic expression in mammalian cells." Journal of Immunological Methods **258**(1-2): 169.

<http://www.sciencedirect.com/science/article/B6T2Y-448BKNV-K/2/e2d03b98bea939838205f13bb42a260d>

The peptide epitope FRHSVV is cryptic in wild-type p53 and is exposed in many types of mutant p53 molecules isolated from various tumors. Mutant p53 marked by this epitope abrogates a tumor-suppressor function of wild-type p53 and possibly contributes to the transforming potential of other oncogenic processes. We report here the construction of a single-chain scFv antibody gene library derived from the mRNA of a mouse immunized with the epitope peptide FRHSVV which mimics the common epitope in p53 mutant protein molecules. The scFv was presented by phage display. The selected antibody gene, named ME1, was found to bind to the mutant p53 protein but not to the wild-type p53 protein. Preliminary studies show that the ME1 gene is expressed in the cytosol of mammalian cells. These findings suggest that the ME1 single-chain antibody may be useful as a tool for clarifying the role of mutant p53 in tumor transformation, especially in cells heterozygous in p53, and possibly for gene therapy of tumors.

Grant, D. D., R. Goldstein, et al. (1999). "Elimination of non-viable 6-thioguanine-sensitive T cells from viable T cells prior to PCR analysis." Journal of Immunological Methods **225**(1-2): 61.

<http://www.sciencedirect.com/science/article/B6T2Y-3WJFCGC-6/2/a96e004dcd5a5436c78e1b0929ca35c9>

The study of T cell clones at the genomic level is expanding our understanding of their role in diseases such as rheumatoid arthritis (RA) and multiple sclerosis (MS). We have been carrying out genotypic analysis by PCR of hypoxanthine phosphoribosyltransferase (hprt) mutations in these cells. Mutant T cells in the population can be cloned on the basis of their resistance to the cytotoxic drug, 6-thioguanine (6-TG). A difficulty is that the majority of primary human T cells are capable of only limited growth ex vivo, even in the presence of 'feeder' cells. PCR analysis of DNA from such clones is made difficult by the limited number of viable mutant (drug-resistant) T

cells and the large number of dead (drug-sensitive) mononuclear cells and feeder cells. DNA from the 'dead' cells remains sufficiently intact for many weeks in culture and can represent a significant source of background in PCR analysis. Here we describe a method employing hypotonic shock and micrococcal nuclease that reliably eliminates non-viable 6-TG-sensitive cells, allowing the study of the hprt gene in <200 T cells by PCR.

Granziero, L., P. Nelboeck, et al. (1997). "Baculovirus cDNA libraries for expression cloning of genes encoding cell-surface antigens." Journal of Immunological Methods **203**(2): 131.

<http://www.sciencedirect.com/science/article/B6T2Y-3PH8M86-F/2/bd85a9c07dafac3b297b84f457bb4a13>

We describe a method for the production of baculovirus-based cDNA libraries. By staining with monoclonal antibodies, single positive cells can be sorted and the virus encoding for the surface epitope can be isolated by limiting dilution. We have used this method to isolate cDNAs encoding several cell-surface antigens.

Guttieri, M. C., C. Bookwalter, et al. (2000). "Expression of a human, neutralizing monoclonal antibody specific to Puumala virus G2-protein in stably-transformed insect cells." Journal of Immunological Methods **246**(1-2): 97.

<http://www.sciencedirect.com/science/article/B6T2Y-41S4SCM-B/2/4cff1fbc5c2fdd8ab8c528110841acea>

We cloned the heavy- and light-chain antibody genes of a human X (human x mouse) trioma secreting a neutralizing, IgG monoclonal antibody to the G2-protein of Puumala virus. The antibody genes were inserted separately into plasmid transfer vector pIEI-4 such that the genes were under control of the baculovirus immediate early gene promoter, IEI. *Trichoplusia ni* (TN) cells were co-transfected with these constructs and a selection plasmid containing a neomycin-resistance gene. Cloned transformants expressing the IgG monoclonal antibody were identified by ELISA of transfected TN cell culture supernatants. TN cell lines were established from four selected clones, of which one was chosen for detailed analysis. Specificity of the insect cell-expressed human antibody was determined by ELISA with Puumala virus-infected cell lysates and by immune-precipitation of radiolabeled Puumala virus proteins. The expressed IgG retained the ability to neutralize Puumala virus in plaque-reduction neutralization assays. Using competitive polymerase chain reaction methods, multiple copies of integrated heavy- and light-chain antibody genes were detected in the insect cell genome. The transformed insect cells were stable and continuously expressed biologically active IgG. We conclude that this methodology provides an alternative eukaryotic source for the generation of human antibodies.

Hein, W. R., T. Barber, et al. (2004). "Long-term collection and characterization of afferent lymph from the ovine small intestine." Journal of Immunological Methods **293**(1-2): 153.

<http://www.sciencedirect.com/science/article/B6T2Y-4D97WW4-1/2/1bfc1c1ee17cd5d7603cce4def7f70ec>

Reliable methods for long-term collection of afferent lymph draining from the small intestine of sheep are described and validated. The procedure was used successfully in normal sheep, in animals infected experimentally with the parasitic intestinal nematode *Trichostrongylus*

colubriformis and in animals infected naturally with *Mycobacterium avium* subsp. paratuberculosis, the causative agent of Johne's disease. Our approach enabled afferent lymph draining from the small intestine to be collected continuously for up to 4 months, without any detrimental effects on the animals. Based on cytokine gene expression profiles of afferent intestinal lymph cells, the two infections induced contrasting regional immune responses, namely, Th2-type immunity in the case of *T. colubriformis* infection and Th1-type immunity in natural cases of Johne's disease. Some immune parameters differed markedly between the two disease models, highlighting the potential value of this approach to gain real-time insights into distinctive host-pathogen interactions as they occur in vivo within the regional immune system of the gastrointestinal tract.

Huang, S.-K., D. M. Essayan, et al. (1994). "Detection of allergen- and mitogen-induced human cytokine transcripts using a competitive polymerase chain reaction." Journal of Immunological Methods **168**(2): 167.

<http://www.sciencedirect.com/science/article/B6T2Y-476KR7K-55/2/8bf55c556b4cf5c532d3984eabffb86c>

Human cytokines, IL-4, IL-5, and IFN- γ play an important role in the regulation of IgE synthesis and atopic diseases. In this communication, we describe the development of a quantitative assay of steady-state cytokine mRNAs (IL-4, IL-5, and IFN- γ) from a variety of cell sources, including peripheral blood mononuclear cells (PBMCs) stimulated with either a mitogen (PHA) or ragweed pollen allergen extract, and cells from allergen-challenged inflammatory sites. Quantitative analysis of IL-5, IL-4 and IFN- γ transcripts was achieved by a competitive reverse transcription-polymerase chain reaction (RT-PCR) technique using internal standard (IS) cRNAs in the presence of specific oligonucleotide primers. Each IS was generated from a plasmid vector containing the respective cytokine cDNA modified by insertion with an SV40-DNA fragment. Both test RNA and IS were reverse-transcribed and subjected to the 'competitive' PCR in the same tube. We first demonstrate the linearity and reproducibility of this technique; second, we apply this competitive PCR assay to analyze quantitatively the expression of IL-4, IL-5, and IFN- γ transcripts in PBMCs before and after stimulation with PHA or crude ragweed allergen. Finally, we analyzed cells isolated from the lung lavage fluids of an atopic subject following allergen challenge, and showed a significant increase of IL-4 and IL-5 transcripts, but not IFN- γ , in the allergen-challenged site when compared to the control. This technique of PCR quantitation provides an easy and efficient tool to study the expression of cytokine genes in allergic inflammatory diseases.

Jena, P. K., A. H. Liu, et al. (1996). "Amplification of genes, single transcripts and cDNA libraries from one cell and direct sequence analysis of amplified products derived from one molecule." Journal of Immunological Methods **190**(2): 199.

<http://www.sciencedirect.com/science/article/B6T2Y-3VXJJDJ-6/2/e7e482b90d1d7358116b3ee9302247ab>

We report a procedure to generate and amplify cDNA libraries and to amplify and sequence genes and single RNA transcript molecules from the same cell without cloning. An absence of cloning steps minimizes potential sources of contamination, which can be especially problematic when working at the single cell level. Potential contamination is further reduced by an absence of any purification step prior to PCR amplification. Amplifications are designed to minimize the production of aberrant molecules in favor of full-length products, which is especially advantageous when generating cDNA libraries. Genes are amplified from isolated single nuclei, which are segregated from cytoplasmic lysates by microcentrifugation. Specific cDNA, total cDNA

or both are synthesized from aliquots of the cytoplasmic lysate, and single cDNA molecules are isolated from others of the same species by limiting dilution prior to PCR amplification. In this way, the frequency of amplified products provides for a direct calculation of cDNA copy number by a Poisson analysis. Incorporation errors by Taq DNA polymerase occur at a low frequency and can be eliminated by sequencing independently amplified cDNA molecules from the same cell. Single molecule amplifications provide sufficient material for numerous (~ 150) direct DNA sequencing reactions. The limiting dilution approach also permits sequence information to be obtained from a single cDNA, when highly related transcripts derived from distinct genes are present in the same cell and simultaneously amplified with the same primers. In sum, this method provides for a maximum amount of nucleic acid information to be extracted from one cell. It has a wide range of applications to studies of the immune system where, to a first approximation, each lymphocyte has a unique receptor identity, where specific states of differentiation may be difficult to assess in a mixed cell population, and where cell immortalization procedures are not always possible nor practical.

Klebs, S. H. G., M. W. Hoffmann, et al. (2003). "Detection of H-2 alleles by PCR-RFLP--an alternative to flow cytometry in the screening of transgenic mice." Journal of Immunological Methods **276**(1-2): 197.

<http://www.sciencedirect.com/science/article/B6T2Y-4816W80-1/2/7b4725a7aba1f71722b0905da3fcef36>

An increasing number of experimental models is based on well-defined transgenic mice in medical and biological research. Particularly in settings in which transgenic recombinants are used, a fast and reliable method is needed to screen for a defined H-2 background. For this purpose, flow cytometry with specific monoclonal antibodies is the standard procedure. However, epitopes of closely related rodent strains show only minor variations affecting the production of specific discriminating antibodies. Therefore, cross-reactivity of antibodies against specific major histocompatibility complex (MHC) leads to unreliable results in settings with closely related strains. In need of a method with high reliability, we have designed a screening assay based on polymerase chain reaction (PCR) followed by restriction fragment length polymorphisms (RFLP) to discriminate the MHC class I antigens H-2Kd, -Kb, -Kk, which are sequence variants of the H-2K gene. A part of the mus musculus MHC gene coding for H-2K--covering exons 4 and 5 with MHC-differentiating restriction sites--was amplified. Subsequent restriction digest of the PCR products allows to discriminate the three aforementioned alleles and to identify homozygous as well as heterozygous haplotypes. To distinguish transgenic mice defined by certain MHC backgrounds, the PCR-RFLP method is simple, cost-effective, specific, and reliable and can be used independently or in addition to other methods in any laboratory.

Kotake, S., J. H. R. Schumacher, et al. (1996). "A simple nested RT-PCR method for quantitation of the relative amounts of multiple cytokine mRNAs in small tissue samples." Journal of Immunological Methods **199**(2): 193.

<http://www.sciencedirect.com/science/article/B6T2Y-3W2XNM3-C/2/69e89ff57295a77936e0385298f4dc41>

It is difficult to quantitate cytokine mRNA profiles in small human tissue specimens obtained by a needle biopsy, even using standard RT-PCR methods, because the amount of mRNA in the specimens is very small. To address this problem, we developed highly sensitive, quantitative, nested RT-PCR techniques to evaluate the expression of multiple cytokine mRNAs in synovial specimens obtained by needle biopsy. To reduce effects of variation of initial RNA concentrations, cDNA from each target RNA sample was normalized, using a simplified

competitive PCR method, to the levels of [beta]-actin cDNA. The first and the second (nested) PCR were performed in the same tube to prevent contamination. The number of PCR-product bands, evident on polyacrylamide gel electrophoresis, was used to quantitate the relative amounts of target cDNA. Using our methods, it was possible to evaluate, in a single synovial tissue specimen obtained by needle biopsy, the relative amounts of mRNAs for 10 cytokines (TNF-[alpha], IL-1[beta], IL-2, IL-4, IL-6, IL-10, IL-12 p40, IL-13, IL-15, IFN-[gamma]) and CD3 [delta] chain. Our methods are particularly valuable if there are multiple target mRNAs, numerous samples, or if the amounts of mRNAs are limited. The methods are applicable to a wide variety of tissues and target mRNAs.

Kramer, K. (2002). "Synthesis of a group-selective antibody library against haptens." Journal of Immunological Methods **266**(1-2): 209.

<http://www.sciencedirect.com/science/article/B6T2Y-45TTVWR-5/2/588d46538d72d8569f5988a74b6ad631>

A generic strategy is described for the generation of libraries comprising hapten-selective antibody genes against a group of structurally related low molecular weight target molecules. Hapten antibody libraries are frequently suffering from high background levels of irrelevant antibody genes as a consequence of the immunization, where the small non-immunogenic target molecule is coupled to a large immunogenic carrier protein. In order to elevate the percentage of hapten-specific genes in the library, B cells harboring antibody genes against the group of triazine herbicides were enriched from 21 individual splenocyte populations by means of immunomagnetic separation (IMS). IMS utilizes the specific binding of membrane-associated immunoglobulin receptors on the B cell surface to hapten-coated paramagnetic beads. The variable genes of the specifically enriched subpopulation were cloned into a phagemid vector. The corresponding library yielded up to 75% triazine binding antibody clones after three rounds of phage selection. At least half of these antibodies (abs) were displaceable by triazines resulting in quantitative assays with nanomolar sensitivities. In contrast, no displaceable clone was obtained at the same selection level in a control library, where IMS was omitted. Due to the elevated percentage of relevant antibody genes, the library can be utilized either for the direct isolation of functional antibodies against various triazine herbicides or as group-specific gene source for evolutionary antibody optimization.

Kruse, N., M. Pette, et al. (1997). "Quantification of cytokine mRNA expression by RT PCR in samples of previously frozen blood." Journal of Immunological Methods **210**(2): 195.

<http://www.sciencedirect.com/science/article/B6T2Y-3SDC31Y-8/2/936b9b326cc81cea002df79555ac6838>

In order to facilitate cytokine mRNA detection in blood cells, we have developed a highly reproducible and easily performed RNA isolation method for use with whole blood. Previously frozen human whole blood samples were lysed in guanidine thiocyanate solution to isolate total RNA. After reverse transcription a PCR method was applied to detect [beta]-actin and cytokine mRNA expression (interleukin-(IL)2, IL4, IL10, tumor necrosis factor alpha (TNF[alpha]) and interferon gamma (IFN[gamma])). The presence of cDNA was confirmed by agarose gel electrophoresis and quantitated on-line using sequence-specific fluorochrome labeled internal oligonucleotide probes. This quantitative method is based on the cleavage of fluorescent dye labeled probes by the 5'->3' endonuclease activity of the Taq DNA polymerase during PCR and measurement of fluorescence intensity by a Sequence Detector System. The signal generated was directly proportional to the starting copy number of target molecules in the sample over 6 log concentrations and quantitative analysis of cDNA concentrations was performed in comparison to

[beta]-actin or cytokine cDNA standards. mRNAs coding for [beta]-actin and TNF[alpha] were readily detectable in cDNAs prepared from the whole blood of eight healthy donors, while the other cytokines were expressed in lower amounts (IFN[gamma], IL10) or were undetectable (IL2, IL4). The assay described is highly reproducible, requires no post PCR manipulation of the amplicons and permits the analysis of several hundred PCR reactions per day. Using this method it is possible to detect and quantify cytokine mRNA expression reliably in small amounts of previously frozen blood even after storage of samples for at least several months.

Lai, J.-P., S. D. Douglas, et al. (1999). "Quantification of substance P mRNA in human mononuclear phagocytes and lymphocytes using a mimic-based RT-PCR." Journal of Immunological Methods **230**(1-2): 149.

<http://www.sciencedirect.com/science/article/B6T2Y-3Y8N90D-H/2/d74bda068a31ad7384a9fb9ad56ce444>

We have recently demonstrated that human monocytes and lymphocytes express the substance P (SP) gene at both the mRNA and protein level [Ho, W.Z., Lai, J.P., Zhu, X.H., Uvaydova, M., Douglas S.D., 1997. Human monocytes and macrophages express substance P and neurokinin-1 receptor. *Journal of Immunology*, 159, p. 5654; Lai, J.P., Douglas, S.D., Ho, W.Z., 1998. Human lymphocytes express substance P and its receptor. *Journal of Neuroimmunology*, 86, p. 80; Lai, J.-P., Douglas, S.D., Rappaport, E., Wu, J., Ho, W.-Z., 1998. Identification of a delta isoform of preprotachykinin mRNA in human mononuclear phagocytes and lymphocytes. *Journal of Neuroimmunology*, 91, p. 121]. Using RT-PCR assay with several specific human SP primer pairs, we were able to differentiate four isoforms of preprotachykinin (PPT-A, the SP precursor) mRNA transcripts on ethidium bromide-stained agarose gels and clone the PCR amplified cDNA of the four isoforms ([alpha], [beta], [gamma], and [delta]) of the PPT-A gene. In an effort to quantitatively measure PPT-A mRNA levels, we have developed a mimic-based RT-PCR assay to analyze total PPT-A mRNA levels in human monocytes and lymphocytes. We designed a specific human SP primer pair (HSP4/HSP3) to amplify a single fragment of cDNA derived from all four isoforms of PPT-A mRNA transcripts, with a sensitivity of 120 molecules per reaction. Thus the PPT-A mRNA transcripts in an unknown sample can be quantitatively analyzed using the mimic-based RT-PCR. The accuracy and reproducibility of this assay were confirmed by the plasmids containing [alpha], [beta], [gamma] and [delta] cDNA inserts and by in vitro synthesized mRNA from a plasmid containing [beta] isoform cDNA insert. Our data indicate that the SP mimic-based RT-PCR assay has potential advantages in studies of SP levels in a variety of human cells as well as in clinical specimens.

Lilley, G. G., O. Dolezal, et al. (1994). "Recombinant single-chain antibody peptide conjugates expressed in *Escherichia coli* for the rapid diagnosis of HIV." Journal of Immunological Methods **171**(2): 211.

<http://www.sciencedirect.com/science/article/B6T2Y-476D471-HJ/2/24fb8737afddf906d0addd04fb5a02e3>

Recombinant single chain Fv (scFv) antibody fragments can form the basis of a rapid, whole-blood diagnostic assay. The scFv described in this study is derived from a monoclonal antibody which has a high affinity for glycophorin A, an abundant glycoprotein on the human red blood cell membrane surface. The prototype reagent built around the scFv was designed to detect, in whole blood samples, the presence of antibodies that have arisen through infection with a foreign organism such as human immunodeficiency virus. The scFv was composed of the antibody heavy-chain variable domain (Vh) joined by a 15 residue linker-(GGGGS)³- to the light-chain variable domain (Vl) terminated by either a C-terminal octapeptide tail (FLAG) or a 35 amino acid segment from the gp41 surface glycoprotein of HIV-1. Constructs were cloned into a *Escherichia*

coli expression vector, pHFA, and expressed in a soluble form into culture supernatant. The product retained anti-glycophorin activity which could be detected directly in culture supernatants by ELISA. Furthermore, the scFv-epitope fusion functioned efficiently in the whole blood agglutination assay and was able to distinguish between HIV-1 positive and negative sera.

Lim, A., V. Baron, et al. (2002). "Combination of MHC-peptide multimer-based T cell sorting with the Immunoscope permits sensitive ex vivo quantitation and follow-up of human CD8+ T cell immune responses." Journal of Immunological Methods **261**(1-2): 177.

<http://www.sciencedirect.com/science/article/B6T2Y-451NPPT-1/2/8d59b4e3613d27d24746e53ace1b5b55>

Identification of MHC-restricted antigens and progress in the induction and control of adaptive cytotoxic immune responses have led to renewed interest in immunotherapy as a treatment for severe pathologies such as cancer and autoimmune diseases. Reliable procedures for detecting and monitoring T cell responses induced by the treatment throughout a clinical trial are needed in order to design rational protocols with increased efficiency. We have attempted to develop such a procedure by combining T cell sorting using HLA-peptide complexes multimerized on magnetic beads together with the quantitative Immunoscope approach. Once a recruited patient has been typed for HLA and target antigens, relevant HLA-peptide multimers can be selected and used for sorting specific peripheral T cells prior to any treatment and at the peak of the expected response to treatment. Clonotypic primers specific for the TCR rearrangements of the specific T cell clones can then be designed and used for measuring the frequency of their TCR transcripts by quantitative PCR on blood samples or T cell subsets throughout the trial. In reconstruction experiments as well as in samples from one rheumatoid arthritis patient, we were readily able to detect and follow several T cell clones with a frequency as low as 10^{-5} among CD8+ T cells. The main advantages of this procedure over other currently available assays are that it does not require any assumptions on the functional status of the specific T cells and it permits the monitoring of individual T cell clones whose phenotypic shift can thus be evaluated.

Litton, M. J., B. Sander, et al. (1994). "Early expression of cytokines in lymph nodes after treatment in vivo with Staphylococcus enterotoxin B." Journal of Immunological Methods **175**(1): 47.

<http://www.sciencedirect.com/science/article/B6T2Y-476MXWH-16H/2/9b850b45027b1664267ef98f2d4f3704>

Excessive cytokine expression induced by superantigen may be one aspect of the pathophysiology associated with Gram positive bacteremia. We have undertaken a study of the kinetics of cytokine production in lymph nodes obtained from in vivo Staphylococcus enterotoxin B (SEB) treated animals. This study was designed to evaluate the short term cytokine profile observed using immunohistochemistry (IHC) in BALB/c mice injected intraperitoneally (i.p.). The observed immunohistochemical kinetic profiles were corroborated using reverse transcription-polymerase chain reaction (RT-PCR) RNA analysis. We report here that TNF, IL-2, and IFN- γ are the principal cytokines which were detected within hours of SEB administration, and that other cytokines such as IL-3, IL-4, IL-5, IL-6, IL-10, GM-CSF and M-CSF were undetectable. TNF and IL-2 appeared very early following SEB priming, and were observed by 1 h. IFN- γ which appeared later (maximally at 14 h) was produced predominantly by CD8+ cells. In contrast, the TNF and IL-2 were produced primarily by CD4+ cells. Identical results were obtained by IHC and RT-PCR; the kinetics of mRNA expression slightly preceded the appearance of protein. The TNF and IFN- γ staining patterns observed in lymph node sections were indicative of Golgi-localized cytokine. The IL-2 staining pattern observed in lymph node sections was distinctive, covering a significant local area of cells. This local regional concentration of IL-2,

which may result from cytokine attached to extracellular binding components, may be an important aspect of the activation phase of a developing immune response. Rapid induction and excessive cytokine production elicited by superantigen in vivo, may ultimately help to explain the shock and death associated with SEB.

Loseke, S., E. Grage-Griebenow, et al. (2003). "Differential expression of IFN-[alpha] subtypes in human PBMC: evaluation of novel real-time PCR assays." Journal of Immunological Methods **276**(1-2): 207.

<http://www.sciencedirect.com/science/article/B6T2Y-483BN53-1/2/216383c3f6377e8658a3b112f8674ff9>

Studies of the human IFN-[alpha] subtype system have been hampered by the lack of efficient procedures to quantify and differentiate the expression of the highly homologous IFN-[alpha] subtypes. Here we evaluate four novel real-time PCR assays for the specific detection and quantification of IFN-[alpha] mRNA for the subtypes [alpha]2, [alpha]6, [alpha]8 and [alpha]1/13 in a combined assay in human peripheral blood mononuclear cells (PBMC). This included (a) the selection of [beta]-glucuronidase (GUS) as a suitable housekeeping gene for relative quantification; (b) verification of the specificity by using human DNA of different IFN-[alpha] subtypes; and (c) comparison of the amplification efficiencies among the different assays. This highly sensitive method allows the detection of low-level, constitutive IFN-[alpha] mRNA and shows differences in the composition of constitutive IFN-[alpha] subtypes compared to other cell types (HeLa and HEp-2). The in vitro stimulation of PBMC with Newcastle disease virus (NDV), Respiratory syncytial virus (RSV) or an inactivated Herpes simplex (HSV) preparation leads to the transcriptional induction of all IFN-[alpha] subtypes investigated but to different expression levels. Among the subtypes detected, IFN-[alpha]13/1 and [alpha]2 are the major transcripts followed by [alpha]8, and finally [alpha]6 as a minor transcribed subtype. Time-kinetics of IFN-[alpha] transcriptional activation also revealed variations in the course of IFN-[alpha] transcription between NDV, RSV or HSV. The data obtained from the real-time PCR assays correlated well with IFN-[alpha]2 protein release. In conclusion, we have demonstrated the suitability and reliability of new real-time PCR assays for the rapid and efficient analysis of IFN-[alpha] subtype expression.

Manfras, B. J., W. A. Rudert, et al. (1997). "Analysis of the [alpha]/[beta] T-cell receptor repertoire by competitive and quantitative family-specific PCR with exogenous standards and high resolution fluorescence based CDR3 size imaging." Journal of Immunological Methods **210**(2): 235.

<http://www.sciencedirect.com/science/article/B6T2Y-3SDC31Y-D/2/f610b6d3366057ae3575e39879797334>

The characterization of the human T-cell receptor (TCR) repertoire in various physiological and pathological conditions has become an important tool in studies of the immune response. Therefore, a number of PCR based strategies for the semiquantitative analysis of the TCR repertoire have been described. Family specific amplification of TCR cDNA has been employed in a number of studies often with contradictory results. We have developed a strategy utilizing exogenous standards with homologous primer binding sites for the quantitative analysis of the [alpha]/[beta] T-cell receptor repertoire. This system allows the detection of even minute differences in T-cell populations based on quantitative PCR (Q-PCR) and competitive PCR (C-PCR). Results presented here demonstrate that expansions of T-cell subsets as defined by the specificity of the variable gene segments can be readily monitored when exceeding 1% of the total repertoire. In addition, the proposed method reveals direct information of CDR3 size heterogeneity and can be used to estimate the T-cell repertoire complexity and monitor clonal

expansions. We discuss variables such as cell number and experimental conditions influencing accuracy and reproducibility of the analyses. We have used this protocol based on non-radioactive techniques for characterization of the fine specificity of the T-cell repertoire in peripheral and organ-infiltrating T-lymphocytes. The analyses revealed information about polyclonal or clonal expansion of T-cells in vivo and in vitro following various stimuli such as superantigenic stimulation of T-cell subsets as well as antigen-driven shaping of the [alpha]/[beta] T-cell repertoire in autoimmune and infectious diseases.

McCarthy, B. J. and A. S. Hill (2001). "Altering the fine specificity of an anti-Legionella single chain antibody by a single amino acid insertion." Journal of Immunological Methods **251**(1-2): 137.

<http://www.sciencedirect.com/science/article/B6T2Y-42PBYP5-G/2/ea0d4902cf877753f22ecc04b91c4fd3>

Antibody engineering provides the potential to clone and manipulate antibody genes to produce fragments with altered specificity. We have produced an anti- Legionella single chain fragment with broader specificity towards Legionella serotypes than the parent monoclonal antibody. Using this relationship between the parent monoclonal and the recombinant antibody derived from it as a model, we attempted to identify those residues responsible for this change in fine specificity. Sequence analysis of this recombinant antibody revealed the deletion of a conserved residue, Asp101, in the CDR-H3 region. Using site-directed mutagenesis, we have created a mutant form of this single chain fragment with an aspartic acid insertion mutation at position 101 of the antibody heavy chain. This mutant scFv demonstrates improved specificity compared to the wild-type recombinant antibody, indicating an important role for Asp101.

Morimoto, K., M. J. Schnell, et al. (2001). "High level expression of a human rabies virus-neutralizing monoclonal antibody by a rhabdovirus-based vector." Journal of Immunological Methods **252**(1-2): 199.

<http://www.sciencedirect.com/science/article/B6T2Y-42WYG14-R/2/f1df8a7b30af3370bbd317029adc3f81>

Humans exposed to rabies virus must be promptly treated by passive immunization with anti-rabies antibody and active immunization with rabies vaccine. Currently, antibody prepared from pooled human serum or from immunized horses is utilized. However, neither of these reagents are readily available, entirely safe, or consistent in their biological activity. An ideal reagent would consist of a panel of human monoclonal antibodies. Such antibodies are now available, their only drawback being the cost of production. Using recombinant technology, we constructed a rabies virus-based vector which expresses high levels (~60 pg/cell) of rabies virus-neutralizing human monoclonal antibody. The vector is a modified vaccine strain of rabies virus in which the rabies virus glycoprotein has been replaced with a chimeric vesicular stomatitis virus glycoprotein, and both heavy and light chain genes encoding a human monoclonal antibody have been inserted. This recombinant virus can infect a variety of mammalian cell lines and is non-cytolytic, allowing the use of cell culture technology routinely employed to produce rabies vaccines.

Mougin, B., P. Garnero, et al. (2001). "A routine assay for the direct analysis of HLA-DR-related shared epitope and B27 alleles in chronic inflammatory arthritis." Journal of Immunological Methods **256**(1-2): 47.

<http://www.sciencedirect.com/science/article/B6T2Y-43RVDJ7-5/2/ed00cb685eb8f12d92d948dd88650dbe>

Knowledge of the genetic background of patients with inflammatory arthritis may be useful for disease management. The main markers are the HLA-DR-associated Shared Epitope (SE) for Rheumatoid Arthritis (RA) and HLA-B27 for ankylosing spondylitis. We have developed a simple molecular biology-based test to provide this essential information. HLA targets are amplified by polymerase chain reaction (PCR), then simultaneously analyzed using 16 individual hybridization reactions in two 8-well ELISA strips with colorimetric detection. Concordance was evaluated using a cohort of RA patients with known genotype. Using this new assay, 100% concordance was observed with conventional genotyping in RA patients both for HLA-DR SE and B27 genotypes. Seventy-three percent of the patients with destructive RA had at least one susceptible allele within SE, compared to 38% of those patients with non-destructive disease. This new assay, which requires minute amount of blood, could be used to determine the genetic background of inflammatory arthritis, particularly in non-specialized settings and for large-scale clinical trials.

Okochi, E., T. Iwahashi, et al. (1995). "Establishment and evaluation of MRK16-magnetic cell sorting assays for detecting low expression of multidrug resistance P-glycoprotein using human leukemia cell lines and peripheral blood cells from healthy donors." Journal of Immunological Methods **187**(1): 127.

<http://www.sciencedirect.com/science/article/B6T2Y-3YB516Y-1W/2/c54687020eb0488e43f8555492e6c1da>

Two types of magnetic cell sorting assays, termed MRK16-MACS and MRK16-MACS-FACS, have been established to detect low expression level of P-glycoprotein (P-gp) using a monoclonal antibody MRK16, which recognizes a cell surface epitope of P-gp. With K-562 and U-937 cell lines, which are known to express low levels of P-gp and hence routinely used as negative control cell lines in conventional flow cytometry, both assays gave significantly positive reactivities indicating improved specificity and sensitivity of these assays. The findings in the dilution test, where P-gp-positive cells were added to P-gp-negative cells at various ratios, demonstrated that the MRK16-MACS assay is quantitative and capable of detecting small numbers of P-gp-positive cells as few as 2.5% of the total cells tested. Furthermore, specific enrichment of P-gp-expressing cells in magnetic cell sorting assays was verified by reverse transcription-polymerase chain reaction (RT-PCR) analysis and functional assay for P-gp with Rhodamine 123. The availability of such magnetic cell sorting assays offer an approach to quantitate low level of P-gp expression.

Pichyangkul, S., P. Saengkrai, et al. (2001). "Isolation and characterization of rhesus blood dendritic cells using flow cytometry." Journal of Immunological Methods **252**(1-2): 15.

<http://www.sciencedirect.com/science/article/B6T2Y-42WXXG14-2/2/e9822a5edc45506a37858dcacb627cfa>

Recognition of dendritic cells (DCs) as initiators and modulators of immune responses and growing use of rhesus monkeys for the preclinical optimization of vaccine formulations prompted characterization of the phenotype and function of isolated rhesus peripheral blood DCs. We developed a flow cytometric method to directly identify and isolate DCs from rhesus peripheral blood whereby a T cell depleted population negative for CD3, CD14, CD16 and CD20 but positive for CD83 yielded a cell population with surface markers, morphology, and a cytokine profile similar to human myeloid DCs. Rhesus blood DCs were more effective than monocytes and B

cells in mixed lymphocyte reactions and in the presentation of recombinant malaria blood stage antigen MSP-1(42) to autologous T cells. The ability to isolate rhesus blood DC from peripheral blood should be a useful tool for immunological investigations.

Snowwhite, I. V., W. E. Jones, et al. (2002). "Comparative analysis of methods for collection and measurement of cytokines and immunoglobulins in cervical and vaginal secretions of HIV and HPV infected women." Journal of Immunological Methods **263**(1-2): 85.

<http://www.sciencedirect.com/science/article/B6T2Y-45F92S9-1/2/893b8b34f2070014867ec417719efc54>

The goal of these studies was to distinguish which of two techniques [cervicovaginal lavage (CVL) and cervical wick (SS)] is the optimal collection method for the measurement of the local immunological response in human papillomavirus (HPV) and HIV infected women. The following parameters were measured in 24 paired samples from 15 women (9 HIV+, 6 HIV-): total protein, immunoglobulin levels, HPV-specific antibodies, and Th1-Th2 cytokines. In addition, relative mRNA levels from CVL cell pellets were compared to protein levels from CVL supernatants. The total protein (2-fold) and IgG concentration (10-fold) are higher in the SS samples, were reproducible (%CVPr>0.28, P18) failed to yield reproducible results for the cytokine assays as compared to the CVL (%CV<5.0). Furthermore, no correlations were found between relative mRNA levels from CVL cell pellet and cytokine protein levels in CVL supernatants. The CVL sample's superior reproducibility in the cytokine assays makes this the better collection method. In addition, cytokine protein level's failure to correlate with mRNA suggests tight regulation of cytokine genes or production from a different cell population.

Tanha, J., G. Dubuc, et al. (2002). "Selection by phage display of llama conventional VH fragments with heavy chain antibody VHH properties." Journal of Immunological Methods **263**(1-2): 97.

<http://www.sciencedirect.com/science/article/B6T2Y-458PF43-2/2/dc88d7722b7bc8b90caf13398e5f3d96>

A llama single domain antibody (dAb) library designed and constructed to contain only heavy chain antibody variable domains (VHHs) also contained a substantial number of typical conventional antibody heavy chain variable sequences (VHs). Panning the library against two carbohydrate-specific antibodies yielded anti-idiotypic dAbs and enriched solely for sequences from the VH subpopulation of the library. The conventional antibody origin of these VHs was confirmed by using oligonucleotide probes, specific for the enriched VHs, to identify the parental sequences in the message employed in library construction. Surprisingly, these VH dAbs, which are produced in high yield in *Escherichia coli*, are highly soluble, have excellent temperature stability profiles and do not display any aggregation tendencies. The very close similarity of these molecules to human VHs makes them potentially very useful as therapeutic dAbs.

Taylor, K. M., J. Paul Luzio, et al. (1994). "A method for in vitro synthesis of unglycosylated recombinant complement component C9." Journal of Immunological Methods **167**(1-2): 129.

<http://www.sciencedirect.com/science/article/B6T2Y-476M0BJ-36R/2/485e59d1d72eb25cfa1a113b06daa5ba>

A method for in vitro synthesis of human complement component C9 has been established in

order to generate unglycosylated normal and mutant proteins without the need to sub-clone. One or two step polymerase chain reaction (PCR) was used to add the T7 RNA polymerase promoter and introduce multiple mutations within the cDNA. The cDNA was then transcribed by T7 RNA polymerase and the mRNA translated in a rabbit reticulocyte lysate or wheat germ system. Successful synthesis was confirmed by: the correct size of PCR product DNA on agarose gel electrophoresis, incorporation of [α]- ^{32} P]UTP into mRNA, and formation of [^{35} S]methionine-labelled protein of the correct molecular mass for full length C9. The wheat germ extract generated up to 1.5 [μ]g of recombinant C9. This unglycosylated C9 had at least 10% of the haemolytic activity of native C9. Unglycosylated C9 polymerised more readily than the native protein. This spontaneous polymerisation was increased by removal of the first 23 amino acids or mutating two cysteines at positions 33 and 36. This therefore provides a rapid method for screening the effect of multiple mutations on the biological activity and polymerisation of pore forming proteins.

Theilgaard-Monch, K., J. Cowland, et al. (2001). "Profiling of gene expression in individual hematopoietic cells by global mRNA amplification and slot blot analysis." Journal of Immunological Methods **252**(1-2): 175.

<http://www.sciencedirect.com/science/article/B6T2Y-42WYG14-N/2/1ed418ccb508e49b8054feb7457f076>

The pattern of expressed genes defines the structure and functional status of cells. Currently, most methods used in gene expression studies depend on large numbers of cells. Thus, their application may be hampered by the heterogeneity of cell populations, and by the low numbers of cells obtainable from in vivo sources. Such drawbacks may be overcome by methods suitable for the profiling of gene expression at the single cell level. We studied whether polymerase chain reaction (PCR) products synthesized from individual cells by global amplification of messenger RNA (mRNA) were suitable as probes for gene expression analysis. For this purpose, cells were subjected to reverse transcription and PCR using sequence independent primers (SIP RT-PCR). The resultant cDNA products were radiolabeled and hybridized to cDNA clones arrayed on a nylon membrane by vacuum slot blotting (a method referred to as slot blot analysis). The SIP RT-PCR procedure was reproducible and allowed the detection of twofold changes in input RNA copies per cell (range: 80-10,000 copies of an in vitro transcribed poly(A)-tailed RNA/cell). Analysis of total RNA and amplified cDNA, obtained from neutrophil granulocytes and the promyelocytic HL-60 cell line, demonstrated comparable gene expression profiles as measured by Northern blot and slot blot analysis. Slot blot analysis of HL-60 cells indicated that individual cells from an apparently homogenous population have varying expression of specific transcripts, which all contribute to the mRNA phenotype of their population. Interestingly, the genes that were detected in some but not all individual HL-60 cells were those found to peak within 2 days of retinoic acid-induced granulocytic differentiation. This study demonstrates the potential of cDNA, synthesized from individual cells by global amplification of mRNA, as probes for cDNA arrays.

Tseng, L.-H., P.-J. Chen, et al. (2002). "Simultaneous genotyping of single nucleotide polymorphisms in the IL-1 gene complex by multiplex polymerase chain reaction-restriction fragment length polymorphism." Journal of Immunological Methods **267**(2): 151.

<http://www.sciencedirect.com/science/article/B6T2Y-460DMW3-2/2/2be180e41b938e530735feaffa93c1b9>

The interleukin-1 (IL-1) gene complex consists of the IL-1[α], IL-1[β] and IL-1 receptor antagonist genes. Single-nucleotide polymorphisms (SNP) in all three genes have been associated with human diseases. In this study, primers containing mismatches at 1-3 nucleotide

positions were designed to incorporate a restriction site for endonuclease AlwNI or XcmI in the presence of allele-specific nucleotides at the polymorphic positions. Based on this technique, a simple and robust multiplex polymerase chain reaction/restriction fragment length polymorphism (multiplex PCR/RFLP) assay was developed to determine simultaneously three to four informative SNPs (IL-1[β]/+3954, IL-1[β]/-511 and IL-1Ra/9261 or IL-1[α]/-889, IL-1[β]/-31, IL-1[β]/5810 and IL-1Ra/11100 SNPs) in the IL-1 gene complex.

Udby, L., J. B. Cowland, et al. (2002). "An ELISA for SGP28/CRISP-3, a cysteine-rich secretory protein in human neutrophils, plasma, and exocrine secretions." Journal of Immunological Methods **263**(1-2): 43.

<http://www.sciencedirect.com/science/article/B6T2Y-45BCSNG-1/2/19928f97a294f1e258e796a786fbabf0>

Specific granule protein of 28 kDa (SGP28), also termed cysteine-rich secretory protein 3 (CRISP-3), is a glycoprotein that belongs to a family of cysteine-rich secretory proteins (CRISPs). SGP28 was originally discovered in human neutrophils, but transcripts are widely distributed in exocrine glands (salivary glands, pancreas, and prostate) and also found at lower levels in epididymis, ovary, thymus, and colon. The function of SGP28/CRISP-3 is not yet known. Similarities to pathogenesis-related proteins in plants and the expression in neutrophils and exocrine glands suggest that SGP28/CRISP-3 may play a role in innate host defense. We describe here the production of a recombinant, C-terminally truncated form of CRISP-3 (rCRISP-3[Δ]) and the generation of polyclonal antibodies against rCRISP-3[Δ] that are useful in immunoblotting and immunocytochemistry. We present a specific, accurate, and reproducible enzyme-linked immunosorbent assay (ELISA) for the measurement of CRISP-3 with a detection limit of 2 ng/ml. We further demonstrate the presence of CRISP-3 protein in human plasma (6.3 [μg/ml]), saliva (21.8 [μg/ml]), seminal plasma (11.2 [μg/ml]), and sweat (0.15 [μg/ml]), and describe the coexistence of two different molecular weight forms of CRISP-3, representing an N-glycosylated and a non-glycosylated form of the mature protein.

van Koningsbruggen, S., H. de Haard, et al. (2003). "Llama-derived phage display antibodies in the dissection of the human disease oculopharyngeal muscular dystrophy." Journal of Immunological Methods **279**(1-2): 149.

<http://www.sciencedirect.com/science/article/B6T2Y-48V7RJ0-2/2/e9d09b00f28394b95f8e074381de24cc>

Functional analysis of the estimated 30,000 genes of the human genome requires fast and reliable high-throughput methods to study spatio-temporal protein dynamics. To explore the suitability of heavy-chain antibodies (HCAbs) for studying mechanisms underlying human disease, we used oculopharyngeal muscular dystrophy (OPMD) as a paradigm for the expanding group of protein aggregation disorders that is characterized by subcellular dislocalization and aggregation of mutant protein. OPMD is caused by a moderate alanine expansion in the poly-A binding protein nuclear 1 (PABPN1) and is associated with intranuclear PABPN1 deposition exclusively in muscle. An experimental approach was designed in which the primary sequence of the PABPN1 gene was employed for generating a prokaryotic expression construct that permitted its expression in the host *Escherichia coli*. The purified product was used for immunization of a llama as well as for the selection of an antigen-specific antibody fragment from the derived phage display library. This single-domain antibody was able to recognize the native gene product in mammalian cell lines and in human muscle tissue by immunocytochemical, immunohistochemical and immunoblot analysis. Our results suggest that phage display derived heavy-chain antibodies can be used in proteomics to study the localization and function of hypothetical gene products,

relevant to human disease.

VanderBorgh, A., A. Van der Aa, et al. (1999). "Identification of overrepresented T cell receptor genes in blood and tissue biopsies by PCR-ELISA." Journal of Immunological Methods **223**(1): 47.

<http://www.sciencedirect.com/science/article/B6T2Y-3VM79Y0-5/2/ccb84eff0224f7c7efc71aeb9c261129>

The analysis of T cell receptor variable (TCR V) gene repertoires in blood or tissues may provide important information when studying immunopathological mechanisms. The overexpression of a TCR gene may indicate the expansion of the corresponding T cell subset. In autoimmune diseases, clonally expanded T cell subsets in the affected organs may represent pathogenic lymphocytes. We describe a simple, rapid and sensitive method to determine the TCR AV and BV gene repertoire using a PCR-ELISA method. RNA is extracted from lymphocytes, transcribed to cDNA, which is then used as a template for PCR with 19 different TCR AV gene and 20 BV gene specific primers as the forward primer, and a digoxigenin (DIG) labeled AC/BC primer as the reverse primer. The DIG labeled PCR amplicons are hybridized with a fluorescein isothiocyanate (FITC) labeled TCR C region specific probe. Finally, the amplicons are quantified by ELISA using anti-FITC coated microtiter plates, and an anti-DIG conjugated peroxidase. Although PCR-ELISA cannot accurately quantify the expression level of a given TCR gene, overrepresented TCR V genes are easily identified by comparing the relative expression levels of each individual V gene in the total V gene repertoire. We demonstrate that this technique can be used to determine TCR profiles in blood and tissue samples containing as few as 50,000 T cells. In combination with CDR3 fragment size analysis, this method is an efficient tool to identify clonally expanded T cell subsets in the synovial biopsies of rheumatoid arthritis patients.

Vidarsson, G., J. G. J. van de Winkel, et al. (2001). "Multiplex screening for functionally rearranged immunoglobulin variable regions reveals expression of hybridoma-specific aberrant V-genes." Journal of Immunological Methods **249**(1-2): 245.

<http://www.sciencedirect.com/science/article/B6T2Y-42D82CM-T/2/1456548fa6dee966be9f4c58b48483b3>

Modification of antibody effector functions is commonly performed by chimerization or humanization. Cloning of antibody variable regions from hybridomas represents a first step that is frequently hampered by the expression of non-functionally rearranged variable regions in hybridoma cells that originate from MOPC21-derived fusion partners. We now present a simple method to clone functionally rearranged V-genes, based on V-gene-specific multiplex PCR screening. Using this method we document the expression of aberrant V-genes that originate from the original B-cell used for the hybridoma generation, not from the fusion partner, and are -- thus -- hybridoma specific.

Vilarino, N. and J. D. MacGlashan (2005). "Transient transfection of human peripheral blood basophils." Journal of Immunological Methods **296**(1-2): 11.

<http://www.sciencedirect.com/science/article/B6T2Y-4DXBS5T-1/2/e5cb290cea20709daf5d306e3c4599cd>

The human basophil has resisted previous attempts at transient transfection. Basophils were

transfected by nucleoporation and to test whether there was sufficient expression to modify cell function, the cells were transfected with a syk kinase tandem SH2 construct linked to GFP. This approach was taken because in RBL cells and murine mast cells syk kinase is known to play a very early role in signal transduction and previous studies in RBL cells demonstrated that expression of the tandem SH2 domains of syk would inhibit signaling, presumably by competition with endogenous syk for binding to ITAMs. Results from basophil transfections with SH2syk were compared to an empty construct. Basophils were stimulated with anti-IgE antibody and analyzed for single cell changes in cytosolic calcium levels. Basophils expressing the empty GFP construct showed a cytosolic calcium response similar to non-expressing cells. In contrast, basophils expressing the GFP-tandem SH2syk construct, on average, showed an anti-IgE-induced calcium response that was completely ablated. The transfection frequency was 8% (median), with an average viable recovery of 12% (n=18). While the procedure is not benign and is not always successful, these studies indicate that with gating techniques, the human basophil, a non-dividing primary leukocyte, can be transiently transfected to express high enough levels of an inhibitory protein to alter an IgE-mediated response.

Weber-Arden, J., O. M. Wilbert, et al. (1996). "Inverse PCR amplification of low-abundance message of [gamma][delta] T cell receptor genes." Journal of Immunological Methods **197**(1-2): 187.

<http://www.sciencedirect.com/science/article/B6T2Y-3W257R7-M/2/2c2d99ba099e15d76f95a65223648442>

The technique of inverse PCR permits the rapid amplification and identification of unknown DNA segments adjacent to well characterized core regions. In the field of immunology anchored PCR and inverse PCR are useful methods for examining junctional diversity and unknown variable gene segments of rearranged T cell receptor genes. We have applied an improved inverse PCR protocol to study the repertoire of [gamma][delta] T cell receptor genes in the developing thymus of the mouse.

Yau, K. Y. F., M. A. T. Groves, et al. (2003). "Selection of hapten-specific single-domain antibodies from a non-immunized llama ribosome display library." Journal of Immunological Methods **281**(1-2): 161.

<http://www.sciencedirect.com/science/article/B6T2Y-49FXMPV-1/2/070291e39a4dd2c040fe5019d43ebd08>

Picloram-specific variable fragments (VHHs) of heavy chain antibodies (HCAbs) were selected from a naive-llama library using ribosome display technology. A cDNA library of VHHs was constructed from lymphocytes of a non-immunized llama and engineered to allow in vitro transcription and translation. With no stop codons present on the transcripts, trimeric complexes of ribosomes, mRNAs and nascent peptides were produced for affinity selection, i.e. panning. After three cycles of panning, seven different VHHs all belonging to the VHH subfamily 1 were isolated. Following another three cycles of selection, only two of the seven VHHs persisted. A comparison of these two sequences with known sequences in the literature suggests that point mutations may have been introduced into the DNA pool during PCR amplification steps of library construction, panning and/or cloning. Three separate point mutations causing three independent amino acid changes (nonsynonymous mutations) accumulated in the same sequence and enriched throughout the selection protocol, suggesting that these changes confer binding advantages. Surface plasmon resonance (SPR) analysis was used to determine binding kinetics of the two clones (3-1D2 and 3-1F6) representing the two different sets of isolated complementarity determining region (CDR)3s. Measured KDs were 3 and 254 [mu]M, respectively. The results indicate that ribosome display technology can be used to efficiently isolate hapten-specific antibody (Ab) fragments from a naive library and concurrently introduce

diversity to the selected pool thereby facilitating molecular evolution. Ribosome display technology can compensate for the limited diversity of a VHH naive library and provide an unlimited source of affinity-matured immunoactive reagents in vitro.

Journal of Infection (6)

Al-Salloom, F. S., A. Al Mahmeed, et al. (2003). "Campylobacter-stimulated INT407 cells produce dissociated cytokine profiles." Journal of Infection **47**(3): 217.

<http://www.sciencedirect.com/science/article/B6WJT-491RP1C-2/2/baa36fb3c0864f794a504ef4f9d227a3>

Objectives. To study the action of factors produced by living *Campylobacter jejuni* (*C. jejuni*) against those present within sonicated and filtrated bacteria on induction of potential cytokines by the human intestinal cell line INT407. **Methods.** We used immunohistochemical technique modified to detect intracellular production of cytokines protein and RT-PCR to read RNA messages for evaluation of de novo cytokine synthesis. **Results.** The data herein display dissociation of cytokine profiles induced on by living *C. jejuni*. Exposure of INT407 cells to 106 live bacteria showed the highest numbers of cytokine producing cells of all examined cytokines. IFN- γ was the highest induced cytokine followed by IL-10, TNF- α and lastly IL-4. Also, abrogation of induction of the proinflammatory cytokines IFN- γ and TNF- α but not the antiinflammatory cytokines IL-4 and IL-10 by sonicated and filtrated bacteria was depicted. At the mRNA level, TNF- α signals were noted in accordance with its protein levels since increased TNF- α mRNA signals were registered only after stimulation with living bacteria. Very low or no induction of TNF- α was registered with non-stimulated cells. **Conclusions.** These results illustrate for the first time a role for factors from living bacteria in directing the immune response towards Th1 type. Characterization of such factors may be essential for future immunotherapeutic interventions during severe bacterial infections.

Ashshi, A. M., P. E. Klapper, et al. (2003). "Detection of human cytomegalovirus, human herpesvirus type 6 and human herpesvirus type 7 in urine specimens by multiplex PCR." Journal of Infection **47**(1): 59.

<http://www.sciencedirect.com/science/article/B6WJT-48JSJH1-1/2/46b03e4f2b4b8c13cc17d86143e26ed7>

Objectives. To develop a sensitive multiplex PCR to detect HCMV, HHV6 and HHV7, to test this PCR on urine specimens sent to the virus diagnostic laboratory and on stored urine samples from HIV-positive patients and their HIV-negative partners and to compare the sensitivity of the multiplex PCR with the diagnostic laboratory's routine service for the detection of HCMV. **Study design.** Primers specific for each of the three viruses were combined in a multiplex PCR that was then optimised for sensitivity. This PCR was applied prospectively to 413 unselected routine urine specimens over a 1 year period and retrospectively to 258 urine specimens from 63 HIV-positive patients and 10 HIV-negative partners. **Methods.** In the prospective study, the multiplex PCR detected 40 specimens positive for HCMV alone, 10 for HHV6, 3 for HHV7 and 3 with a dual infection of HCMV and HHV6. The sensitivity for HCMV was 93.5% by multiplex PCR compared to 28.3% by culture. HHV6 DNA was detected in 6 neonates (2-21 days) and HHV7 DNA in 2

neonates (4 and 20 days). In the retrospective study of HIV patients, HCMV was the most commonly detected virus (55.6%) compared to HHV6 (7.9%) and HHV7 (4.8%). Conclusions. The multiplex PCR was significantly more sensitive than non-DNA based procedures for the detection of HCMV. Urine may be a useful non-invasive specimen for the detection of HHV6 and HHV7 and their presence in neonates suggest perinatal transmission or the possibility of in utero infection.

Bakhiet, M., F. S. Al-Salloom, et al. (2004). "Induction of [alpha] and [beta] chemokines by intestinal epithelial cells stimulated with *Campylobacter jejuni*." *Journal of Infection* **48**(3): 236.

<http://www.sciencedirect.com/science/article/B6WJT-4BDM2K7-1/2/cc5d7040b208975927e4039ca0875a22>

Objectives. To investigate the production of dynamic [alpha] and [beta] chemokines represented by interleukin-8 (IL-8) as [alpha] chemokine and CCL2 (monocyte-chemoattractant protein-1, CCR2 ligand), CCL4 (macrophage-inflammatory protein-1[beta], CCR5 ligand), CCL3 (macrophage-inflammatory protein-1[alpha], CCR1/5 ligand), (CCL5, regulated upon activation, normal T-cell expressed and secreted (RANTES, CCR5 ligand) as [beta] chemokines by the human intestinal cell line INT407 stimulated with factors produced by living *Campylobacter jejuni* (C. jejuni) and those present within sonicated and filtrated bacteria. **Methods.** We used immunohistochemical technique modified to detect intracellular production of cytokines protein and RT-PCR to read RNA messages for evaluation of de novo cytokine synthesis. **Results.** Living bacteria induced increased numbers of IL-8, CCL4 and CCL2 but not CCL3 or CCL5 producing cells. Low numbers of IL-8, CCL4 and CCL2 producing cells were detected with filtrated supernatant compared to living and sonicated bacteria. A non-significant low number of chemokine producing cells was noted when comparing numbers of chemokine producing cells stimulated with living C. jejuni to those stimulated with sonicated bacteria, indicating that the triggering factors involved in stimulation with living bacteria were still active after sonication, but they were largely lost upon filtration. The mRNA signals for IL-8 were noted in conformity with its protein levels as increased IL-8 mRNA signals were registered after stimulation with living and sonicated bacteria but not with filtrated supernatant. **Conclusions.** Preferential production of chemokines probably induced by membrane associate factors of C. jejuni acting on intestinal epithelial cells is presented. These chemokines are suggested to be part of an inflammatory network affecting cell types that contribute to initiation and/or resolution of the infection.

Boone, S. A. and C. P. Gerba "The occurrence of influenza A virus on household and day care center fomites." *Journal of Infection In Press, Corrected Proof*

<http://www.sciencedirect.com/science/article/B6WJT-4DPYJMT-2/2/1dd74cc83157ae5332b07fc981d46d29>

Objective The goal of this study was to evaluate the prevalence of influenza A virus on surfaces in day care and home settings to better assess the potential role of fomites in the transmission of influenza. **Methods** During two and a half years, 218 fomites were tested from 14 different day care centers. Ten different fomites from bathrooms, kitchens and play areas were sampled. In addition, 92 fomites from eight different homes with children were tested over 6 months. Fourteen different household fomites from bathrooms, kitchens and living areas were sampled. Influenza A viral RNA was detected using reverse transcriptase-polymerase chain reaction. **Results** Influenza was detected on 23% of day care fomites sampled during the fall and 53% of fomites sampled during the spring. Spring and fall sample data was determined to be statistically different at the 0.05 [alpha]-level by Chi-square analysis $PP=0.00002$. There was no statistical difference found between moist and dry fomites (Chi square $P=0.13998$). No influenza was detected on home fomites sampled during the summer. In contrast, influenza was detected on 59% of home fomites sampled during March. **Conclusions** Influenza A virus was detected on over 50% of the fomites

tested in homes and day care centers during influenza season.

Xu, J., J. E. Moore, et al. (2003). "Identification of a novel [alpha]-proteobacterium causing bacteraemia in an immunocompetent patient." Journal of Infection **47**(2): 167.

<http://www.sciencedirect.com/science/article/B6WJT-48S4KR9-1/2/a2e902ae243a0ac54f03fd8e41d71>

An 89-year male with pyrexia and suspected bacteremia was admitted to hospital, where a Gram-negative rod was identified from blood culture. The organism was difficult to identify phenotypically and the resulting sequencing of a 559 bp section of the 16S rRNA gene did not have a high homology score (>97.0%) with any deposited GenBank accession numbers and hence was not able to be assigned to a species within any genus. Given that the isolate was a member of the alpha subclass of the Proteobacteria but did not fall into any of the known genera with more than 93.7% homology (*Brucella*, *Rhizobium*, *Ochrobactrum*, *Agrobacterium*), we believe this isolate to represent a novel [alpha]-proteobacterium, which was the cause of bacteraemia in this patient.

Yee, E. K., L. J. Melkerson-Watson, et al. (2004). "Genomic analysis of penicillin-resistant *Streptococcus pneumoniae* in Southeastern Michigan." Journal of Infection **49**(2): 126.

<http://www.sciencedirect.com/science/article/B6WJT-4C604VR-1/2/0be762e87f0f3e339a3778882e328e39>

Objective. The emergence of multidrug resistance within *Streptococcus pneumoniae* population was analysed, correlating penicillin resistance Pen(R) with secondary antibiotic resistance, capsular serotype, and genetic diversity among isolates. **Methods.** DNA fingerprinting, following macro-restriction enzyme digestion and pulse field gel electrophoresis (PFGE), and restriction fragment analysis of the PBP 2b gene, following PCR amplification, were performed on the Pen(R) *S. pneumoniae*, among 377 clinical isolates obtained from the clinical microbiology laboratory (University of Michigan Medical Center). **Results.** Overall 35% of the isolates were Pen(R) of which 45% demonstrated high-level penicillin (Pen(R)-R, MIC>1). Respiratory isolates were more likely to be Pen(R). **Conclusion.** The emergence of multidrug resistance in the *S. pneumoniae* population in SE Michigan is not due to expansion of a single or limited number of resistant clones, is occurring most frequently in the paediatric population and is associated with a decreased susceptibility to penicillin.

Journal of Inorganic Biochemistry (2)

Colangelo, D., A. Ghiglia, et al. (2005). "Water-soluble benzoheterocycle trisium clusters as potential inhibitors of telomerase enzyme." Journal of Inorganic Biochemistry **99**(2): 505.

<http://www.sciencedirect.com/science/article/B6TGG-4DXTHPN-7/2/937bb71f23ed2a348438fe6da7121712>

We have studied the ability of several bioorganometallic clusters $[(\mu\text{-H})\text{Os}3(\text{CO})9(\text{L})([\mu\text{-}3\text{-}\eta^2\text{-}(\text{Q-H}))]$, where $\text{L} = [\text{P}(\text{C}_6\text{H}_4\text{SO}_3\text{Na})_3]$ or $[\text{P}(\text{OCH}_2\text{CH}_2\text{NMe}_3)_3]$, and $\text{Q} =$ quinoline, 3-aminoquinoline, quinoxaline or phenanthridine, of inhibiting telomerase, a crucial enzyme for cancer progression. In general, quinolines have shown interesting biological properties, especially in inhibiting enzymes. For example, the 2,3,7-trichloro-5-nitroquinoxaline (TNQX) exhibited strong anti-telomerase activity in vitro. Among the quinoline-clusters under study, only the negatively charged ones (by virtue of the sulfonated phosphines) exhibited good anti-telomeric activity on semi-purified enzyme in a cell-free assay, while they were ineffective in vitro on Taq, a different DNA-polymerase. On the contrary, the treatment of breast cancer MCF-7 cell line did not evidence any activity of these clusters, suggesting a low aptitude for crossing cell membrane. Furthermore, all clusters exhibited non-specific, acute cytotoxicity, probably due to accumulation on cell membranes by virtue of their amphiphilic character. A detailed study of Os uptake and accumulation in MCF-7 cells supported this hypothesis.

Kawakami, J., H. Imanaka, et al. (2000). "In vitro selection of aptamers that act with Zn^{2+} ." Journal of Inorganic Biochemistry **82**(1-4): 197.

<http://www.sciencedirect.com/science/article/B6TGG-41ST2C3-X/2/fd87e0f1cccc6bbc6c2ed2f74dbad108>

An in vitro selection was carried out with Zn^{2+} to isolate novel RNA molecules, zinc-dependent aptamers, that bind to HIV-1 Tat protein. RNAs bound to Tat were collected by using a nitrocellulose filter from a library of random RNAs and regenerated to the next generation of the RNA library by subsequent reverse transcription, polymerase chain reaction, and transcription. Sequences of the selected RNAs were determined after 6 and 12 rounds of the selection. The control clones after normal selection procedure with Mg^{2+} had a consensus UUG that resembled essential sequences of TAR or Arg aptamers. On the other hand, many unique sequences were revealed from a library selected with Zn^{2+} and the RNA with most abundant sequence (clone 31) bound to Tat tightly only when Zn^{2+} existed. The secondary structure of clone 31 RNA was predicted by using a computational prediction with our thermodynamic parameters and enzymatic scission of the RNA. Several model RNAs were prepared and the binding property of these RNAs to Tat were investigated. As a result, all the model RNAs did not reproduce the binding property of clone 31. Therefore, the Tat aptamer that acts with Zn^{2+} should require a relatively longer region of the sequence which is able to offer tertiary cooperation of several motifs for the binding.

Journal of Laboratory and Clinical Medicine (8)

Casonato, A., M. G. Cattini, et al. (2004). "A new L1446P mutation is responsible for impaired von Willebrand factor synthesis, structure, and function." Journal of Laboratory and Clinical Medicine **144**(5): 254.

<http://www.sciencedirect.com/science/article/B6WJW-4DW8K9J-D/2/6a41bcd30b2a10d3c6aed5a14feade47>

We report on a new mutation (4337T-->C) in exon 28 of the von Willebrand factor (VWF) gene, resulting in a substitution of L with P at residue 1446 (L1446P) of pre-pro-VWF. The defect is transmitted as a dominant trait and induces a reduced VWF synthesis, an abnormal VWF

multimer pattern and a deficient VWF-platelet glycoprotein Ib interaction. The proband had low plasma and platelet VWF antigen levels, a reduced VWF collagen-binding capacity, and a disproportionately low VWF ristocetin cofactor activity, associated with the absence of ristocetin-induced platelet aggregation. Multimer analysis showed that the smaller multimers were slightly low, whereas the larger ones were significantly reduced or absent, with a clear cutoff between the two patterns. Similar hemostatic findings were observed in the proband's sister and nephew. Desmopressin administration restored VWF levels to near normal, but this was not so for VWF ristocetin cofactor activity or ristocetin-induced platelet aggregation. VWF multimers improved after desmopressin, moreover, with the larger forms restored and the smaller ones still relatively more represented. Recombinant P1446 VWF synthesis was reduced at heterozygous level, and its multimer pattern was similar to that observed in plasma VWF. These findings confirm the role of L1446P mutation in determining the von Willebrand disease (VWD) phenotype observed in our patients. Given the lack of large and intermediate VWF multimers, and the fact that the VWF-platelet interaction defect appears to be partially independent of multimer pattern, the VWD associated with L1446P mutation may belong to the type 2A/2M VWD variant.

Chang, J.-M., M.-C. Kuo, et al. (2004). "1-[alpha],25-Dihydroxyvitamin D3 regulates inducible nitric oxide synthase messenger RNA expression and nitric oxide release in macrophage-like RAW 264.7 cells." Journal of Laboratory and Clinical Medicine **143**(1): 14.

<http://www.sciencedirect.com/science/article/B6WJW-4BHJ5WF-B/2/ce9dc20cfac369f9eb6ffce6024aea4c>

The expression of inducible nitric oxide synthase (iNOS) expression and release of nitric oxide (NO) from macrophages are markedly increased in granulomatous infections. Activation of macrophages 1[alpha]-hydroxylase results in an increase of 1[alpha],25-dihydroxyvitamin D3 [1,25(OH)2D3]. However, the significance of this increased production is not completely understood. In this study, we analyzed 1,25(OH)2D3 and NO production in patients with tuberculosis infection and hypercalcemia and used lipopolysaccharide (LPS) to stimulate RAW 264.7 cells in an attempt to assess iNOS expression and gaseous NO production regulated by 1,25(OH)2D3. Peroxynitrite (OONO-) production and lactate dehydrogenase activity were also examined. Without additional stimulation, peripheral-blood mononuclear cells (PBMCs) from patients with tuberculosis converted more 25-hydroxyvitamin D3 to 1,25(OH)2D3 than did those from normal controls. These PBMCs released less NO than did those from control subjects, at baseline and in the stimulated state. We found that 1,25(OH)2D3 dose-dependently inhibited iNOS messenger RNA expression of the LPS-stimulated RAW 264.7 cells and also significantly reduced the gaseous NO release and OONO- production. Paralleling the 1,25(OH)2D3-induced inhibition of NO release were reductions in OONO- and LDH production. In conclusion, 1,25(OH)2D3 inhibited iNOS expression and reduced NO production by LPS-stimulated macrophages in the range of physiological doses. Inhibition of the NO surge was coupled with a reduction in OONO- and LDH production. Increased 1,25(OH)2D3 production and decreased release of NO from the PBMCs of patients with tuberculosis and hypercalcemia were also noted. We propose that 1,25(OH)2D3 production by macrophages may protect themselves against oxidative injuries caused by the NO burst. In the case of tuberculosis infection, increased 1,25(OH)2D3 synthesis may further contribute to the development of an unwanted phenomenon--hypercalcemia.

Denomme, G. A., T. E. Warkentin, et al. (1997). "Activation of platelets by sera containing igg1 heparin-dependent antibodies: an explanation for the predominance of the Fc[gamma]rIIa "low responder" (his131) gene in patients with heparin-induced thrombocytopenia." Journal of Laboratory and Clinical Medicine **130**(3): 278.

<http://www.sciencedirect.com/science/article/B6WJW-4CT0YW9-12/2/b94a26f373b3bb0a31fb19f161dbd4e9>

Heparin-induced thrombocytopenia (HIT) is a prothrombotic disorder caused by heparin-dependent IgG (HIT-IgG) that recognizes a complex of heparin and platelet factor 4 (PF4), leading to platelet activation via the platelet Fc[gamma]IIa receptors (Fc[gamma]RIIa). Not all patients who generate HIT-IgG in response to heparin develop HIT, however, possibly because of observed differences in the ability of platelets from healthy individuals to be activated by HIT sera. It is known that a polymorphism in the platelet Fc[gamma]RIIa plays an important role in determining platelet reactivity to murine platelet-activating monoclonal antibodies of the IgG1 subclass: homozygous arg131 ("high responder" or HR) platelets respond well, and homozygous his131 ("low responder" or LR) platelets respond poorly, respectively, to these murine monoclonal antibodies. We sought to determine whether the differing risk for HIT among patients who receive heparin, as well as the variable platelet reactivity to HIT sera, could be explained by preferential activation by HIT-IgG of platelets bearing a particular Fc[gamma]RIIa phenotype. We found that the LR Fc[gamma]RIIa gene frequency was significantly overrepresented among 84 HIT patients, compared with that of 264 control subjects (0.565 versus 0.471; $p = 0.03$). We studied the subclass distribution of HIT-IgG against its major antigen, heparin/PF4 complexes, and found that 55 of 61 (90%) HIT sera expressed IgG1 antibodies either alone ($n = 47$) or in combination with IgG2 ($n = 5$) or IgG3 ($n = 3$). We then compared the platelet-activating profile of HIT sera with murine platelet-activating monoclonal antibodies. As expected, the murine IgG1 monoclonal antibodies preferentially activated platelets from homozygous HR individuals. In contrast, however, the LR homozygous platelets exhibited the greatest reactivity to HIT sera that contained predominantly anti-heparin/PF4 antibodies of the IgG1 subclass. We conclude that the significant overrepresentation of the LR (his131) gene among patients with HIT may be explained by the preferential activation of LR Fc[gamma]RIIa platelets by HIT antibodies of the IgG1 subclass, which is the predominant immunoglobulin subclass generated in HIT.

Giannini, C., F. Giannelli, et al. (1999). "Prevalence of mixed infection by different hepatitis C virus genotypes in patients with hepatitis C virus--related chronic liver disease." Journal of Laboratory and Clinical Medicine **134**(1): 68.

<http://www.sciencedirect.com/science/article/B6WJW-4CYXVS6-32/2/f0248820896baad3d457b0b2e9666162>

Multiple infection by different hepatitis C virus (HCV) genotypes may be of great clinico-pathologic interest. In this study we determined the effective prevalence of coinfections by two or more HCV genotypes in 213 subjects with HCV-positive chronic hepatitis by using genotype-specific polymerase chain reaction (PCR), genotype-specific probe hybridization, and direct sequencing. The most prevalent genotype was HCV-1b (54%). HCV-2 (a/c) was also prevalent (27%), and types 1a and 3a were found in 5% and 3% of patients, respectively. A mixed infection was detected in 23 patients (10.8%): 4 out of 23 were coinfecting by types 1a + 1b, while the remaining 19 patients had a 1b + 2 (a/c) mixed infection. Further analysis based on restriction fragment length polymorphism (RFLP) on type-specific PCR products was used to verify genotyping results. Only four coinfections (1a + 1b in 2 patients and 1b + 2 (a/c) in the remaining 2 patients, respectively) were confirmed by enzyme cleavage. All patients with true coinfection had long-lasting infection and liver cirrhosis. Both true and false mixed infections resulting from RFLP analysis were confirmed by direct sequencing of type-specific amplification products. We also determined a recurrent C/T transversion at position 618 in all sequenced samples. In 4 cases another point mutation (G/A at position 626) was found, reducing the number of mismatches between HCV-2 and HCV-1b from 4 to 3 (or 2). Interestingly, all HCV-2 isolates sequenced showed the highest degree of nucleotide homology with HCV-2 subtype c, confirming the relatively high prevalence of this subtype in Italy. In conclusion, we showed the possibility of multiple infection by different HCV types in the general population of chronically infected patients

without particular risk factors, even if in a low percentage of cases. Further studies are needed to assess the clinical relevance of chronic HCV infection with multiple genotypes.

Lipscomb, D., C. Bourne, et al. (1999). "DNA sequence of the canine platelet [beta]3 gene from cDNA: Comparison of canine and mouse [beta]3 to segments that encode alloantigenic sites and functional domains of [beta]3 in human beings." Journal of Laboratory and Clinical Medicine **134**(3): 313.

<http://www.sciencedirect.com/science/article/B6WJW-4CYXVWK-4C/2/f889685b677da7879eb34709a3a2a0b7>

The platelet glycoprotein complex [alpha]IIb[beta]3 is required for platelet-fibrinogen binding and platelet aggregation. This study was designed to characterize the nucleotide sequence of the canine platelet [beta]3 gene from cDNA. The nucleotide and deduced amino acid sequences of the canine [beta]3 gene were 92% and 96% homologous, respectively, with the sequences previously established for the [beta]3 gene of human beings. Within the [beta]3 gene, the nucleotide sequence of cDNA prepared from canine platelets shared homology of 89% for the cytoplasmic domain, 93% for the transmembrane domain, 92% for the extracellular domain, 94% for the arginine-glycine-aspartic acid (RGD) binding domain, and 97% for the region associated with Ca²⁺-dependent stabilization of the [alpha]IIb[beta]3 fibrinogen-binding pocket. The deduced amino acid sequence of canine [beta]3 was 100%, 97%, 96%, and 95% homologous with the cytoplasmic, transmembrane, extracellular, and RGD-binding domains, respectively, and was 100% homologous with the region associated with Ca²⁺-dependent stabilization of the [alpha]IIb[beta]3 fibrinogen-binding pocket of [beta]3 in human beings. The canine platelet cDNA signal peptide segment of the [beta]3 gene encodes for 22 amino acids, as compared with 26 amino acids previously reported for human beings. The deduced amino acid sequence of canine [beta]3 corresponds to the high-frequency allelic form for five of the six alloantigenic sites reportedly associated with human platelets: Leu33Leu40Pro407Arg489Arg636. The apparent amino acid residue in position 143 (Pen alloantigen) of canine platelet [beta]3 is histidine compared with arginine in human beings. Knowledge of the [beta]3 gene nucleotide sequence of normal dogs will facilitate the understanding of platelet [alpha]IIb[beta]3 structure-function relationships.

Mackenzie, R. K., N. Topley, et al. (1997). "Staphylococcal exoproducts down-regulate cyclooxygenase 1 and 2 in peritoneal macrophages." Journal of Laboratory and Clinical Medicine **129**(1): 23.

<http://www.sciencedirect.com/science/article/B6WJW-4CMYYM6-6B/2/aff53ced3b61ae386471386e629d4440>

Peritoneal macrophages (PMOs) are important components of the host defense against microbial infection in patients undergoing continuous ambulatory peritoneal dialysis (CAPD). Incubation of human PM[osol]s with cell-free supernatant (BFS), prepared from *Staphylococcus aureus*, inhibited prostaglandin E₂ (PGE₂) and thromboxane B₂ (TXB₂) production. Slot-blot analysis of cyclooxygenase-1 (Cox-1) and Cox-2 demonstrated a decrease in both Cox-1 (29%) and, to a greater extent, Cox-2 (65%) protein expression after BFS stimulation. When competitive polymerase chain reaction (PCR) was used, the peak levels of Cox-1 and Cox-2 messenger ribonucleic acid (mRNA) in unstimulated PMOs were 0.304 +/- 0.13 pmol/L and 9.61 +/- 2.84 pmol/L (mean +/- SEM, N = 3), respectively. After exposure of samples to BFS for 30 minutes, the level of Cox-2 mRNA was reduced to 0.59 +/- 0.449 pmol/L (16-fold reduction, p < 0.05). In contrast, these same PM[osol]s showed an increased expression of IL-6 mRNA and increased secretion of IL-6 protein. These results indicate that prostaglandin production in PM[osol]s is regulated by alterations in both immunoreactive Cox-1 and Cox-2. The down-regulation of Cox

metabolism in these cells is primarily related to the delayed and depressed increase in the Cox-2 gene product.

Takahashi, M., K. Tsuchiya, et al. (1997). "A role for Na/K adenosine triphosphatase in the pathogenesis of cyst formation in experimental polycystic kidney disease." Journal of Laboratory and Clinical Medicine **129**(5): 517.

<http://www.sciencedirect.com/science/article/B6WJW-4CN5N73-VI2/54bef7858315321dca6e69e8f1934558>

Multiple cyst formation with fluid retention is a characteristic structural abnormality in polycystic kidney disease (PKD). Na/K adenosine triphosphatase (ATPase) is a major transporting membrane protein that is ubiquitous in the epithelial cell, which has been thought to be involved in cystogenesis. We have investigated the molecular and histologic basis of Na/K ATPase activity in experimental PKD in vivo. Rats were treated with diphenylthiazole (100 mg/100 gm body weight), and cyst formation was examined histologically. Na/K ATPase activity was measured enzymatically by using a fluorometric method, and reverse transcription-competitive polymerase chain reaction (RT-PCR) analysis was used to quantitate mRNA levels in the isolated single nephron segment. Kidneys were immunostained with subunit-specific antibodies to determine the localization of Na/K ATPase in the epithelial cell. The enzyme activity increased in the cortical collecting duct from 25.9 $\pm$ 3.5 mmol/Lpmol/mm/min to 72.9 $\pm$ 6.8 pmol/mm/min and in the outer medullary collecting duct from 13.0 $\pm$ 3.9 mmol/Lpmol/mm/min to 58.5 $\pm$ 9.8 pmol/mm/min ($n = 6$, $p < 0.01$); however, all other segments showed no significant changes. No significant alternation in α 1- and β 1-subunits of Na/K ATPase mRNA levels was observed by competitive PCR assay in either segment. The enzyme was stained at the basolateral membrane even in the cystic tubules. Na/K ATPase activity was up-regulated in the cyst-formed kidney, but this was not accompanied with transcriptional up-regulation. Increased Na/K ATPase activity at normal locations may play a role in abnormal net fluid transport in the development and progression of experimental PKD.

Tang, D. C., S. Fucharoen, et al. (2001). "Rapid differentiation of five common α -thalassemia genotypes by polymerase chain reaction." Journal of Laboratory and Clinical Medicine **137**(4): 290.

<http://www.sciencedirect.com/science/article/B6WJW-45SJFPK-1J2/ea3abf80689aa421b51873c1c9140b92>

The α -thalassemias are common genetic disorders that arise from reduced synthesis of the α -globin chains. At present, large-scale carrier screening and clinically valuable antenatal detection programs have not been established for the congenital disorder α -thalassemia (α -thal). We have developed a simple nonradioactive polymerase chain reaction (PCR) approach that can detect and differentiate several common α -globin gene deletional α -thals regardless of the break points. When three primer sets were used—two gene-specific sets for the α 1- and α 2-globin genes and one set for the β -actin gene (serving as an internal control)—PCR products from genomic DNA were simultaneously amplified and analyzed after coamplification and gel electrophoresis. The number of α -globin genes present in the subjects was determined by the intensity of α 1 and α 2 bands normalized with that of β -actin when using densitometry. Our results demonstrate that five common genotypes of deletional α -thal are differentiated by the ratios of α 1/ β -actin and α 2/ β -actin. We also examined the feasibility of coupling this allele-specific amplification to a color-complementary assay. This easy and reproducible PCR assay is suitable for identifying α -thal carriers in screenings of large populations and improving genetic counseling. (J Lab Clin Med

Journal of Molecular and Cellular Cardiology (7)

Cremers, B., M. Flesch, et al. (2003). "Modulation of myocardial contractility by lysophosphatidic acid (LPA)." Journal of Molecular and Cellular Cardiology **35**(1): 71.

<http://www.sciencedirect.com/science/article/B6WK6-47S6CWX-2/2/17362036670bae78358631ef196759ac>

Lysophosphatidic acid (LPA) is a phospholipid messenger, which is released from activated platelets and leukocytes. This study examined the effects of LPA on myocardial contractility and characterized the signal transduction pathway involved in these effects. Functional effects of LPA were determined in isolated, electrically driven human myocardial preparations and rat cardiac myocytes. In human atrial and ventricular myocardial preparations, LPA (100 [μ]mol/l) decreased isoprenaline (0.03 [μ]mol/l) enhanced force of contraction by 17 $\pm$ 2% and 28 $\pm$ 3%, respectively. The effect of LPA was attenuated by suramin (1 mmol/l). In isolated rat cardiomyocytes, LPA (1-100 [μ]mol/l) concentration dependently abolished isoprenaline (0.03 [μ]mol/l) induced increase in cell shortening. This antiadrenergic effect was blunted after pretreatment with pertussis toxin (5 [μ]g/ml, 12 h). Forskolin (10 [μ]mol/l) stimulated adenylyl cyclase activity was inhibited by LPA in human myocardial membranes. PCR analysis of human atrial and ventricular cDNAs revealed the expression of two cognate LPA receptors: EDG-2 and EDG-7. Our results suggest that LPA exerts antiadrenergic effects on force of contraction in human and rodent myocardium via a G[α]i/o protein-mediated mechanism, most probably by LPA binding to the mammalian LPA receptors EDG-2 and/or EDG-7. This newly discovered action of LPA might be of pathophysiological importance in conditions like myocardial ischemia or inflammatory disorders when LPA release is enhanced.

Fodstad, H., H. Swan, et al. (2004). "Loss-of-function mutations of the K⁺ channel gene KCNJ2 constitute a rare cause of long QT syndrome." Journal of Molecular and Cellular Cardiology **37**(2): 593.

<http://www.sciencedirect.com/science/article/B6WK6-4CTCV39-3/2/3a379b3db65a7b788b42df5d0f1e0552>

Mutations of the KCNJ2 gene encoding the potassium channel Kir2.1 were previously shown to cause Andersen's syndrome (AS), a multisystem disease manifesting with developmental abnormalities, cardiac arrhythmias and periodic paralyses. We conducted a search for KCNJ2 mutations among 188 unrelated patients suspected to have long QT syndrome (LQTS). The screening was performed by denaturing high-performance liquid chromatography (dHPLC) and DNA sequencing. Two novel mutations of the KCNJ2 gene were detected: a missense threonine to alanine mutation (T75A) in the N-terminal region (family 1) and an in-frame deletion of two amino acids ([Δ]FQ163-164) in the M2 transmembrane region (family 2). In addition, a previously described silent polymorphism C1146T was detected. In family 1, some of the affected family members had a history of periodic muscle weakness characteristic of AS, but no dysmorphic features. The mean QTc interval of the affected members were 444 $\pm$ 24 ms (family 1, n=7) and 456 $\pm$ 8 ms (family 2, n=2). The mutations affect functionally important regions of the KCNJ2 channel protein: upon injection of the *Xenopus* oocytes with the wild type and mutant

KCNJ2 constructs, the channel proteins were correctly synthesized and localized to the cell surface, but no measurable inward K⁺ current could be detected for the mutant KCNJ2 constructs. In conclusion, we report two novel loss-of-function mutations of the KCNJ2 channel, affecting different domains of the channel protein. Mutations of the KCNJ2 gene should be considered in genetic subclassification of LQTS patients, even in the absence of overt manifestations of AS.

Gabel, S. A., V. R. Walker, et al. (2005). "Estrogen receptor beta mediates gender differences in ischemia/reperfusion injury." Journal of Molecular and Cellular Cardiology **38**(2): 289.

<http://www.sciencedirect.com/science/article/B6WK6-4F94YNH-4/2/77c721bb386528b4560ad87294814331>

Under hypercontractile conditions associated with increased intracellular calcium, male hearts show enhanced ischemia/reperfusion injury compared to female hearts. Our aim in this study was to identify the specific estrogen receptor involved in this gender difference. Following brief treatment with isoproterenol, isolated mouse hearts were subjected to ischemia and reperfusion. Postischemic contractile function and infarct size were measured in wild-type (WT) male and female hearts, and female hearts lacking functional alpha estrogen receptor ([alpha]ERKO), or the beta estrogen receptor ([ss]ERKO). WT male hearts exhibited significantly less functional recovery and more necrosis than WT females. [alpha]ERKO female hearts exhibited ischemia/reperfusion injury similar to that observed in WT females, whereas [ss]ERKO females exhibited significantly less functional recovery than WT females and were similar to WT males. These data suggest that estrogen, through the [ss]-estrogen receptor, plays a role in the protection observed in the female heart. Furthermore, we identified genes that were differentially expressed in [ss]ERKO female hearts compared to [alpha]ERKO and WT female hearts, and found altered expression of a number of metabolism genes, which may be important in ischemic injury. We further showed that WT female hearts have increased ratio of carbohydrate to fatty acid metabolism relative to WT males.

Gao, P. J., Y. Li, et al. (2003). "Differentiation of vascular myofibroblasts induced by transforming growth factor-[beta]1 requires the involvement of protein kinase C[alpha]." Journal of Molecular and Cellular Cardiology **35**(9): 1105.

<http://www.sciencedirect.com/science/article/B6WK6-493P6N1-2/2/7ada2bfeccc1fc2cfb4bb22007339cd9>

In response to vascular injury, adventitial fibroblasts can modulate their phenotype to myofibroblasts, cells that participate in arterial remodeling. However, the signaling mechanisms underlying the vascular myofibroblast differentiation remain unknown. Since protein kinase C (PKC) is a key enzyme for cell differentiation, we examined whether PKC isoforms were involved in the vascular myofibroblast differentiation. The association between PKC[alpha] and myofibroblast differentiation was investigated in cultured rat aortic fibroblasts treated with transforming growth factor-[beta]1 (TGF[beta]1). Confocal immunofluorescence microscopy indicated that fibroblasts expressed [alpha]-smooth muscle actin ([alpha]-SM actin) after TGF[beta]1 treatment. Moreover, TGF[beta]1 stimulation increased both PKC[alpha] mRNA expression (measured by real-time quantitative RT-PCR) and PKC activity (determined by histone-like pseudosubstrate phosphorylation) in adventitial fibroblasts. Western blot analysis indicated that PKC[alpha] protein expression was higher in TGF[beta]1-treated fibroblasts than in untreated cells. TGF[beta]1-induced expression of [alpha]-SM actin was inhibited in a dose-dependent manner by treating cells with a PKC inhibitor, calphostin C, and was abolished by depleting PKC[alpha] with antisense PKC[alpha] oligodeoxynucleotides. Our results demonstrate

that TGF[β]1 induces adventitial myofibroblast differentiation via a PKC[α]-dependent process.

Nakajima-Taniguchi, C., H. Matsui, et al. (1995). "Novel missense mutation in [alpha]-tropomyosin gene found in Japanese patients with hypertrophic cardiomyopathy." Journal of Molecular and Cellular Cardiology **27**(9): 2053.

<http://www.sciencedirect.com/science/article/B6WK6-4BSF46K-X/2/8770d569c5d38aad5bc2295ca89b99c5>

We have searched for mutations in [alpha]-tropomyosin gene in 50 Japanese patients with hypertrophic cardiomyopathy (HCM) by means of polymerase chain reaction (PCR)-single strand conformation polymorphism (SSCP) analysis. Two missense mutations of the [alpha]-tropomyosin gene were detected in Japanese patients with familial HCM. Sequencing analysis revealed a C to T transition at codon 63 leading to a replacement of Ala with Val residue, and a G to A transition with replacement of Asp by Asn at codon 175. These missense mutations were found at residues which were markedly conserved across the species, and have been reported to interact with troponin T. This is the first report on a mutant [alpha]-tropomyosin gene in a Japanese Population. Familial HCM is a genetically heterogeneous disease in Japanese patients, similar to that reported in Caucasian kindreds.

Tang, Z., B. S. McGowan, et al. (2004). "Gene expression profiling during the transition to failure in TNF-[alpha] over-expressing mice demonstrates the development of autoimmune myocarditis." Journal of Molecular and Cellular Cardiology **36**(4): 515.

<http://www.sciencedirect.com/science/article/B6WK6-4BYRS5M-1/2/defba54e050b9e64e7204dcd6b22e11d>

Transgenic mice with cardiac-specific over-expression of tumor necrosis factor-[alpha] (TNF1.6) progress to dilated heart failure. A significant inflammatory response precedes functional deterioration, and may contribute to cardiac damage in this model. To evaluate the underlying molecular mechanisms, we assessed the gene expression in six groups of mouse hearts defined by age, gender, and phenotype (n = 3/group) using Affymetrix microarray analysis. Phenotype was defined as compensated (in young TNF1.6) or decompensated (in older TNF1.6) via echocardiogram. Of the >1000 transcripts altered in the compensated hearts (fold change > 2, P < 0.05), 50 genes were differentially regulated, including seven immunoglobulin genes. Real-time reverse transcriptase-polymerase chain reaction and cDNA microarray confirmed the Affymetrix data. Mac3⁺ macrophages, CD4⁺ T and CD45/B220⁺ B-cells were identified in both compensated and decompensated hearts. However, a large amount of IgG was found deposited in areas devoid of B-lymphocytes in the myocardium of decompensated TNF1.6 mice; no such accumulation was seen in the compensated or age-matched controls. Furthermore, nuclei density analyses showed a two-fold increase in the myocardium of both compensated and decompensated TNF1.6 mice (vs. WT). This study suggests that TNF-[alpha] over-expression activates not only the inflammatory response, but also humoral immune responses within the transgenic hearts. The autoimmune response occurs concomitantly with cardiac decompensation and may participate in triggering the transition to failure in TNF1.6 mice.

Yu, C.-M., K. Wing-Hon Lai, et al. (2004). "Normalization of renal aquaporin-2 water channel expression by foscarnil, valsartan, and combination therapy in congestive heart failure: a new mechanism of

action." Journal of Molecular and Cellular Cardiology **36**(3): 445.

<http://www.sciencedirect.com/science/article/B6WK6-4BJ0TXN-1/2/5ee3172d3cee0ac68ce3d81d3818b38a>

This study investigated the effect of fasinopril (Fos), valsartan (Val), and combination of both drugs (Fos + Val) on the cardiac and renal expression of aquaporin-1 (AQP1) and aquaporin-2 (AQP2) in congestive heart failure (CHF). A rat model of CHF was created by ligating the left anterior descending coronary artery to induce acute myocardial infarction (AMI). Rats were treated by Fos, Val, or Fos + Val for 4 weeks. In renal medulla and cortex, AMI was associated with 2.2- and 1.8-fold increase in AQP2 mRNA expression when compared with Sham-operated rats (medulla: 23.6 $\pm$ 2.8 vs. 52.3 $\pm$ 8.7%; P P P = NS vs. Sham) and in the cortex (Fos, 21.2 $\pm$ 6.7%; Val, 20.4 $\pm$ 6.0%; Fos + Val, 18.9 $\pm$ 7.5%; P = NS vs. Sham). Similarly, the AQP2 protein expression increased by 2.1-fold after CHF (P P P < 0.05). The renal and cardiac AQP1 gene and protein expressions were unaltered in CHF or by medical therapy. There was no observed cardiac AQP2 expression in all the study groups. Treatment with Fos, Val, or combination therapy was effective in preventing the upregulation of renal AQP2 gene and protein expressions in CHF rats caused by AMI.

Journal of Neuroimmunology (78)

Bauer, J., U. Ganter, et al. (1993). "Effects of interleukin-1 and interleukin-6 on metallothionein and amyloid precursor protein expression in human neuroblastoma cells: Evidence that interleukin-6 possibly acts via a receptor different from the 80-kDa interleukin-6 receptor." Journal of Neuroimmunology **45**(1-2): 163.

<http://www.sciencedirect.com/science/article/B6T03-476WCFH-WM/2/9f385781b125e3e5aeebd6441f7ef5bf>

Since immunohistochemical studies indicated the presence of interleukin-6 in the cortices of patients with Alzheimer's disease, we were interested in the eventual biological effects of this cytokine on neuronal cells. We found that interleukin-6 and interleukin-1 induced metallothionein expression in a human neuronal (SH-SY5Y neuroblastoma) cell line. In contrast to metallothionein, amyloid precursor protein expression was unaffected by both cytokines. When searching in the same cell line for the expression of the classical 80-kDa interleukin-6 binding protein, which is part of the dimeric interleukin-6 receptor, we were unable to detect the respective mRNA. Our findings either indicate that the interleukin-6 receptor in these cells is expressed in extremely low levels or that interleukin-6 may act upon neuronal cells via a different, yet unknown neuronal receptor.

Bencsik, A., C. Malcus, et al. (1996). "Selective induction of cytokines in mouse brain infected with canine distemper virus: structural, cellular and temporal expression." Journal of Neuroimmunology **65**(1): 1.

<http://www.sciencedirect.com/science/article/B6T03-3VXN38M-1/2/58491b94d58ac7369728972e5406dad0>

We have previously shown that, in experimentally inoculated mice, canine distemper virus (CDV), a neurotropic virus, selectively infects certain brain structures (hypothalamus, hippocampus, monoaminergic nuclei, etc). Here we demonstrate that tumor necrosis factor (TNF)-[alpha], interleukin (IL)-1 [beta] and IL-6 transcripts are selectively expressed in these CDV-targeted structures, except in the dentate gyrus, where cytokines are induced without prior CDV replication. The time-course of TNF-[alpha] expression vs. viral replication in the hypothalamus was different from that in hippocampus. In addition, we show that a substantial number of neurons express TNF-[alpha] and IL-6. These findings provide new insights into the possible participation of cytokines in the neurological disorders triggered by CDV infection.

Blanco-Jerez, C., J. F. Plaza, et al. (2002). "Increased levels of IL-15 mRNA in relapsing-remitting multiple sclerosis attacks." Journal of Neuroimmunology **128**(1-2): 90.

<http://www.sciencedirect.com/science/article/B6T03-45Y6MNW-1/2/09a8a6a5fc44d41e36826545f77b7584>

IL-15 is a proinflammatory cytokine which has recently been implicated in multiple sclerosis (MS) pathogenesis, where it may play a role in the initiation and/or progression of the disease. We have used reverse transcriptase-polymerase chain reaction (RT-PCR) to study IL-15 mRNA levels in peripheral blood mononuclear cells (PBMC) from healthy controls and relapsing-remitting MS (RRMS) patients in a stable phase of the disease and during a bout, both before and after corticosteroid treatment (CST). IL-15 mRNA expression was found to be similar in controls and stable patients. We have detected an increased level of IL-15 mRNA in PBMC of patients with a relapse, which was maintained after CST. We have also found an inverse correlation between PBMC IL-15 mRNA levels at the onset of the relapse and the time elapsed since the previous attack, as well as an absence of correlation between IL-15 mRNA levels and the patient demographic and clinical characteristics. Results in the present work further suggest a role for IL-15 in MS pathophysiology.

Braun, A., P. Wiebe, et al. (1999). "Differential modulation of human immunoglobulin isotype production by the neuropeptides substance P, NKA and NKB." Journal of Neuroimmunology **97**(1-2): 43.

<http://www.sciencedirect.com/science/article/B6T03-3WS61MX-7/2/10ed0fa9c335c765b28aef507c095727>

The modifying effects of tachykinins substance P, neurokinin A and neurokinin B on immunoglobulin production were analyzed in an in vitro culture system. Purified human T- and B-cells were stimulated with TGF[beta]2 and IL-5 to induce preferential IgA production. Neuropeptides had the following effects. (1) The levels of IgA and IgG4 production were enhanced by IL-5 and TGF[beta]2; IgA levels remained constant or were slightly augmented by neuropeptides, whereas IgG4 was further augmented. (2) IL-5 and TGF[beta]2 did not alter IgG3 production, but neuropeptides stimulated secretion of this subclass. (3) IgG1 and IgM production were inhibited by IL-5 and TGF[beta]2. This effect was prevented by neuropeptides. (4) Other isotypes including IgG2 and IgE remained unaffected. Except for IgM, these effects were blocked by specific receptor antagonists indicating specificity. The tachykinin receptor NK-1 mRNA was detected in B- and T-cells, whereas NK-3 mRNA was only present in T- and B-cell coculture following activation. Furthermore, neuropeptide effects depended on cytokine co-stimulation and the presence of T-cells. These results suggest that neuropeptides are potent modifiers of preferential IgA synthesis.

Breijl, E. C. W., W. L. van der Pol, et al. (2003). "No association of Fc[gamma]RIIa, Fc[gamma]RIIIa and Fc[gamma]RIIIb polymorphisms with MS." Journal of Neuroimmunology **140**(1-2): 210.

<http://www.sciencedirect.com/science/article/B6T03-491GS5M-8/2/eafc30d47bdc4254bfdc5c5b86ba2cf7>

Anti-myelin IgGs occur in the cerebrospinal fluid (CSF) and serum of multiple sclerosis (MS) patients, and can induce inflammatory effector functions in leukocytes by crosslinking IgG receptors (Fc[gamma]R). The efficiency of Fc[gamma]R-mediated inflammatory processes is affected by functional polymorphisms of three Fc[gamma] receptors (Fc[gamma]RIIa, Fc[gamma]RIIIa, Fc[gamma]RIIIb). The relevance of Fc[gamma]R polymorphisms in MS was evaluated by studying the distribution of Fc[gamma]RIIa, Fc[gamma]RIIIa and Fc[gamma]RIIIb genotypes in 432 MS patients and 515 healthy controls. No significant differences were found between MS patients and controls, or between subgroups of patients. We conclude that Fc[gamma] receptor polymorphisms influence neither susceptibility nor clinical disease course of MS.

Carlson, K. A., J. Limoges, et al. (2004). "OTK18 expression in brain mononuclear phagocytes parallels the severity of HIV-1 encephalitis." Journal of Neuroimmunology **150**(1-2): 186.

<http://www.sciencedirect.com/science/article/B6T03-4C09KS6-1/2/79a3e718c365989b2d693ad4b037a167>

OTK18 was isolated by mRNA differential display of human monocyte-derived macrophages (MDM) infected with human immunodeficiency virus type one (HIV-1). Northern blot and real-time reverse transcription polymerase chain reaction showed low levels of OTK18 expression in human tissue, which markedly increased during advanced HIV-1 encephalitis (HIVE). Immunocytochemistry, using rabbit polyclonal antisera, showed OTK18 localized to brain mononuclear phagocytes (MP) in moderate to severe HIV-1 encephalitis. OTK18 expression was selective and not found in HIV-1-infected brain tissue with limited neuropathological abnormalities, nor in cytomegalovirus encephalitis, multiple sclerosis, Alzheimer's disease, or uninfected control brains. Thus, OTK18 expression in brain mononuclear phagocytes is a signature for advanced HIV-1 encephalitis.

Charles, P. C., K. S. Weber, et al. (1999). "Cytokine, chemokine and chemokine receptor mRNA expression in different strains of normal mice: implications for establishment of a Th1/Th2 bias." Journal of Neuroimmunology **100**(1-2): 64.

<http://www.sciencedirect.com/science/article/B6T03-3YC05J7-9/2/6dda8cfd3b491dfc1490c8ab514194e9>

The resistance or susceptibility of inbred strains of mice to various pathogens and autoimmune diseases such as EAE has been linked to differences in the balance between cytokines associated with Th1- and Th2-type immune responses. Previous work from this laboratory on the mouse strain specific resistance to mouse adenovirus type I (MAV-1)-induced encephalopathy revealed subtle differences in the transcription rates of several immunologically important molecules that was evident prior to infection. In this study, we show striking differences in cytokine, chemokine and chemokine receptor mRNA expression in the spleens of normal, immunologically naive C57BL/6J, BALB/cJ and SJL/J mice. Messenger RNAs for interferon (IFN)-[gamma] and the chemokine IFN [gamma] inducible protein (IP)-10 were preferentially expressed in C57BL/6J spleens, whereas in BALB/cJ spleens mRNAs for lymphotoxin-[beta], interferon-

[beta], transforming growth factor-[beta], and the chemokine receptors CCR3 and CXCR4 predominated. A unique profile of chemokine receptors was found in spleens from normal SJL/J mice that correlated with the presence of polymorphisms within the CCR-3 gene. The patterns of gene expression fit well into the Th1/Th2 paradigm for C57BL/6J and BALB/cJ strains and suggest an important role for chemokines, as well as cytokines, in contributing to the genetic basis of the immune response.

Chataway, J., S. Sawcer, et al. (1999). "A screen of candidates from peaks of linkage: evidence for the involvement of myeloperoxidase in multiple sclerosis." Journal of Neuroimmunology **98**(2): 208.

<http://www.sciencedirect.com/science/article/B6T03-3WXNYG7-H/2/a11f7977dd34eebeb084cd06f0ba4f00>

We tested 11 microsatellite markers for evidence of transmission distortion in 744 trio families with multiple sclerosis. Ten of the markers lie within or near to candidate genes selected on the basis that they map within the regions of potential linkage identified in our previously reported linkage genome screen, while the eleventh is an anonymous marker which had previously shown modest evidence for transmission distortion in our sibling pair families. Only the marker related to the myeloperoxidase (MPO) gene revealed tentative evidence for linkage disequilibrium and further work on this gene is clearly needed in order to resolve the status of this region in conferring susceptibility to multiple sclerosis.

Chataway, J., S. Sawcer, et al. (1999). "No evidence for association of multiple sclerosis with the complement factors C6 and C7." Journal of Neuroimmunology **99**(1): 150.

<http://www.sciencedirect.com/science/article/B6T03-3Y9H4FG-M/2/006eb49aaead687e5912560850d8ed38>

Four genome screens in multiple sclerosis have been completed and each has identified evidence for linkage in the pericentromeric region of chromosome 5. This region encodes a number of candidate genes including those for the complement components C6, C7 and C9. We have used a multiplexed oligoligation assay (OLA) to test single nucleotide polymorphisms (SNPs) from the C6 and C7 genes for evidence of association with multiple sclerosis in our sibling pair families. There was no statistically significant difference in the allele frequencies of these polymorphisms in the index cases from our families when compared with locally derived controls. No evidence for transmission distortion was seen with any of the polymorphisms, or with the haplotype built from the three SNPs from the C7 gene. Despite offering themselves as potential candidates these complement genes appear not to confer susceptibility to multiple sclerosis.

Cross, A. H., R. M. Keeling, et al. (1996). "Inducible nitric oxide synthase gene expression and enzyme activity correlate with disease activity in murine experimental autoimmune encephalomyelitis." Journal of Neuroimmunology **71**(1-2): 145.

<http://www.sciencedirect.com/science/article/B6T03-3R867H0-N/2/398eccff619d17193412e3b089c7f4d>

Messenger RNA encoding inducible NO synthase (iNOS) was measured by competitive reverse transcriptase polymerase chain reaction (cRT-PCR) and ribonuclease protection assays in spinal cords from mice at varying stages of experimental allergic encephalomyelitis (EAE) and from

control mice. iNOS mRNA was increased in spinal cords from mice with acute EAE. cRT-PCR assays revealed a 10-20-fold increase in iNOS mRNA in spinal cords during acute EAE compared with the level observed in normal mouse spinal cords. Functional iNOS activity, as assessed by assay of calcium-independent citrulline production, was also significantly increased in spinal cords from mice with acute EAE in comparison to normal controls. The correlation of functional iNOS expression with active disease in EAE is consistent with a pathogenic role for excess NO in this model of cell-mediated central nervous system autoimmunity.

DeVry, C. G., M. Valdez, et al. (2004). "RDP58, a novel immunomodulatory peptide, ameliorates clinical signs of disease in the Lewis rat model of acute experimental autoimmune encephalomyelitis." Journal of Neuroimmunology **152**(1-2): 33.

<http://www.sciencedirect.com/science/article/B6T03-4CPP63V-4/2/48b93709658bd511b2083b1e6b810abe>

The therapeutic value of a novel immunomodulatory peptide, RDP58, was investigated in the acute experimental autoimmune encephalomyelitis (EAE) model of Multiple Sclerosis (MS). RDP58 is a 10-amino acid peptide with two major activities: (i) inhibition of inflammatory TH1 cytokines such as TNF[alpha], IFN[gamma], and IL12 and (ii) up-regulation of heme oxygenase-1 (HO-1) expression. Experiments in which EAE-induced Lewis rats exhibit an acute monophasic episode of disease demonstrated that a single intracerebroventricular injection of RDP58 is effective in preventing clinical signs of disease. The therapeutic effect on disease activity was observed at all pre-onset administration times and at all doses tested. Consistent with disease activity in vivo, RDP58-treated animals had reduced cellular infiltration within the spinal cord along with decreased TNF[alpha] expression levels. The data in this proof of concept study support the premise that RDP58, as a platform molecule, may be a promising new therapeutic intervention in autoimmune and inflammatory diseases.

Elovaara, I., U. Utz, et al. (1995). "Limited T cell receptor usage by HTLV-I tax-specific, HLA class I restricted cytotoxic T lymphocytes from patients with HTLV-I associated neurological disease." Journal of Neuroimmunology **63**(1): 47.

<http://www.sciencedirect.com/science/article/B6T03-4002J2F-M/2/26443ea37a654ce0141374195444a3b5>

T cell receptor (TCR) V[alpha] and V[beta] chain usage of HTLV-I tax-specific, HLA class I restricted CD8+ cytotoxic T cells (CTL) was determined from lymphocytes obtained from peripheral blood of patients with HTLV-I associated neurological disease. To characterize TCR repertoire, CD8+ lymphocytes from peripheral blood were cloned in limiting dilution, and the resulting wells were screened for HTLV-I-specific precursor CTL activity. RNA was isolated from HLA-A2 restricted HTLV-I tax peptide-specific (tax 11-19; LLFGYPVYV) CD8+ CTL lines and cDNA was analyzed by PCR amplification using V[alpha] and V[beta] chain family-specific oligonucleotide primers. The results indicate that CD8+ cytotoxic T cell lines from HLA-A2 HAM/TSP patients express a limited repertoire of T cell receptor chains which may correlate with duration and severity of disease. The restricted use of TCR genes expressed by antigen-specific CTL may play a critical role in the pathogenesis of HAM/TSP and may be of value in developing immunotherapeutic strategies that focus on eliminating these cells or inhibiting their activity.

Floris, S., S. R. Ruuls, et al. (2002). "Interferon-[beta] directly influences monocyte infiltration into the

central nervous system." Journal of Neuroimmunology **127**(1-2): 69.

<http://www.sciencedirect.com/science/article/B6T03-45NGR1M-1/2/4f80169144df9e63ab2767cdcf88c273>

Interferon-[beta] (IFN-[beta]) has beneficial effects on the clinical symptoms of multiple sclerosis (MS) patients, but its exact mechanism of action is yet unknown. We here suggest that IFN-[beta] directly modulates inflammatory events at the level of cerebral endothelium. IFN-[beta] treatment resulted in a marked reduction of perivascular infiltrates in acute experimental allergic encephalomyelitis (EAE), the rat model for MS, which was coupled to a major decrease in the expression of the adhesion molecules ICAM-1 and VCAM-1 on brain capillaries. In vitro, IFN-[beta] reduced the mRNA levels and protein expression of adhesion molecules of brain endothelial cell cultures and diminished monocyte transendothelial migration. Monocyte adhesion and subsequent migration was found to be predominantly regulated by VCAM-1. These data indicate that IFN-[beta] exerts direct antiinflammatory effects on brain endothelial cells thereby contributing to reduced lesion formation as observed in MS patients.

Fujii, T., N. Ushiyama, et al. (2002). "Effects of human antithymocyte globulin on acetylcholine synthesis, its release and choline acetyltransferase transcription in a human leukemic T-cell line." Journal of Neuroimmunology **128**(1-2): 1.

<http://www.sciencedirect.com/science/article/B6T03-45V716F-1/2/e7bf261425e7842c25a4ec782ac473ce>

Lymphocytes possess an independent, nonneuronal cholinergic system. In the present study, we investigated the short- and long-term effects of antithymocyte globulin (ATG)-Fresenius (ATG-F), a human antithymocyte globulin that binds to CD2, CD7 and CD11a, on acetylcholine (ACh) synthesis and transcription of choline acetyltransferase (ChAT) in CCRF-CEM cells, a human leukemic T-cell line. In the short-term (6 h), ATG-F enhanced ACh release, likely through transient increases in intracellular Ca^{2+} ($[Ca^{2+}]_i$) mediated by CD7, which led to declines in intracellular ACh content. By 48 h, however, the ACh content had increased as compared to control due to up-regulation of ChAT expression mediated by CD11a.

Fujii, T., S. Yamada, et al. (1998). "Induction of choline acetyltransferase mRNA in human mononuclear leukocytes stimulated by phytohemagglutinin, a T-cell activator." Journal of Neuroimmunology **82**(1): 101.

<http://www.sciencedirect.com/science/article/B6T03-3SR9W9C-G/2/5fb9e082b826e109d86bc5dd93d250a8>

The induction of mRNA for choline acetyltransferase (ChAT), which catalyzes acetylcholine (ACh) synthesis was investigated in human mononuclear leukocytes (MNL) stimulated by phytohemagglutinin (PHA), a T-cell activator, using the reverse transcription-polymerase chain reaction. Stimulation of MNL by PHA induced the expression of ChAT mRNA, and potentiated ACh synthesis. ChAT mRNA induction required more time than the induction of interleukin-2 mRNA. Expression of the gene encoding the vesicular ACh transporter, which mediates ACh transport in cholinergic neurons, was not observed in PHA-stimulated MNL, suggesting that the mechanisms controlling ACh release from T-lymphocytes differ from those in cholinergic neurons. These findings demonstrate that activation of T-lymphocytes up-regulates ACh synthesis in the blood, and suggest that ACh plays an important role as a neuroimmunomodulator besides its role as a neurotransmitter.

Fyhr, I.-M., A.-R. Moslemi, et al. (1998). "T cell receptor [beta]-chain repertoire in inclusion body myositis." Journal of Neuroimmunology **91**(1-2): 129.

<http://www.sciencedirect.com/science/article/B6T03-3V5VDX7-K/2/5022ebb62f3b601e7ec172e1bb382457>

Inclusion body myositis (IBM) is the most common muscle disease affecting individuals over 50 years of age. The inflammatory reaction is characterized by cell infiltrates predominated by CD8+ cytotoxic T cells. To analyze clonality of muscle infiltrating lymphocytes, we studied the complementarity determining region 3 (CDR3) length distribution of the T cell receptor (TCR). Muscle infiltrating lymphocytes were studied in three IBM patients and compared with peripheral blood lymphocytes (PBL) in two of these patients. The study was performed by reverse transcription polymerase chain reaction (RT-PCR) of RNA extracted from muscle tissue and PBL followed by analysis of fragment length distribution of the CDR3 region in each of 24 different V[beta] families. There was a restricted usage of TCR V[beta] gene families in muscle infiltrating T cells in all three patients. Some of the TCR V[beta] gene families showed oligoclonal expansions but polyclonal patterns were dominating. The CDR3 distribution of most V[beta] families differed between muscle infiltrating lymphocytes and PBL indicating that T cells have expanded locally or selectively accumulated in muscle.

Goldmuntz, E. A., R. L. Wilder, et al. (1993). "The origin of the autoimmune disease-resistant LER rat: An outcross between the Buffalo and autoimmune disease-prone Lewis inbred rat strains." Journal of Neuroimmunology **44**(2): 215.

<http://www.sciencedirect.com/science/article/B6T03-476W9PJ-7C/2/e5c0f182c0482ff214cd8266eeedd8d5>

The Lewis (LEW) rat strain is highly susceptible to a large number of experimentally induced inflammatory and autoimmune diseases. The Lewis resistant (LER) rat strain, which reportedly arose as a spontaneous mutation in a closed colony of LEW rats, is resistant to many of these disorders. The mechanism of resistance is not yet clear. We report the analysis of 19 simple dinucleotide repeat polymorphisms in 13 rat strains including the LEW/N and LER/N rat strains. The LEW/N and LER/N alleles were the same in only 42% of cases. For all of the other polymorphisms, the LER/N and Buffalo (BUF/N) rat strain alleles were identical. These data provide evidence that the LER strain did not arise as a spontaneous mutation in the LEW strain but is the result of an outcross between the LEW and BUF rat strains. The LER rat strain is now a recombinant inbred rat strain. This information should facilitate the genetic analysis of the loci responsible for resistance to experimental autoimmune disease in the LER rat.

Guo, C.-J., J.-P. Lai, et al. (2002). "Substance P up-regulates macrophage inflammatory protein-1[beta] expression in human T lymphocytes." Journal of Neuroimmunology **131**(1-2): 160.

<http://www.sciencedirect.com/science/article/B6T03-46WV6NX-6/2/4406c82190b5431c55606624ffa4d78b>

Substance P (SP) is an important modulator of neuroimmunoregulation. We have demonstrated that human T lymphocytes express SP and neurokinin-1 receptor (NK-1R), a primary SP receptor. In the present study, we investigated whether SP stimulates synthesis of macrophage inflammatory protein-1[beta] (MIP-1[beta]) in human T lymphocytes. SP significantly enhanced

MIP-1[beta] expression at both the mRNA and protein level in a human T cell line (Jurkat) containing the SP receptor gene (J-SPR) as determined by real-time PCR and ELISA assays. SP-induced MIP-1[beta] expression is abrogated by the specific NK-1R antagonist (CP-96,345). The supernatants from SP-stimulated J-SPR T cell cultures enhanced T lymphocyte chemotaxis in vitro, indicating functional activity of SP-induced MIP-1[beta]. In addition, SP augmented secretion of MIP-1[beta] from primary cultures of peripheral blood lymphocytes (PBL) isolated from some of the donors. This donor variability was due to differential expression of the primary SP receptor (NK-1R) on PBL from different donors. PBL from two of seven donors that did not respond to SP stimulation had undetectable NK-1R expression. Our mechanistic studies showed that SP activated NF-[kappa]B promoter-directed luciferase activity, which may be responsible for its effect on MIP-1[beta] expression in human T cells. Our data provide a potential mechanism by which SP selectively influences cellular immune responses such as [beta]-chemokine expression in human T lymphocytes through NK-1R, which may have an important in vivo implication in inflammatory diseases.

Hackstein, H., A. Bitsch, et al. (2001). "Analysis of interleukin-4 receptor [alpha] chain variants in multiple sclerosis." *Journal of Neuroimmunology* **113**(2): 240.

<http://www.sciencedirect.com/science/article/B6T03-427JWJW-9/2/128f9e707281c5647e0ffc1b474a8027>

A recent candidate gene study employing microsatellite markers suggested a possible linkage of multiple sclerosis (MS) with the interleukin-4 receptor (IL4R) gene. Consequently, we investigated the association of different IL4R variants with MS in 341 german MS patients and 305 healthy controls. Analysis of the first 100 MS patients for six IL4R variants showed an increased frequency of the R551 variant in MS patients versus healthy controls and carriage of the same IL4R variant was weakly associated with myelin oligodendrocyte glycoprotein (MOG) autoantibody production. However, further analysis of all 341 MS patients did not confirm the finding that this IL4R variant represents a general genetic risk factor for MS but revealed an increased frequency of the R551 variant in MS patients with primary progressive MS (PPMS, n=48) as compared to patients with relapsing remitting MS or secondary progressive MS (RR/SPMS n=284; P=0.005 for genotype differences) and to 305 healthy controls (P=0.001 for genotype differences). This association was statistically independent of the presence of the well-known MS susceptibility allele HLA-DRB1*15. After correction for multiple comparisons only the genotype differences between PPMS patients and healthy controls remained statistically significant. These results indicate, that the IL4R variant R551 may influence the genetic predisposition for PPMS but does not represent a general genetic risk factor for MS.

Hardin-Pouzet, H., P. Giraudon, et al. (1996). "Cytokines are increased in the rat hippocampus after serotonergic neuron degeneration and upregulate the expression of GDH, an enzyme involved in glutamate detoxification." *Journal of Neuroimmunology* **69**(1-2): 117.

<http://www.sciencedirect.com/science/article/B6T03-49GTP2H-19/2/6932d464541a8f40161aff277bcc5a8a>

The degeneration of serotonergic neurons increases the expression of glutamate dehydrogenase (GDH) in hippocampal astrocytes. This process was demonstrated to be independent of the serotonin level. At the same time, upregulation of tumor necrosis factor (TNF) α and interleukin (IL)-1[alpha] mRNA were observed, whereas levels of transforming growth factor (TGF) [beta]1 mRNA remained unchanged. The level of GDH mRNA was increased in primary cultures of hippocampal astrocytes treated with TNF[alpha] and IL-1[alpha] suggesting that these cytokines act on the GDH metabolism. TNF α and IL-1[alpha] induced an increase in GDH promoter activity

in C8S (an astrocytic cell line) transfected with constructs containing 5' flanking genomic sequences of GDH driving the expression of a reporter gene. These observations suggest that cytokines may be signals that upregulate the astrocytic GDH expression in response to the degeneration of serotonergic terminals in the hippocampus.

He, B., V. Navikas, et al. (1995). "Tumor necrosis factor [alpha]-308 alleles in multiple sclerosis and optic neuritis." Journal of Neuroimmunology **63**(2): 143.

<http://www.sciencedirect.com/science/article/B6T03-4002J1P-5/2/2c722b17a858484652efeaefdf753399>

Tumor necrosis factor-[alpha] (TNF-[alpha]), a proinflammatory cytokine, is believed to play an important role in multiple sclerosis (MS) pathogenesis. A bi-allelic polymorphism in the TNF-[alpha] promoter region (TNF[alpha]-308), has been reported to influence levels of TNF-[alpha] production. In the present study, we investigated the TNF[alpha]-308 polymorphism in 93 patients with MS, 17 patients with optic neuritis (ON) and 95 healthy individuals using an allele-specific PCR technique. Allelic genotype was compared with TNF-[alpha] mRNA expression levels and HLA class II phenotypes. No significant difference regarding the TNF[alpha]-308 polymorphism was observed between MS patients and controls. Specifically, the less common allele, TNF2, which is associated with higher expression levels of TNF-[alpha], was somewhat less frequent among MS patients. In fact, analysis of 19 patients homozygous for the MS associated HLA-DR-DQ haplotype HLA-Dw2 showed that this haplotype does not carry the TNF2 allele. In addition, in 47 patients, the TNF-[alpha] alleles did not correlate with expression levels measured as numbers of TNF-[alpha] expressing cells. Thus, we found no evidence for an important role of TNF[alpha]-308 polymorphism for genetic susceptibility to MS.

Hewett, S. J., T. P. Misko, et al. (1996). "Murine encephalitogenic lymphoid cells induce nitric oxide synthase in primary astrocytes." Journal of Neuroimmunology **64**(2): 201.

<http://www.sciencedirect.com/science/article/B6T03-3VXHJX1-D/2/eeb3c53904ce561b0cb253ab169fe335>

A cytokine-inducible form of nitric oxide synthase (iNOS), capable of producing large quantities of nitric oxide (NO), can be induced in many cell types. We demonstrate that conditioned medium from encephalitogenic myelin basic protein-sensitized lymphoid cells (MBP-CM) induces the expression of iNOS in primary cultures of murine astrocytes in a time- and concentration-dependent manner. iNOS mRNA was detected by reverse transcriptase-polymerase chain reaction (RT-PCR) as early as 3 h post-exposure. Accumulation of nitrite into the astrocyte culture medium, an indirect measure of NO, was measurable 3 h post-exposure, plateaued at 24 h, and was prevented by the simultaneous administration of the NOS inhibitors, -NG-nitroarginine methyl ester, NG-nitro--arginine or aminoguanidine. Astrocyte expression of iNOS protein, detected by immunohistochemistry and immunoprecipitation/Western blot, was prevented by inhibitors of RNA or protein metabolism, consistent with its dependence on de novo protein synthesis.

Horiuchi, Y., T. Fujii, et al. (2003). "The endogenous, immunologically active peptide apelin inhibits lymphocytic cholinergic activity during immunological responses." Journal of Neuroimmunology **144**(1-2): 46.

<http://www.sciencedirect.com/science/article/B6T03-49W2KP6-4/2/b9052cc9ad50e308cefb0615d3f156b6>

We investigated the effects of apelin, an immunologically active peptide ligand for orphan receptor APJ, on acetylcholine (ACh) synthesis in MOLT-3 human leukemic T cells. We initially confirmed expression of APJ mRNA in several human T- and B-cell lines by reverse transcription-polymerase chain reaction (RT-PCR). We also found that in phytohemagglutinin (PHA)-stimulated MOLT-3 cells, an active apelin fragment, apelin-13, down-regulates expression of choline acetyltransferase (ChAT) mRNA and significantly reduces ChAT activity and cellular ACh content and release. It thus appears that apelin inhibits lymphocytic cholinergic activity via APJ during immunological responses.

House, S. D., X. Mao, et al. (2001). "Chronic morphine potentiates the inflammatory response by disrupting interleukin-1[beta] modulation of the hypothalamic-pituitary-adrenal axis." Journal of Neuroimmunology **118**(2): 277.

<http://www.sciencedirect.com/science/article/B6T03-43N673R-B/2/69b056794a9c791b29b84ae0c65a8b1e>

Interleukin-1-beta (IL-1[beta]) can promote inflammation by up-regulating vascular adhesion molecules and inhibit inflammation by activating the hypothalamic-pituitary-adrenal (HPA) axis to produce anti-inflammatory glucocorticoids. In this study, chronic morphine was shown to suppress IL-1[beta]-induction of corticotropin releasing factor (CRF) mRNA and plasma corticosterone levels. Leukocyte-endothelial adhesion (LEA) in rat mesenteric venules increased during IL-1[beta]- and FMLP-induced inflammation. Chronic morphine potentiated the LEA response to either IL-1[beta] or FMLP alone, and greatly enhanced LEA in response to combined IL-1[beta] and FMLP. Thus, it appears that chronic morphine exposure may promote a potentially damaging inflammatory reaction by disrupting the balance between IL-1[beta]-mediated local inflammation and the anti-inflammatory effects of the HPA axis.

Hvas, J., J. R. Oksenberg, et al. (1993). "[gamma][delta] T cell receptor repertoire in brain lesions of patients with multiple sclerosis." Journal of Neuroimmunology **46**(1-2): 225.

<http://www.sciencedirect.com/science/article/B6T03-47DKV NK-62/2/8821bee32de713ea71f69b1ea6917720>

The identification of activated T cells in the brains of patients with multiple sclerosis (MS) suggests that these cells are critical in the pathogenesis of this disease. Recently we have used the PCR method to analyse rearrangements of V[alpha] and V[beta] genes of the T cell receptor (TCR) in samples of MS and control brains. The results of these studies showed that TCR V gene usage in MS brains may be restricted and in particular that V[beta] genes may be preferentially rearranged in certain HLA haplotypes associated with susceptibility to MS. In view of the recent evidence that T lymphocytes bearing the [gamma][delta] chains may have autoreactive potential, we have assessed whether or not such TCR-bearing lymphocytes were also present in chronic MS lesions. TCR V[gamma] and V[delta] were analysed by the PCR method using a panel of V[gamma] and V[delta] primers paired with C[gamma] or C[delta] primers in 12 MS brains, as well as in brain samples of ten normal post-mortem cases and three neurological controls. TCR V[gamma]---C[gamma] and V[delta]---C[delta] rearrangements were confirmed using Southern blotting and hybridisation of the PCR products with specific C[gamma] and C[delta] probes. Only one to four rearranged TCR V[gamma] and V[delta] transcripts were detected in each of the 23 brain samples obtained from 12 MS patients, with the majority of [gamma][delta] T cells

expressing the V[gamma]2 and V[delta]2 chains. In marked contrast, V[gamma] and V[delta] transcripts could only be found in one of the ten non-neurological control brains analysed. To assess the clonality of V[gamma]2 and V[delta]2 T cell receptor chains in the brain samples of MS patients, we have sequenced the junctional regions of the TCR V[gamma]-N-J[gamma]-C[gamma] and V[delta]-N-D[delta]-N-J[delta]-C[delta] segments amplified from brain tissues, CSF and spleens of two MS patients and from the spleen of two control subjects. The sequence analysis obtained so far shows no compelling evidence of an MS specific expansion of one or more clones expressing particular types of [gamma][delta] T cell receptors. In contrast, a clonal expansion of a different population of TCR [gamma][delta]-bearing T cells was found in the spleen of both an MS patient and one of the control individuals. These results suggests that the [gamma][delta] T cell response at the site ohronic lesions, involve a number of clones, possibly in response to several inflammatory antigenic stimuli. Whether or not [gamma][delta] T cells are involved in the initiation of or in the chronicity of the disease remains to be elucidated.

Jander, S. and G. Stoll (1998). "Differential induction of interleukin-12, interleukin-18, and interleukin-1[beta] converting enzyme mRNA in experimental autoimmune encephalomyelitis of the Lewis rat." Journal of Neuroimmunology **91**(1-2): 93.

<http://www.sciencedirect.com/science/article/B6T03-3V5VDX7-D/2/80b7055b9e70e206cee62e3634637763>

Experimental autoimmune encephalomyelitis (EAE) is a model of autoimmune central nervous system (CNS) disease that is mediated by autoreactive Th1 cells secreting the proinflammatory cytokine interferon (IFN)-[gamma]. Interleukin (IL)-12 in its heterodimeric p35/p40 isoform and the recently described cytokine IL-18 potently induce T cell production of IFN-[gamma]. Interleukin-1[beta] converting enzyme (ICE) is required to convert IL-18 precursor protein into its biologically active mature form. In this study, we used semiquantitative reverse transcriptase-polymerase chain reaction to determine steady state levels of IL-12, IL-18, and ICE mRNA in the spinal cord of Lewis rats at different stages of EAE. In control rats, we found significant IL-18, ICE, and IL-12p35, but not IL-12p40 mRNA expression. IL-18 mRNA increased during the acute stage of EAE together with a marked induction of ICE mRNA. IL-12p35 mRNA levels did not change significantly throughout the course of EAE. Surprisingly, the peak expression of IL-12p40 mRNA was delayed by several days relative to the peak of T cell infiltration and IFN-[gamma] mRNA synthesis. Our data implicate the IL-18/ICE pathway in the amplification of Th1-mediated immune responses in the CNS but suggest a different, so far undefined role of endogenous IL-12 in the late effector phase of EAE.

Kim, J. S., S. C. Gautam, et al. (1995). "Expression of monocyte chemoattractant protein-1 and macrophage inflammatory protein-1 after focal cerebral ischemia in the rat." Journal of Neuroimmunology **56**(2): 127.

<http://www.sciencedirect.com/science/article/B6T03-3YYTF9M-2/2/2d2963a185399a09cb5d5220b36fcc35>

Chemoattractant cytokines, the chemokines, play an important role in early events of inflammation at the site of tissue damage. We examined the expression of mRNA and the protein products of two such chemokines; i.e. monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1[alpha] (MIP-1[alpha]) in the ischemic brain tissue following middle cerebral artery occlusion (MCAo). The mRNA transcripts of MCP-1 and MIP-1[alpha] were detected by Northern hybridization and reverse transcriptase polymerase chain reaction (RT/PCR), respectively, and the anatomic distribution of specific proteins was analyzed by immunohistochemistry. We found that MCP-1 mRNA was not expressed in the brains of normal

rats or rats sacrificed 2 h after MCAo. 6 h after the induction of cerebral ischemia, weak expression of both mRNAs was detected in the ischemic tissue. mRNAs were expressed up to 48 h, and were markedly attenuated at 96 h. In the rats subjected to MCA occlusion, MCP-1 immunoreactivity was diffusely expressed and localized to the ischemic area, and was most intense at 48 h after MCA occlusion. Endothelial cells and macrophage-like cells expressed MCP-1 protein in the ischemic brain. The distribution and morphology of MIP-1[alpha] immunoreactive cells were identical with activated astrocytes. We conclude that MCP-1 and MIP-1[alpha] mRNAs and proteins are induced after cerebral ischemia in the rat. They may have a role in promoting inflammatory and/or repair processes in the ischemic brain, possibly by attracting or modulating inflammatory cells in the ischemic area.

Knezevic-Cuca, J., K. B. Stansberry, et al. (2000). "Neurotrophic role of interleukin-6 and soluble interleukin-6 receptors in N1E-115 neuroblastoma cells." Journal of Neuroimmunology **102**(1): 8.

<http://www.sciencedirect.com/science/article/B6T03-3Y9HBD2-2/2/8c2f02e244f65b8cde0a084abafd72b5>

Interleukin 6 (IL-6) plays a role in physiological and pathophysiological processes in neuronal cells. We studied whether IL-6 plays a role in neuroblastoma cells in culture. These studies demonstrate that N1E-115 cells constitutively express IL-6 but not IL-6R. Exogenous IL-6 stimulated neuronal proliferation in a dose-dependent manner. Under serum-free conditions soluble IL-6 receptors (sIL-6R) alone or in combination with IL-6 exerted significant proliferative effects, while IL-6 alone failed to promote cell proliferation. Neutralizing anti-IL-6 antibody caused a 30-40% reduction in IL-6 mediated proliferation. Our results suggest the importance of IL-6/sIL-6R for proliferation and survival of N1E-115 adrenergic neuroblastoma cells.

Kuhlmann, T., M. Glas, et al. (2002). "Investigation of bax, bcl-2, bcl-x and p53 gene polymorphisms in multiple sclerosis." Journal of Neuroimmunology **129**(1-2): 154.

<http://www.sciencedirect.com/science/article/B6T03-4689C4N-2/2/acf5a393657dde644ca220d75d448102>

Clinical course, outcome, radiological features, severity, and histopathology are heterogenous in multiple sclerosis (MS). Since MS is considered to be a polygenic disease, the genetic background may at least partly be responsible for this variability. Some MS cases are histopathologically characterized by a dramatic oligodendrocyte loss that is in part caused by apoptosis. A dysregulated apoptotic elimination of self-reactive T cells may also contribute to disease susceptibility. To analyze genetic differences in the apoptosis regulating factors bcl-2, bax, bcl-x and p53 we investigated polymorphisms of these genes in 105 patients with a relapsing remitting disease course and 99 controls by PCR-SSCP and direct sequencing. We identified so far unpublished sequence alterations in the promotor region of the bcl-x gene, in exon 7 of the p53 gene, and in exon 1 of the bax gene. No differences were observed between MS patients and controls. Additional known polymorphisms were found in intron 3 of the bax gene and in exon 6 of the p53 gene. No significant differences in the frequency of gene sequence variations were found between MS patients and controls. The apoptosis genes studied here therefore appear less likely to be important effector genes in MS.

Lai, J.-P., S. D. Douglas, et al. (1998). "Human lymphocytes express substance P and its receptor." Journal of Neuroimmunology **86**(1): 80.

<http://www.sciencedirect.com/science/article/B6T03-3SY8T41-9/2/daebd5f4ebc8841ef3dcd3420b6936fc>

We present data demonstrating the gene expression of substance P (SP) and its receptor in human peripheral blood-isolated lymphocytes. Using reverse transcribed polymerase chain reaction (RT-PCR) assay, preprotachykinin-A (substance-P) mRNA is detected in human peripheral blood-isolated lymphocytes. Among the [alpha], [beta] and [gamma] transcripts of the SP gene, only the [beta] and [gamma] transcripts are detectable in these cells. These RT-PCR amplified transcripts are recognized by Southern blot assay using a specific SP probe. Direct DNA sequence analysis of the RT-PCR products from lymphocytes also confirmed the structure of these transcripts which are identical to those found in human neuronal cells. At the protein level, human lymphocytes produced endogenous SP as determined by an enzyme immunoassay. Capsaicin, a vanillyl fatty acid amide (ingredient of hot pepper), released preformed SP from lymphocytes. In addition, using RT/nested-PCR analysis, we identified the presence of mRNA for neurokinin-1 receptor (the receptor for SP) in human peripheral blood-isolated lymphocytes, which was confirmed by Southern blot and DNA sequencing analysis. The demonstration that human lymphocytes express SP and its receptor support the notion that SP is biologically involved in regulating the functions of these cells in an autocrine fashion.

Lai, J.-P., S. D. Douglas, et al. (1998). "Identification of a [delta] isoform of preprotachykinin mRNA in human mononuclear phagocytes and lymphocytes." *Journal of Neuroimmunology* **91**(1-2): 121.

<http://www.sciencedirect.com/science/article/B6T03-3V5VDX7-J/2/b2b9491ec410b96ceefaa977379f576>

We have characterized preprotachykinin (PPT-A) gene transcript splicing products and identified a fourth isoform of PPT-A mRNA transcript in human peripheral blood-isolated monocytes and PBL. Using RT-PCR, Southern blot analysis and nucleotide sequencing analysis, we have identified the four isoforms of PPT-A transcripts ([alpha], [beta], [gamma] and [delta]) in human peripheral blood-isolated monocytes and PBL. The [delta]-PPT transcript present in the immune cells lacks exons 4 and 6. The sequences of exons 3, 5 and 7 of [delta]-PPT transcript completely match those of [beta]-PPT transcript. The [alpha]-PPT and [beta]-PPT sequences in these cells are identical to those obtained by Tan and Too (GenBank accession number U37539) and Harmar et al. (Genbank accession number X54469), but differ by a single nucleotide from another entry by Chiwakata et al. (Genbank accession number M68906). In comparison to this latter sequence, there was a C->T change at amino acid position 87 (CCT->CTT) which may result in a Pro to Leu change. Identification of the new SP mRNA transcript in both human CNS and immune cells supports the concept of an important biological link between CNS and immune system.

Lai, J.-P., W.-Z. Ho, et al. (2002). "A non-peptide substance P antagonist down-regulates SP mRNA expression in human mononuclear phagocytes." *Journal of Neuroimmunology* **128**(1-2): 101.

<http://www.sciencedirect.com/science/article/B6T03-46086CK-1/2/c6e2b0035303983d1751235ad108c96c>

Substance P (SP), a potent modulator of neuroimmunoregulation, exerts its activity by binding to the neurokinin-1 receptor (NK-1R). The SP-NK-1R interaction is important in inflammation and viral infections, including HIV infection of human immune cells. We recently demonstrated that SP modulates HIV replication and that a non-peptide SP antagonist CP-96,345 inhibits HIV replication in human monocyte-derived macrophages (MDM) by affecting the SP-NK-1R

interaction. In order to examine the effect of the SP antagonist on SP mRNA expression, MDM was incubated with or without CP-96,345 in the presence or absence of HIV infection. SP mRNA expression in these cells was then determined by real-time PCR technology. The effect of CP-96,345 on chemokine gene expression was also investigated by using a cDNA array assay. CP-96,345 down-regulated SP mRNA expression and antagonized exogenous SP-enhanced SP expression at the mRNA level, suggesting that SP autocrine regulation was interrupted by CP-96,345. CP-96,345 inhibited HIV replication in MDM, associated with down-regulated SP mRNA expression in comparison to HIV infection controls. In parallel with down-regulated SP and CCR5 mRNA expression, cDNA array assays indicated that CP-96,345 treatment also inhibited IL-8 gene expression, while enhancing expression of fractalkine and monocyte chemoattractant protein-3 (MCP-3). Since SP plays an important role in inflammation and viral infections, these studies may have potential applications for therapeutic intervention of inflammation and viral infection of immune cells.

Ledeboer, A., A. Wierinckx, et al. (2003). "Regional and temporal expression patterns of interleukin-10, interleukin-10 receptor and adhesion molecules in the rat spinal cord during chronic relapsing EAE." *Journal of Neuroimmunology* **136**(1-2): 94.

<http://www.sciencedirect.com/science/article/B6T03-481D7XS-6/2/faf7f318ae98dc163cc89f64ea767164>

Adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) mediate leukocyte infiltration into the CNS, in experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis (MS). Because exogenous interleukin-10 (IL-10) inhibits ICAM-1 and VCAM-1 expression and clinical EAE, we hypothesize that endogenous IL-10 signaling may suppress expression of adhesion molecules. In a rat model of chronic relapsing EAE, expression levels of IL-10 and its receptor (IL-10R1), ICAM-1 and VCAM-1 mRNA in the spinal cord are markedly increased, whereas levels of IL-10 mRNA remain relatively low. The temporal pattern of mRNA and protein expression showed marked differences between spinal cord levels. During relapse, IL-10, IL-10R1, ICAM-1, VCAM-1 mRNA levels and neurological scores show positive correlations. We conclude that endogenous IL-10 is not a crucial factor inhibiting adhesion molecule expression in this model.

Li, Y., S. D. Douglas, et al. (2001). "Substance P enhances HIV-1 replication in latently infected human immune cells." *Journal of Neuroimmunology* **121**(1-2): 67.

<http://www.sciencedirect.com/science/article/B6T03-448FVSN-3/2/e120ffb63d3a1f11c1363831dd406bb2>

Substance P (SP) is a potent modulator of neuroimmunoregulation. SP receptors are present on human monocytes and T lymphocytes, and SP alters the function of these immune cells. We investigated the effects of SP on HIV-1 replication in latently infected human immune cells. SP significantly enhanced HIV-1 replication in the latently infected promonocytic cell line (U1) and T lymphocyte line (ACH-2) stimulated with tumor necrosis factor (TNF-[alpha]). When added to these cells in combination with TNF-[alpha], SP also enhanced HIV-1 gag gene expression in U1 and ACH-2 cells. This stimulatory effect of SP was associated with the activation of HIV-LTR (long terminal repeat) driven chloramphenicol acetyltransferase (CAT) gene expression, and could be blocked by pretreatment of U1 and ACH-2 cells with an SP receptor antagonist RP-67,580, indicating specific SP receptor-mediated regulation. Furthermore, the addition of SP to the cultures of latently infected peripheral blood mononuclear cells isolated from HIV-1-infected patients enhanced HIV-1 gag gene expression. Thus, SP may play a potentially important role as a positive regulator of HIV-1 replication in latently infected monocytes and lymphocytes. These

observations may have significant implications toward understanding the role of neuropeptide SP in the immunopathogenesis of HIV-1 infection and AIDS.

Liu, H.-C., J. K. Anday, et al. (2004). "Dual effects of morphine on permeability and apoptosis of vascular endothelial cells: morphine potentiates lipopolysaccharide-induced permeability and apoptosis of vascular endothelial cells." Journal of Neuroimmunology **146**(1-2): 13.

<http://www.sciencedirect.com/science/article/B6T03-4B4S3HG-1/2/902aea9ea50d23c1cda6c7cd54ab12c2>

Vascular endothelial cells (VEC) provide an essential protective barrier between the vascular system and underlying tissues. Using VEC barrier models of human coronary artery cells and human and rat brain microvascular endothelial cells, we investigated the mechanism by which morphine affects lipopolysaccharide (LPS)-induced VEC permeability. We demonstrated that co-administration of morphine and LPS induced greater VEC apoptosis and permeability than morphine or LPS alone. The extent of induced apoptosis appeared to be cell-type dependent. Furthermore, RT-PCR analysis revealed that morphine and LPS up-regulated Fas expression. These data suggest potential crosstalk between the signaling pathways that mediate morphine- and LPS-triggered apoptosis in brain VEC.

Maier, C. C., R. D. LeBoeuf, et al. (1993). "The structure of a myelin basic protein-associated idiotope." Journal of Neuroimmunology **46**(1-2): 235.

<http://www.sciencedirect.com/science/article/B6T03-47DKV NK-63/2/34c8e5bc7369ca1dc148997476723333>

A cross-reactive idiotope (CRI) has been previously described on monoclonal antibodies (mAbs) specific for encephalitogenic peptides from myelin basic protein (MBP). The anti-CRI mAb, F25F7, binds an idiotope (Id) localized to the light chains of an anti-MBP peptide 1-9 mAb, denoted F23C6, and an anti-MBP peptide 80-89 mAb, denoted 845D3. It is the purpose of this study to further delineate the CRI being recognized by F25F7. To this end, we have found a structural correlation between the CRI and the antigen, a small synthetic peptide, denoted PBM 9-1, used to elicit the anti-Id mAb. Sequence comparison between the light chain of F23C6 and PBM 9-1 reveals a region of homology in CDR 2/FWK 3. The configuration of this site in the VL, as determined by comparison with a mAb, HyHEL-10, whose structure has been determined and is 97% homologous to the light chain of F23C6, conforms to the rules used to define antigenic determinants or Ids. A synthetic peptide having the F23C6 VL CDR 2/FWK 3 sequence inhibited the binding of F25F7 to F23C6 and 845D3. Taken together, these data suggest the Id recognized by F25F7 is defined, in part, by the PBM 9-1-like sequence of F23C6.

Makarenkova, V. P., G. V. Shurin, et al. (2003). "Lung cancer-derived bombesin-like peptides down-regulate the generation and function of human dendritic cells." Journal of Neuroimmunology **145**(1-2): 55.

<http://www.sciencedirect.com/science/article/B6T03-4B3DT8W-5/2/d16ed0c758f9f3264d818f4596979c80>

Development of tumors is regulated by tumor-derived neuroendocrine factors, including bombesin-like peptides (BLP). We have evaluated neuroendocrine regulation of dendritic cell

(DC) maturation and function by both tumor-derived and purified bombesin (BOM), neuromedin B (NMB), gastrin-releasing peptide (GRP), and a BOM antagonist -Phe-bombesin (DPB). BOM, NMB and GRP dose-dependently inhibited maturation of DC assessed as down-regulation of CD40, CD80 and CD86 expression on DC. BOM and GRP also inhibited interleukin-12 (IL-12) production by DC and their ability to activate T cells. DPB partly abrogated immunosuppressive effect of tumor cells on DC. These data are a first evidence for the role of BLP in the regulation of DC maturation and function, demonstrating that BLP inhibit DC maturation and longevity in the lung cancer microenvironment. This suggests a new mechanism of tumor escape and provides new targets for the immunopharmacological correction of immune effectors in cancer.

Matsubara, S., T. Kitaguchi, et al. (2001). "Experimental allergic myositis in SJL/J mouse. Reappraisal of immune reaction based on changes after single immunization." Journal of Neuroimmunology **119**(2): 223.

<http://www.sciencedirect.com/science/article/B6T03-442XPR6-9/2/942b312a2a6e902590e3561b9fedd268>

SJL/J mice have been subjected to immunization with wide varieties of antigens to produce models of autoimmune disorders including experimental myositis. They also have a defect in dysferlin gene and spontaneously develop muscle fiber degeneration, a condition akin to limb-girdle type muscular dystrophy and Miyoshi myopathy. To know whether muscle inflammation of SJL mice after immunization with muscle fractions really represents immune-mediated myositis or no more than an epiphenomenon of muscle degeneration due to dysferlin defect, we studied immunological parameters after immunization with rabbit myosin B fraction. Initial infiltration of macrophages and CD4+ lymphocytes on day 11 was followed by increase in number of CD8+ cells. Such increase was not observed in the nontreated and adjuvant controls. Some infiltrating cells were interferon gamma (IFN-[gamma]) positive. Furthermore, increased expression of the signal transducers and activator of transcription 1 (STAT-1) and interferon regulatory factor 1 (IRF-1) mRNA was shown in the first 2 weeks. These results indicate Th1 system activity in the muscle, rather than simple dysferlin deficiency, particularly 1-3 weeks after immunization. Thus it is concluded that an immune-mediated myositis is taking place at this stage. This model can be helpful in understanding pathomechanisms involved in the early stage of human myositides. It has also important implications concerning immune reactions associated with transplantation or gene therapy for muscular dystrophies.

Matsumoto, Y., G. Kim, et al. (2000). "Characterization of T cell receptor associated with the development of P2 peptide-induced autoimmune neuritis." Journal of Neuroimmunology **102**(1): 67.

<http://www.sciencedirect.com/science/article/B6T03-3Y9HBD2-9/2/c724221608fce2ee1ed62e728471e419>

To characterize experimental autoimmune neuritis (EAN)-inducing T cells in more detail, we performed CDR3 spectratyping analysis and found oligoclonal expansion of several V[beta] spectratypes in nerve-infiltrating T cells. V[beta]5 expansion was observed all the stages examined, whereas V[beta]8.2 and V[beta]17 expansion was mainly found at the peak and preclinical stages, respectively. Since V[beta]5 expansion persists throughout the course of the disease, V[beta]5+ T cells are judged to be the main effector cells. V[beta]8.2+ and V[beta]17+ T cells may also be pathogenic but are not the main effectors because expansion of these spectratypes was found at a limited period of time. Sequence analysis revealed that V[beta]5, V[beta]8.2 and V[beta]17 spectratype-derived TCR clones possess their own dominant sequences in the CDR3 region with no homology among the clones. These findings suggest that polyclonally activated T cells are involved in the formation of the nerve lesion. Furthermore,

vaccination with V[beta]5 DNA, but not with V[beta]10 DNA, suppressed the development of EAN significantly. Collectively, these findings indicate that determination of autoimmune disease-associated TCR by CDR3 spectratyping provides useful information for designing TCR-based immunotherapy for the disease.

Misko, T. P., J. L. Trotter, et al. (1995). "Mediation of inflammation by encephalitogenic cells: interferon [gamma] induction of nitric oxide synthase and cyclooxygenase 2." Journal of Neuroimmunology **61**(2): 195.

<http://www.sciencedirect.com/science/article/B6T03-3YYV2B2-B/2/3e1c7e2d7e5d1107b0ddf10e3a975d1d>

Experimental autoimmune encephalomyelitis (EAE) is a T cell-mediated inflammatory demyelinating disorder of the central nervous system (CNS) which serves as a prime animal model for the human disease multiple sclerosis. Previous studies from these laboratories demonstrated excess nitric oxide (NO) in the CNS of EAE-affected mice, and amelioration of EAE with a selective inhibitor of the inducible nitric oxide synthase (iNOS). Recent studies from other laboratories have indicated that prostaglandin PGE₂ is increased in CNS tissues of EAE-affected rodents and that EAE is prevented by the inhibition of cyclooxygenase activity. The present study investigated the ability of encephalitogenic lymphoid cells to induce NOS and cyclooxygenase (COX-2) in the murine macrophage line, RAW 264.7. In order to mimic the extracellular milieu present in EAE lesions, conditioned medium (CM) of activated EAE-inducer cells was added to this macrophage line. CM caused a time-dependent increase in nitrite, indicating NO production. Reverse-transcriptase PCR demonstrated iNOS mRNA in RAW 264.7 cells, first detected at 3 h, and Western blots confirmed the induction in RAW cells of the 130-kDa iNOS protein. Production of nitrite by CM-exposed RAW 264.7 cells was blocked by inhibitors of NOS (-N-methylarginine or aminoguanidine) or by antibodies to murine IFN-[gamma] or IL-1[beta]. CM of activated encephalitogenic cells induced production of PGE₂ by RAW 264.7 cells, as determined by ELISA, and Western blots identified the presence of the 70-80-kDa inducible COX (COX-2) protein. Induction of COX-2 could be inhibited by antibody to IFN-[gamma]. Thus, encephalitogenic cells are capable of inducing the expression of the inflammatory enzymes iNOS and COX-2 in a murine macrophage line via the T cell cytokine IFN-[gamma], alone or in combination with IL-1[beta].

Niino, M., S. Kikuchi, et al. (2003). "Genetic polymorphisms of osteopontin in association with multiple sclerosis in Japanese patients." Journal of Neuroimmunology **136**(1-2): 125.

<http://www.sciencedirect.com/science/article/B6T03-47S6Y27-2/2/b63cf65b11e7e4d06b9f0ae7f6439f01>

Osteopontin (OPN) exhibits pleiotropic functions and abundant transcripts for OPN are present in brains of patients with multiple sclerosis (MS). The aim of this study was to investigate the role of OPN genes in the pathogenesis of MS. Polymorphisms at the 8090th, 9250th and 9583rd positions in OPN were detected by PCR-RFLP from DNAs of 116 MS Japanese patients and 124 healthy controls. The C/C genotype at the 8090th position in exon 6 was more prevalent in MS than in control (p=0.01) and A/A (age; 25.2±8.9 years, p=0.01) genotypes. There were no significant correlations between OPN gene polymorphisms and disease progression. Our results suggest that the 8090th polymorphism might be associated with susceptibility to MS, while the 9583rd polymorphism might be associated with age of onset of MS.

Omari, K. I. M. and K. Dorovini-Zis (2001). "Expression and function of the costimulatory molecules B7-1 (CD80) and B7-2 (CD86) in an in vitro model of the human blood-brain barrier." Journal of Neuroimmunology **113**(1): 129.

<http://www.sciencedirect.com/science/article/B6T03-41Y28FP-G/2/d5a182e24ffe4bbb2470d32971e36a9e>

The interaction of B7 molecules with their ligand provides important accessory signals for optimal T cell activation and proliferation. In this study the in vitro expression of B7-1 and B7-2 by human brain microvessel endothelial cells (HBMEC) was investigated by semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) and immunocytochemistry. In addition, the contribution of B7 molecules to T cell proliferation on cerebral endothelial cells was studied by coincubating purified CD4+ T cells with resting or cytokine activated HBMEC. Untreated cultures constitutively expressed B7-2 RNA and surface protein, but lacked B7-1 expression. Treatment with TNF-[alpha] and IFN-[gamma] upregulated B7-2 and induced de novo expression of B7-1. Monoclonal blocking antibodies to B7-1 or B7-2 and human CTLA-4Ig chimeric protein significantly reduced the ability of HBMEC to support [alpha]-CD3-induced proliferation of CD4+ T lymphocytes. Expression of B7 glycoproteins and the ability to provide secondary signals for T cell proliferation suggest a potential role of the human cerebral endothelium in T cell activation during the early stages of central nervous system inflammation.

Omari, K. M. and K. Dorovini-Zis (2003). "CD40 expressed by human brain endothelial cells regulates CD4+ T cell adhesion to endothelium." Journal of Neuroimmunology **134**(1-2): 166.

<http://www.sciencedirect.com/science/article/B6T03-47CXW39-2/2/7eada645c7068bf95570aeb37140eaf8>

Recent evidence suggests that interactions between CD40 on antigen presenting cells (APC) and CD40L on T cells generate signals that result in the activation of APC. In this study, the expression and function of CD40 was investigated in primary cultures of human brain microvessel endothelial cells (HBMEC). Results revealed constitutive expression of CD40 on untreated HBMEC. Stimulation with TNF-[alpha], IFN-[gamma], LPS or combination of TNF-[alpha] and IFN-[gamma] significantly upregulated CD40. The majority of CD40 molecules were localized on the apical surface of EC. Incubation of HBMEC with soluble CD40L resulted in increased expression of the adhesion molecules E-selectin, VCAM-1 and ICAM-1. Consequently, the adhesion of both resting and anti-CD3 activated CD4+ T lymphocytes to CD40L treated HBMEC was significantly increased compared to unstimulated EC. The expression of CD40 by cerebral endothelium, and endothelial cell activation following binding of CD40 to its ligand, CD40L, suggest a potential mechanism by which activated CD40L expressing T cells could enhance adhesion and migration of inflammatory cells across the blood-brain barrier (BBB) to sites of inflammation in the human central nervous system (CNS).

Palma, C. and S. Manzini (1998). "Substance P induces secretion of immunomodulatory cytokines by human astrocytoma cells." Journal of Neuroimmunology **81**(1-2): 127.

<http://www.sciencedirect.com/science/article/B6T03-3S4PGS1-H/2/cf62cd33062e1a6661c493fd9e912715>

In human astrocytoma cell lines, substance P (SP) stimulated interleukin (IL)-8, IL-6, granulocyte macrophage colony-stimulating factor and leukemia inhibitory factor protein secretion. These SP effects were blocked by a specific NK1 tachykinin receptor antagonist. Further, SP stimulation

increased the half-life of IL-6 and IL-8 messenger RNAs, suggesting that the synthesis of these cytokines is also regulated post-transcriptionally. SP-induced cytokine release was inhibited by staurosporine and phorbol 12-myristate 13-acetate desensitization suggesting protein kinase C involvement. The demonstration that SP affects cytokine production in glioma cells might be of relevance for the biology of such tumors.

Palma, C., F. Urbani, et al. (1995). "Interleukin-6 production by U373 MG, a human astrocytoma cell line: different pathways involved in substance P and lipopolysaccharide activation." Journal of Neuroimmunology **59**(1-2): 155.

<http://www.sciencedirect.com/science/article/B6T03-3YYTVJM-K/2/07176691abda11a44b8feb2e534caf73>

Substance P (SP) and lipopolysaccharide (LPS) stimulated interleukin-6 (IL-6) gene expression, as well as IL-6 protein secretion in the human astrocytoma cell line U373 MG. Staurosporine, an inhibitor of protein kinase C (PKC), entirely blocked SP- but not LPS-induced IL-6 release. In addition, the down regulation of PKC inhibited the SP response and only marginally altered LPS activation. Differently from SP, LPS-induced IL-6 release was markedly reduced by W7, a calmodulin antagonist. Moreover, SP interacted in a synergistic manner with LPS. Thus, neural (SP) and bacterial (LPS) mediators stimulate U373 MG IL-6 release via distinct, though not antagonistic, activation pathways.

Petitto, J. M. and Z. Huang (1995). "Molecular cloning of the coding sequence of an interleukin-2 receptor [alpha] subunit cDNA in murine brain." Journal of Neuroimmunology **59**(1-2): 135.

<http://www.sciencedirect.com/science/article/B6T03-3YYTVJM-H/2/b54e85b6fcf908dd0ca891bb412fad69>

Interleukin-2 (IL-2) has various trophic and neuromodulatory actions in the mammalian central nervous system (CNS). The interleukin-2 receptor [alpha] (IL-2R[alpha]) is an accessory subunit of the IL-2 receptor heterotrimer complex which is essential for 'high' affinity IL-2 binding. Although an IL-2R[alpha] (or IL-2Ra-like) epitope has been localized in brain by immunohistochemistry, it was unknown whether the IL-2R[alpha] subunit expressed in-brain was derived from the same or a different gene than the lymphocyte IL-2R[alpha]. Therefore, in the present study, the cDNA comprising the full length coding region was cloned and sequenced from saline-perfused forebrain. The brain IL-2R[alpha] cDNA was found to be 100% homologous with the corresponding lymphocyte IL-2R[alpha] cDNA sequence. IL-2R[alpha] mRNA was expressed at very low levels in saline-perfused forebrain of non-challenged BALB/c mice as well as in saline-perfused forebrain from severe combined immunodeficiency (SCID) mice. The present data, demonstrating IL-2R[alpha] gene expression in both well-perfused normal and SCID mouse forebrain from which no CD3[gamma] gene expression was detected by PCR, provides evidence that the IL-2R[alpha] clones isolated are from resident brain cells and not from blood lymphocytes (e.g. T lymphocytes). Thus, these findings demonstrate that the protein coding sequence of the mouse brain IL-2R[alpha] is derived from the same gene coding sequence as the lymphocyte IL-2R[alpha], and indicate that previously reported differences in the size of their respective mRNA transcripts appear to be due to differences in untranslated regions.

Petitto, J. M., Z. Huang, et al. (1994). "Molecular cloning of NPY-Y1 receptor cDNA from rat splenic lymphocytes: Evidence of low levels of mRNA expression and [125I]NPY binding sites." Journal

of *Neuroimmunology* **54**(1-2): 81.

<http://www.sciencedirect.com/science/article/B6T03-47DKSSM-13/2/387a5ebe6f988fcbc17da518f07694d5>

The present study addressed the question of whether the effects of neuropeptide Y (NPY) on parameters of cellular immune activity are mediated by the direct action of this neuropeptide on lymphocyte NPY receptors. A partial cDNA corresponding to bp 3-585 of the NPY-Y1 receptor coding sequence was cloned from rat splenic lymphocytes and found to have 100% nucleotide sequence homology with that segment of the NPY-Y1 receptor in brain. Basal levels of NPY-Y1 mRNA expression and [¹²⁵I]NPY binding sites of rat splenic lymphocytes were markedly lower than in frontal cortex. These data provide the first direct evidence that cells of the immune system possess NPY receptors, and suggest that further study will be necessary to determine their physiological significance.

Piraino, P. S., T. A. Yednock, et al. (2002). "Prolonged reversal of chronic experimental allergic encephalomyelitis using a small molecule inhibitor of [alpha]4 integrin." *Journal of Neuroimmunology* **131**(1-2): 147.

<http://www.sciencedirect.com/science/article/B6T03-46WV6NX-5/2/d20dbd5381c8a439d0c599c6e358282f>

CNS leukocytic invasion in experimental allergic encephalomyelitis (EAE) depends on [alpha]4[beta]1 integrin/vascular cell adhesion molecule-1 (VCAM-1) interactions. A small molecule inhibitor of [alpha]4[beta]1 integrin (CT301) was administered to guinea pigs in the chronic phase (>d40) of EAE for 10, 20, 30 or 40 days. CT301 elicited a rapid, significant improvement in the clinical and pathological scores that was maintained throughout the treatment period. A progressive loss of cells in the spinal cord of treated animals confirmed the resolution of inflammation associated with clinical recovery. Therefore, prolonged inhibition of [alpha]4[beta]1 integrin caused a sustained reversal of disease pathology in chronic EAE and may be similarly useful in MS.

Poluektova, L., T. Moran, et al. (2001). "The regulation of alpha chemokines during HIV-1 infection and leukocyte activation: relevance for HIV-1-associated dementia." *Journal of Neuroimmunology* **120**(1-2): 112.

<http://www.sciencedirect.com/science/article/B6T03-449TY7W-G/2/4e3b51812120860e70b47c74102e8f2c>

Cellular immunity against human immunodeficiency virus type 1 (HIV-1)-infected brain macrophages serves to prevent productive viral replication in the nervous system. Inevitably, during advanced disease, this antiretroviral response breaks down. This could occur through virus-induced dysregulation of lymphocyte trafficking. Thus, we studied the production of non-ELR-containing [alpha]-chemokines and their receptor (CXCR3) expression in relevant virus target cells. Macrophages, lymphocytes, and astrocytes secreted [alpha]-chemokines after HIV-1 infection and/or immune activation. Lymphocyte CXCR3-mediated chemotactic responses were operative. In all, [alpha]-chemokine-mediated T cell migration continued after HIV-1 infection and the neuroinflammatory events operative during productive viral replication in brain.

Reiseter, B. S., G. T. Miller, et al. (1998). "Treatment of murine experimental autoimmune encephalomyelitis with a myelin basic protein peptide analog alters the cellular composition of leukocytes infiltrating the cerebrospinal fluid." Journal of Neuroimmunology **91**(1-2): 156.

<http://www.sciencedirect.com/science/article/B6T03-3V5VDX7-P/2/516c59d6881d06c6ef64fe9006bf325e>

Experimental autoimmune encephalomyelitis (EAE) can be effectively treated during disease exacerbation by administration of a peptide corresponding to the major T cell epitope of myelin basic protein (MBP), but the mechanism by which T cell tolerance leads to clinical improvement is not well-defined. Acute exacerbations of EAE are accompanied by an infiltration of blood-borne leukocytes into the brain and spinal cord, where they mediate inflammation and demyelination. To investigate peptide effects on infiltrating cells, we collected cerebrospinal fluid (CSF) from (PL/JxSJL)F1 mice with MBP-induced EAE. Pleiocytosis by lymphocytes, neutrophils, and macrophages was seen throughout the course of relapsing-remitting disease. A single administration of the MBP peptide analog, Ac1-11[4Y], reduced disease severity, accompanied by a dramatic and selective loss of neutrophil pleiocytosis. A longer course of peptide therapy resulted in complete recovery from clinical signs of disease, and decreased pleiocytosis by all cell types. Clinical severity throughout the course of disease and therapy was directly related to the degree of infiltration by neutrophils and macrophages, and the clinical improvement following peptide therapy was accompanied by decreased central nervous system (CNS) expression of chemoattractants for these cell types. These observations support a model of disease exacerbation mediated by phagocytic cellular infiltration under the ultimate control of T cell-derived factors, amenable to treatment by down-regulation of the T cell activation state.

Ringheim, G. E., K. L. Burgher, et al. (1995). "Interleukin-6 mRNA expression by cortical neurons in culture: Evidence for neuronal sources of interleukin-6 production in the brain." Journal of Neuroimmunology **63**(2): 113.

<http://www.sciencedirect.com/science/article/B6T03-4002J1P-2/2/128eb0f2ac84f4279cd746e0cad090b1>

In this study, we investigated the capacity of murine cortical neurons to express interleukin-6 (IL-6) mRNA and protein in culture. Using in situ hybridization techniques, IL-6 mRNA was localized to neuronal cells in these cultures. Moreover, IL-6 mRNA expression as measured by in situ and PCR was shown to be upregulated by the proinflammatory cytokines interleukin-1[beta] (IL-1[beta]) and tumor necrosis factor-[alpha] (TNF-[alpha]). This was consistent with the dose and time-dependent increases in IL-6 secreted protein observed from cultures stimulated with IL-1[beta] and TNF-[alpha]. Taken together, the data suggest that neurons are capable of participating more directly in the CNS cytokine network than previously thought and may play an important role in the inflammatory response activities in the brain.

Rinner, I., K. Kawashima, et al. (1998). "Rat lymphocytes produce and secrete acetylcholine in dependence of differentiation and activation." Journal of Neuroimmunology **81**(1-2): 31.

<http://www.sciencedirect.com/science/article/B6T03-3S4PGS1-4/2/37cf97457a69535fa892f82f0abb6ce4>

Previous data from this laboratory suggested for the first time that immune cells of the immune system of different species are capable to synthesize the neurotransmitter acetylcholine. In the present study we detected the RNA message for choline acetyltransferase in thymic, splenic and

peripheral blood lymphocytes of rats using RT-PCR. Furthermore, using a sensitive radioimmunoassay, we measured acetylcholine in thymic, splenic and peripheral blood lymphocytes. T-cells were found to contain about three times the amount of acetylcholine as compared to B-cells, and CD4⁺ cells showed significantly higher levels as compared to CD8⁺ cells. Mitogenic stimulation with PHA increased the acetylcholine levels in lymphoid cells as well as the release into the supernatants.

Rosche, B., S. Cepok, et al. (2004). "The role of the Polio Virus Receptor and the Herpesvirus entry mediator B genes for the development of MS." Journal of Neuroimmunology **156**(1-2): 171.

<http://www.sciencedirect.com/science/article/B6T03-4D4D5VY-1/2/d50cc01ad92cea05ca6032c1eff9f0d2>

Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease of the central nervous system (CNS). Although the cause of MS is still uncertain, it is well accepted that both genetic and environmental factors are important for the development of disease. In this study, we focused on the Polio Virus Receptor (PVR) and Herpesvirus entry mediator B (HVEB) receptor genes, which are located on chromosome 19q13, a region previously linked to MS. Both receptors are expressed in the brain and immune system and play an important role for inter-cellular adhesion and entry of neurotropic viruses to the brain. We identified four new polymorphisms in the PVR gene, which were located in the promoter region and three different exons. All exonic polymorphisms altered the amino acid sequence of the receptor. No new polymorphisms were found in the HVEB gene, but we confirmed a previously identified intronic polymorphism. We analyzed the frequency of the polymorphisms by RFLP analysis in sporadic MS patients, MS families, and healthy controls and determined the surface expression of HVEB and PVR on peripheral blood monocytes. We did not find differences in the frequency of the polymorphisms or surface expression between MS patients and controls. Overall, our findings do not support a role of HVEB and PVR genes in the development of MS.

Roupe van der Voort, C., C. J. Heijnen, et al. (2000). "Stress induces increases in IL-6 production by leucocytes of patients with the chronic inflammatory disease juvenile rheumatoid arthritis: a putative role for [alpha]1-adrenergic receptors." Journal of Neuroimmunology **110**(1-2): 223.

<http://www.sciencedirect.com/science/article/B6T03-41C2RJC-W/2/17d44ec4c8811b10166b07f41bd657ca>

Juvenile rheumatoid arthritis (JRA) is characterized by chronic inflammation of the joints. In the present study we demonstrate that exposure of JRA patients to a noradrenergic stressor (cold pressor test) results in enhanced LPS-induced IL-6 production by peripheral blood cells of these patients. Healthy, age-matched controls had the same rise in norepinephrine, but do not respond with changes in IL-6 production after exposure to the cold pressor test. Moreover, PBMC of patients with JRA express mRNA encoding [alpha]1-adrenergic receptors (AR), predominantly of the [alpha]1d-AR subtype. In contrast, we could not detect mRNA encoding for [alpha]1-AR in PBMC of healthy controls. The results of this study suggest that expression of [alpha]1-AR mRNA in PBMC during chronic inflammation is associated with altered responses of the immune system to stress.

Roupe van der Voort, C., A. Kavelaars, et al. (1999). "Neuroendocrine mediators up-regulate [alpha]1b- and [alpha]1d-adrenergic receptor subtypes in human monocytes." Journal of Neuroimmunology

95(1-2): 165.

<http://www.sciencedirect.com/science/article/B6T03-3W4B1W0-M/2/3ce0eaf25fca4acc3d71a5e0c63d5a7b>

[beta]2- and [alpha]2-adrenergic receptors (AR) are thought to be the main AR subtypes to exert the effects of catecholamines on the immune system. However, in the present study, we demonstrate that another subtype of AR can be induced in human monocytes. Expression of [alpha]1b- and [alpha]1d-AR mRNA can be obtained by culturing freshly isolated human peripheral blood monocytes with the neuroendocrine mediators dexamethasone or the [beta]2-AR agonist terbutaline. Using the human monocytic cell line THP-1, we demonstrate that increased levels of [alpha]1b- and [alpha]1d-mRNA are accompanied by increased levels of receptor protein as determined by Western blot analysis and radioligand binding assays. This study describes for the first time regulated expression of [alpha]1-AR subtypes in human monocytes.

Roupe van der Voort, C., A. Kavelaars, et al. (2000). "Noradrenaline induces phosphorylation of ERK-2 in human peripheral blood mononuclear cells after induction of [alpha]1-adrenergic receptors." *Journal of Neuroimmunology* **108**(1-2): 82.

<http://www.sciencedirect.com/science/article/B6T03-40R5BF6-C/2/7f8f3b439bff5ea69989c29fc7c53c5>

[alpha]1-Adrenergic receptors (ARs) are not expressed by peripheral blood mononuclear cells (PBMCs) of healthy human individuals. However, in the present study we show that [alpha]1-ARs can be induced in lymphocytes after culturing with either the mitogen PHA or the glucocorticoid dexamethasone. Moreover, incubation of these activated PBMCs with noradrenaline (NA) results in enhanced phosphorylation of ERK-2, a kinase involved in the activation of many immune functions. Similar induction of [alpha]1-AR mRNA with concomitant NA-induced activation of ERK-2 occurs in monocytes after culture with LPS. Our results demonstrate that functional [alpha]1-ARs can be induced on PBMCs and that these [alpha]1-ARs mediate NA-induced activation of ERK-2.

Roy, S., R. G. Charboneau, et al. (1999). "Morphine synergizes with lipopolysaccharide in a chronic endotoxemia model." *Journal of Neuroimmunology* **95**(1-2): 107.

<http://www.sciencedirect.com/science/article/B6T03-3W4B1W0-C/2/d135b0b84fd31c2d702400ce37fcb24>

Emergent or elective surgical procedures may be complicated by sepsis, resulting in critical illness that can lead to organ failure and death. The opioid drug, morphine is widely used to alleviate pain in post-surgical patients; however, it is well documented that chronic treatment of mice with morphine affects the proliferation, differentiation and function of immune cells. Thus, morphine might be expected to exacerbate the effects of sepsis, which also compromises the immune system. To test this notion, we investigated the effect on several immune functions of a clinical dose of morphine (4 mg/kg) superimposed upon a lipopolysaccharide (LPS)-induced infection model. Our results show that this relatively low dose of morphine, though generally having no effects on immune parameters by itself, significantly augmented LPS responses. A clinical dose of morphine (4 mg/kg body weight) superimposed upon an animal model of sepsis resulted in a significant increase in mortality at 48 h. In the absence of the drug, most septic animals died after 96 h. Phenotypic responses such as, decreased thymic cellularity, compromised mitogenic response and inhibition of IL-2 synthesis that are evident at 48-72 h after

LPS injection appear as early as 24 h in animals that receive morphine in addition to LPS. In addition, our results show that in T cells there is a shift from TH1 type cytokine elaboration to a TH2 type cytokine elaboration in animals that receive both LPS and morphine.

Sato, S., S. L. Reiner, et al. (1997). "Central nervous system cytokine mRNA expression following Theiler's murine encephalomyelitis virus infection." Journal of Neuroimmunology **76**(1-2): 213.

<http://www.sciencedirect.com/science/article/B6T03-3PSB1XD-Y/2/aeffadb4e512d063b5dc934a0010bbe3>

DA strain of Theiler's murine encephalomyelitis virus (TMEV) produces a biphasic disease with an initial self-limited acute gray matter polioencephalomyelitis in all strains of mice followed by, in the case of certain susceptible strains of mice, a chronic inflammatory demyelination of the spinal cord with a persistent virus infection. A pathogenic role for T-helper 1 (Th1) cells during the demyelinating phase of disease has been proposed. We characterized the cytokine mRNA expression in the brain and spinal cord of susceptible and resistant strains of mice during the early encephalomyelitic disease and the late demyelination, using a semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) assay. At the time of the encephalomyelitis, both resistant and susceptible mice expressed proinflammatory cytokine mRNAs followed by T-cell derived mRNAs; susceptible mice expressed more IL-12 p40 mRNA than resistant mice. During this early disease, there was no significant difference in Th1 cytokine mRNA expression in the brain and spinal cord among the four strains and relatively little Th2 type cytokine upregulation above levels seen in mock-infected controls. During the late demyelinating disease, susceptible but not resistant mice had evidence of viral genome and a continuous expression of Th1 type cytokine mRNAs. The expression of Th2 cytokine mRNAs varied among the different strains and did not correlate with susceptibility or resistance. The results indicate the complexity of cytokine mRNA expression following TMEV infection and the dependence of the expression on disease pathology, the time following infection and the genetics of the host.

Schaecher, K. E., J.-M. Goust, et al. (2001). "The effects of calpain inhibition upon IL-2 and CD25 expression in human peripheral blood mononuclear cells." Journal of Neuroimmunology **119**(2): 333.

<http://www.sciencedirect.com/science/article/B6T03-442XPR6-R/2/32a66410440e29c426f84df9f0015f0e>

Calcium is an important contributor to T cell activation; it is also the major factor in the activation of the calcium-activated neutral proteinase, calpain. For this reason, we wanted to investigate if calpain has a role in T cell activation and what aspects of this activation calpain affects. As measured by semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR), calpain inhibition decreased interleukin-2 (IL-2) and CD25 mRNA expression in a dose-dependent manner, at early time points following the initial activation, and over extended periods of time in activated human peripheral blood mononuclear cells (PBMCs). Using an enzyme-linked immuno-sorbent assay (ELISA) specific for human IL-2, we found that calpain inhibition decreased IL-2 secretion in a dose-dependent manner, shortly after activation, and continuously over time. Inhibiting calpain caused a dose-dependent inhibition of CD25 cell surface expression and also inhibited expression shortly after activation and for at least 48 h. This study showed that calpain has an integral role in the synthesis of the two important T cell activation factors, IL-2 and CD25.

Schwarz, H., P. M. Villiger, et al. (1994). "Neuropeptide Y is an inducible gene in the human immune system." Journal of Neuroimmunology **51**(1): 53.

<http://www.sciencedirect.com/science/article/B6T03-476HJ7V-12/2/4c93ee2395ce3483427fcc6e784c3be8>

This study reports on neuropeptide Y (NPY) mRNA expression in human peripheral blood mononuclear cells (PBMC) and lymphoid tissues. By reverse transcription polymerase chain reaction (RT-PCR) it is shown that activated human PBMC of normal blood donors expressed the NPY gene. The PCR products had the expected size and Northern blotting demonstrated the presence of the 0.8-kb NPY mRNA. To define the subpopulations of mononuclear cells expressing this neuropeptide, purified monocytes, B cells and T cells were stimulated with specific activators. Monocytes and in vitro matured macrophages expressed 3PY mRNA in response to phorbol myristate acetate (PMA). B lymphocytes expressed NPY mRNA following stimulation with antibody to surface immunoglobulin and PMA. In order to analyze whether these cell types express NPY under physiological conditions in vivo, human bone marrow, tonsil and thymus were analyzed. In situ hybridization of bone marrow revealed a small number of cells containing high levels of NPY mRNA which was also detected in RNA extracts of human thymus and tonsil. In summary, NPY is an inducible gene in human lymphocytes and monocytes and it is expressed at sites where these cells are activated in vivo.

Sciacca, F. L., C. Ferri, et al. (2000). "Association study of a new polymorphism in the PECAM-1 gene in multiple sclerosis." Journal of Neuroimmunology **104**(2): 174.

<http://www.sciencedirect.com/science/article/B6T03-3YS33NS-B/2/0e7bb4c477810d5342fd1251f9f3aa10>

Genetic polymorphisms of immunorelevant genes may modulate occurrence or clinical features of multifactorial diseases. PECAM-1 is an adhesion molecule crucial for transmigration of cells from blood to tissues, but its genetic contribution to multifactorial diseases has never been investigated. We have identified and characterized a tetranucleotide repeat polymorphism within the third intron of PECAM-1. In a cohort of healthy controls (HC), we found 10 alleles. An assessment of the association of this polymorphism with multiple sclerosis (MS) showed similar allele and genotype frequencies in HC and MS patients as well as in MS patients differing for the gravity of their disease course. We conclude that although potentially able to affect organ-specific autoimmune diseases, this new PECAM-1 polymorphism, does not seem to contribute to the genetic background of MS.

Sedqi, M., S. Roy, et al. (1996). "Expression cloning of a full-length cDNA encoding delta opioid receptor from mouse thymocytes." Journal of Neuroimmunology **65**(2): 167.

<http://www.sciencedirect.com/science/article/B6T03-3W496PR-B/2/bd214dd7346ef81c9cd6e57fbff89d3c>

A delta opioid receptor complementary DNA (cDNA) was cloned by expression of cDNA library from activated thymocytes in Cos 7 cells. The deduced amino acid sequence of this receptor was similar to that described in the brain. As analyzed by southern blot hybridization, the delta opioid receptor transcripts are constitutively expressed in unactivated thymocytes. In addition, neither [kappa] nor [mu] opioid receptor transcripts were detected in thymocytes, suggesting tissue-specific opioid receptor gene expression in the immune system. The studies represent the first report of a full-length opioid receptor in the immune system.

Selmaj, K., C. Kowal, et al. (2000). "Naked DNA vaccination differentially modulates autoimmune responses in experimental autoimmune encephalomyelitis." Journal of Neuroimmunology **111**(1-2): 34.

<http://www.sciencedirect.com/science/article/B6T03-41J66JG-5/2/c2d1bce2466dedd2629dc569c1cbaf96>

Vaccination with naked DNA represents a therapeutic strategy currently under consideration in multiple sclerosis (MS). In this study, we tested the potential therapeutic effect of vaccination with a naked DNA construct encoding proteolipid protein (pRc/CMV-PLP) upon the outcome of subsequent sensitization for experimental autoimmune encephalomyelitis (EAE) actively-induced in SJL mice with PLP139-151 peptide in adjuvant. Intramuscular vaccination with the naked DNA pRc/CMV-PLP construct led to PLP expression in local muscle tissue that persisted for about 8 weeks. Early sensitization for EAE (4 weeks after DNA vaccination) caused recipient mice to develop a severe, exacerbated form of disease (in comparison to control mice), while late sensitization (>10 weeks) resulted in a milder, ameliorated form. In the groups sensitized 10 weeks post-DNA vaccination led to peripheral tolerance as evidenced by a decrease in T cell proliferation and cytotoxic T cell response, no Th2 response, and no increase in apoptosis. These data are novel in that they demonstrate a differential effect of DNA vaccination and have important implications for its use as a mechanism to enhance or modulate immune reactivity.

Shahabi, N. A., Y. Daaka, et al. (1999). "Delta opioid receptors expressed by stably transfected jurkat cells signal through the map kinase pathway in a ras-independent manner." Journal of Neuroimmunology **94**(1-2): 48.

<http://www.sciencedirect.com/science/article/B6T03-3VNPV73-5/2/661a1db953b9c8a9bbdf0073e3480e3e>

Delta opioid receptors (DOR) are G-protein coupled 7-transmembrane receptors (GPCR), expressed by thymic and splenic T cells, that modulate interleukin (IL)-2 production and proliferation in response to concanavalin A or crosslinking the TCR. Mitogen-activated protein kinases (MAPKs) are involved in mediating intracellular responses to TCR crosslinking. In addition, MAPKs can be activated by signaling cascades that are initiated by the release of G-proteins from GPCRs. To determine whether DORs expressed by T cells signal through the MAPKs, extracellular-regulated kinases (ERKs) 1 and 2, two delta opioid peptides, deltorphin and [-Ala2,-Leu5]-enkephalin (DADLE), were studied in Jurkat cells that had been stably transfected with DOR (DOR-Ju.1). These peptides rapidly and dose-dependently induced ERK phosphorylation; pretreatment with naltrindole (NTI), a selective DOR antagonist, abolished this. Pertussis toxin (PTX) also inhibited phosphorylation, indicating the involvement of the Gi/o proteins. Herbimycin A, a protein tyrosine kinase (PTK) inhibitor, reduced the DADLE-induced ERK phosphorylation by 68%. ERK phosphorylation was inhibited by Bisindolylmaleimide 1 (GF109203X), an inhibitor of PKC, and by pretreatment with PMA prior to DADLE. A GTP/GDP exchange assay was used to assess the potential role of Ras in the pathway leading to ERK phosphorylation; DADLE failed to stimulate GTP/GDP exchange in comparison to PMA. Additional studies showed that DADLE stimulated an increase in cfos mRNA; this was reduced by the inhibitor of MAPK/ERK kinase (MEK), PD98059. Therefore, in DOR-Ju.1 cells, DOR agonists stimulate ERK phosphorylation in a Ras independent and PKC-dependent manner; PTKs appear to be involved. MAPKs mediate the increase in cfos mRNA induced by DOR agonists.

Sharp, B. M., N. Shahabi, et al. (1997). "Detection of basal levels and induction of delta opioid receptor mRNA in murine splenocytes." Journal of Neuroimmunology **78**(1-2): 198.

<http://www.sciencedirect.com/science/article/B6T03-3R7J7TN-T/2/30b2caf1bc1a471b250fc774b93494c6>

Activation of delta opioid receptors (DOR) modulates calcium mobilization, interleukin-2 production, chemotaxis and proliferation of T-lymphocytes. Recent reports indicate that lymphocytes and mononuclear cells may express mRNA transcripts for DOR. The investigations reported herein show that low levels of DOR were consistently detected by RT-PCR amplification of RNA from freshly obtained Balb/c murine splenocytes, both weanling and adult. Culturing cells without stimulation increased DOR levels and concanavalin A apparently reduced this; DOR was preferentially expressed in a T-cell-enriched fraction. Thus, the expression of DOR mRNA by unactivated splenocytes is modulated by culture and con A in the T-cell fraction.

Shrikant, P., C. Il Yup, et al. (1994). "Regulation of intercellular adhesion molecule-1 gene expression by tumor necrosis factor-[alpha], interleukin-1[beta], and interferon-[gamma] in astrocytes." Journal of Neuroimmunology **51**(2): 209.

<http://www.sciencedirect.com/science/article/B6T03-476WCJ3-X/7/2/10c3570a28de34dab7faadb50d3e5e68>

Intercellular adhesion molecule-1 (ICAM-1) is a cell surface glycoprotein which can be induced on astrocytes, the major glial cell of the central nervous system (CNS). In this study, we examined the effect of three proinflammatory cytokines, tumor necrosis factor-alpha (TNF-[alpha]), interleukin-1 beta (IL-1[beta]), and interferon-gamma (IFN-[gamma]), on the expression of ICAM-1 by primary rat astrocytes. Astrocytes constitutively express ICAM-1 mRNA and protein, which is enhanced by treatment with TNF-[alpha], IL-1[beta] and IFN-[gamma]. TNF-[alpha] is the most potent inducer of ICAM-1 expression, followed by IL-1[beta], then IFN-[gamma]. Kinetic analysis demonstrated optimum ICAM-1 mRNA expression after a 1-h exposure to TNF-[alpha], 2 h exposure to IL-1[beta], and 4 h exposure to IFN-[gamma]. Peak ICAM-1 protein expression was detected 12-16 h after treatment with TNF-[alpha] or IL-1[beta], and after a 24-h exposure to IFN-[gamma]. Nuclear run-on analysis demonstrated that the ICAM-1 gene is transcribed under basal conditions in astrocytes, and that both TNF-[alpha] and IL-1[beta] enhance transcriptional activation of the ICAM-1 gene. ICAM-1 mRNA stability studies determined that basal ICAM-1 mRNA has a half-life of about 1 h, and that TNF-[alpha], IL-1[beta] and IFN-[gamma] have a modest effect on stabilization of basal ICAM-1 mRNA expression. These results indicate that under inflammatory conditions in the CNS, such as multiple sclerosis (MS) and experimental allergic encephalomyelitis (EAE), astrocytes can be induced to express the adhesion molecule ICAM-1, which can contribute to inflammatory events within the CNS.

Sierra-Honigsmann, A. M., S. A. Rubin, et al. (1993). "Borna disease virus in peripheral blood mononuclear and bone marrow cells of neonatally and chronically infected rats." Journal of Neuroimmunology **45**(1-2): 31.

<http://www.sciencedirect.com/science/article/B6T03-476WCFH-W1/2/a38e1296003ebdc5af5e57da7ccb38ff>

Borna disease virus (BDV) establishes a persistent infection in cells of the nervous system in rats. The response, or lack thereof, of the immune system to BDV infection of neurons is responsible for the presence or absence, respectively, of Borna disease. We recently demonstrated

transmission of BDV by bone marrow cells from neonatally infected rats. Our findings suggested the possibility of a heretofore unsuspected interaction between BDV and the immune system, that of direct effects of BDV infection on the cells of the immune system. This report enlarges upon the previous findings and confirms the presence of BDV RNA in bone marrow cells of neonatally infected rats, using a reverse transcription-polymerization chain reaction-enzyme immunosorbent assay (RT-PCR-EIA). In addition, we detected BDV RNA in peripheral blood mononuclear cells of neonatally infected rats, and in rats inoculated as adults in the chronic, but not the acute, stage of infection. In addition, the RT-PCR-EIA technique identified BDV RNA in cerebrospinal fluid, nasal secretions, saliva, urine and stool. BDV-sequences were not detected in the plasma of infected animals nor in the body fluids and tissues of normal rats.

Skorupa, A. F., S. C. G. Brezinski, et al. (2001). "Expression of Golli mRNA during development in primary immune lymphoid organs of the rat." Journal of Neuroimmunology **119**(1): 64.

<http://www.sciencedirect.com/science/article/B6T03-43T7TV1-8/2/1e290e509c09a562efdfb4492b89a213>

The gene-of-the-oligodendrocyte lineage (Golli)-MBP transcription unit contains three Golli-specific exons together with eight exons of the "classical" myelin basic protein (MBP) gene, yielding alternatively spliced proteins which share amino acid sequence with MBP. Unlike MBP, a late antigen expressed only in the nervous system, Golli gene products are expressed pre- and post-natally at many sites. In this study, we determined the sequence of Golli in rat by RT-PCR and 5' RACE and showed that Golli sequences are expressed in primary lymphoid organs as early as e16.5, which could explain the anergic rat T cell response we previously observed in Golli-induced meningitis.

Stinissen, P., J. Zhang, et al. (1998). "[gamma][delta] T cell responses to activated T cells in multiple sclerosis patients induced by T cell vaccination." Journal of Neuroimmunology **87**(1-2): 94.

<http://www.sciencedirect.com/science/article/B6T03-3TCFKBS-D/2/7d104c3ce2ce497a7136eaa09c53491d>

To explore the hypothesis that [gamma][delta] T cells may regulate activated [alpha][beta] T cells, we studied [gamma][delta] T cell responses to [alpha][beta] T cell clones in Multiple Sclerosis (MS) patients who received attenuated autologous autoreactive T cells. We recently conducted a pilot study of T cell vaccination with myelin basic protein reactive T cells in MS. Since T cell vaccination upregulates the anti-vaccine T cell responses, we evaluated [gamma][delta] T cell reactivity towards the vaccine in the vaccinated patients. Lymphocytes were stimulated in vitro with irradiated vaccine cells and the responding lines were checked for the presence of [gamma][delta] T cells. Our data demonstrate that in the majority of vaccinated MS patients [gamma][delta] T cells expand upon stimulation with the vaccine cells. The responding [gamma][delta] T cells were predominantly V[delta]1+/V[gamma]1+, and represented diverse clonal origins. The [gamma][delta] T cells could not inhibit in vitro proliferation of the vaccine T cells and displayed low cytotoxic reactivity towards the vaccine clones. However, they produced high levels of IL2, TNF[alpha] and IL10. These results indicate that [gamma][delta] T cells can be stimulated by activated [alpha][beta] T cells, and that these [gamma][delta] T cell responses are upregulated after T cell vaccination. These findings suggest that [gamma][delta] T cells are involved in peripheral mechanisms to control activated autoreactive T cells.

Storto, M., U. de Grazia, et al. (2000). "Expression of metabotropic glutamate receptors in murine thymocytes and thymic stromal cells." Journal of Neuroimmunology **109**(2): 112.

<http://www.sciencedirect.com/science/article/B6T03-416BXKJ-8/2/77eb587eee3257eb4dd9fa07a1292290>

RT-PCR combined with immunoblotting showed the expression of group-I (mGlu1 and 5) and group-II (mGlu2 and 3) metabotropic glutamate receptors in whole mouse thymus, isolated thymocytes and TC-1S thymic stromal cell line. Cytofluorimetric analysis showed that mGlu-5 receptors were absent in CD4-/CD8- but present in more mature CD4+ CD8+ and CD4+CD8- thymocytes. mGlu-1a receptors showed an opposite pattern of expression with respect to mGlu5, whereas mGlu2/3 receptor expression did not differ between double negative and double positive cells. mGlu receptors expressed in both thymic cell components were functional, as indicated by measurements of polyphosphoinositide hydrolysis or cAMP formation. These data suggest a possible role for mGlu receptor signalling in the thymus.

Szczepanik, A. M., S. Funes, et al. (2001). "IL-4, IL-10 and IL-13 modulate A[beta](1-42)-induced cytokine and chemokine production in primary murine microglia and a human monocyte cell line." Journal of Neuroimmunology **113**(1): 49.

<http://www.sciencedirect.com/science/article/B6T03-41Y28FP-6/2/53671e21c5a94a9775f9364e7a06b2fe>

A hallmark of the immunopathology associated with Alzheimer's disease (AD) is the presence of activated microglia surrounding senile plaque deposits of [beta]-amyloid (A[beta]) peptides. A[beta] peptides have been shown to be potent activators of microglia and macrophages, but little is known about endogenous factors that may modulate their responses to amyloid. We investigated whether the 'anti-inflammatory' cytokines IL-4, IL-10 and IL-13 could regulate A[beta]-induced production of the inflammatory cytokines IL-1[alpha], IL-1[beta], TNF-[alpha], IL-6 and the chemokine MCP-1. A[beta](1-42) time- and dose-dependently induced the production and secretion of these inflammatory proteins in the human THP-1 monocyte cell line and in primary murine microglia, similar to what was observed for lipopolysaccharide (LPS) stimulated cells. IL-10 was found to suppress all A[beta] and LPS-induced inflammatory proteins measured (IL-1[alpha], IL-1[beta], IL-6, TNF-[alpha] and MCP-1) in both cell types with the exception of LPS-induced MCP-1 in THP-1 cells where no change was observed. In contrast to the inhibition observed for IL-10, both IL-4 and IL-13 enhanced MCP-1 secretion. IL-4 and IL-13 reduced IL-6 secretion, but effects on IL-1[alpha], IL-1[beta] or TNF-[alpha] were dependent on cell type and stimulus conditions. Additional experiments using RT-PCR showed that IL-4, IL-10 and IL-13 mRNA is found to be present in human brain tissue. These results show that IL-4, IL-10, and IL-13 differentially regulate microglial responses to A[beta] and may play a role in the inflammation pathology observed surrounding senile plaques.

van der Meulen, M. F. G., D. F. van Wichen, et al. (2002). "Evidence for heterogeneity of T cell expansion in polymyositis and inclusion body myositis." Journal of Neuroimmunology **133**(1-2): 198.

<http://www.sciencedirect.com/science/article/B6T03-473MDJN-8/2/62fb9bfc2d78098e014bddcf8d4a5a7f>

V[beta] usage of muscle-infiltrating T lymphocytes in polymyositis (PM) and sporadic inclusion body myositis (s-IBM) was correlated with clinical and histopathological features. Immunohistochemical analysis was combined with complementarity-determining region 3 (CDR3)

length analysis in nine muscle biopsies of eight PM patients and six biopsies of five s-IBM patients. V[beta] usage was heterogeneous in seven patients. Four of these patients had definite PM with endomysial located T cell infiltrates, but T cells specifically surrounding and invading individual non-necrotic fibers were not found. In two s-IBM patients, V[beta] 2 usage was increased. In one of them, a repeat biopsy showed a heterogeneous V[beta] usage. We conclude that clonal expansion of muscle-infiltrating T cells could only be detected in part of the patients. Explanations may be that clonal expansion does not take place in all disease phases and that PM is a heterogeneous disease with respect to pathogenesis.

van Sorge, N. M., W. L. van der Pol, et al. "Severity of Guillain-Barre syndrome is associated with Fc[gamma] Receptor III polymorphisms." *Journal of Neuroimmunology* **In Press, Corrected Proof** <http://www.sciencedirect.com/science/article/B6T03-4FNDRX7-1/2/3be0aafac7e642d89d6a15eb0ea3822a>

Macrophages and ganglioside-specific IgG are involved in the pathogenesis of Guillain-Barre syndrome (GBS). Leukocyte IgG receptors (Fc[gamma]R) confer potent cellular effector functions to the specificity of IgG. The efficacy of IgG-mediated cellular inflammatory responses is determined by functional polymorphisms of three Fc[gamma]R subclasses (Fc[gamma]RIIa: H131/R131; Fc[gamma]RIIIa: V158/F158; Fc[gamma]RIIIb: NA1/NA2). Fc[gamma]R genotype distributions were determined in a Dutch, and British cohort of GBS patients and controls. In addition, a meta-analysis incorporating all previously published data, encompassing a total of 345 GBS patients and 714 healthy controls, was performed. Results suggest that Fc[gamma]RIII genotypes may represent mild disease-modifying factors in GBS.

Vandenbroeck, K., G. Martino, et al. (1997). "Occurrence and clinical relevance of an interleukin-4 gene polymorphism in patients with multiple sclerosis." *Journal of Neuroimmunology* **76**(1-2): 189.

<http://www.sciencedirect.com/science/article/B6T03-3PSB1XD-V/2/9689c82c112f673f33b3397b3975017e>

An epistatic gene interaction has been advocated to explain disease susceptibility in multiple sclerosis (MS). Cytokine genes are possible candidates due to the central role played by cytokines in the regulation of the immune-mediated pathogenetic process leading to central nervous system demyelination in these patients. Since interleukin (IL)-4 gene polymorphisms have been associated with immune-mediated diseases, we have analysed the relationship between a variable number of tandem repeat polymorphism of the IL-4 gene and clinical and physiological features of 256 sporadic MS patients and 146 healthy controls. Genotype frequencies were similar between the MS group and healthy controls. However, in MS patients a positive and significant correlation ($r=0.91$; $p<0.001$) was found between the carriage rate of the IL-4 B1 allele (from 0.21 to 0.36) and age of disease onset. No association was found between IL-4 alleles and disease progression, sex or ethnic background of the patients. Our results show that the IL-4 B1 allele is associated with late onset of MS and therefore might represent a modifier of age of onset rather than a susceptibility factor for patients with MS.

Yamabe, T., G. Dhir, et al. (1994). "Cytokine-gene expression in measles-infected adult human glial cells." *Journal of Neuroimmunology* **49**(1-2): 171.

<http://www.sciencedirect.com/science/article/B6T03-476HK1W-7D/2/bb5d439bf483a77e9c61e55e06575557>

The expression of interleukin (IL)-1[beta], IL-6 and tumor necrosis factor (TNF) [alpha] transcripts in cultured human glial cells was examined using reverse transcription followed by polymerase chain reaction (PCR) amplification and Southern blot quantitation. Microglial cultures derived from brain biopsy specimens from three different individuals expressed transcripts for the three cytokines under basal culture conditions. This expression was enhanced in response to measles virus (MV) infection (IL-1[beta], 2.2-8.8-fold; IL-6, 2.5-8.4-fold; TNF[alpha], 2.2-3.2-fold). Neither IL-1[beta] nor TNF[alpha] transcripts were detectable in undissociated brain tissue from two individuals, suggesting that the basal expression of these cytokines in culture may have been induced by tissue dissociation or by the culture conditions. Oligodendrocytes did not express cytokine transcripts under basal culture conditions, and IL-1[beta] and IL-6 but not TNF[alpha] transcripts could be induced by MV. Similarly, meningeal fibroblasts expressed IL-1[beta] and IL-6 but not TNF[alpha] in response to MV-infection, suggesting that the production of TNF[alpha] is more cell type-restricted than either IL-1[beta] or IL-6. The results indicate that adult human microglia can participate in the inflammatory response to MV infection in the CNS by producing cytokines that contribute to inflammation and demyelination. In addition, besides their role in myelination, oligodendrocytes can potentially influence immunoreactivity in the CNS by producing IL-1[beta] and IL-6.

Yamakuni, H., N. Kawaguchi, et al. (2002). "ATP induces leukemia inhibitory factor mRNA in cultured rat astrocytes." *Journal of Neuroimmunology* **129**(1-2): 43.

<http://www.sciencedirect.com/science/article/B6T03-468VMH4-2/2/8c2a1e213354cd968c18c909df8a7b36>

Leukemia inhibitory factor (LIF) is a cytokine involved in the survival and differentiation of the neural cells in the central and peripheral nervous systems. In the present study, we examined the effects of various neurotransmitter receptor agonists on LIF mRNA expression in cultured rat astrocytes, microglia and neurons to elucidate the cell types producing LIF and to clarify the neurotransmitter(s) regulating the mRNA expression. The results demonstrated that the expression of LIF mRNA was intensely induced by ATP in the cultured astrocytes. Experiments using ATP, UTP and related compounds showed the involvement of P2Y2 and P2Y4 purinoceptors in the expression induced by ATP.

Ye, S.-M. and R. W. Johnson (2001). "Regulation of interleukin-6 gene expression in brain of aged mice by nuclear factor [kappa]B." *Journal of Neuroimmunology* **117**(1-2): 87.

<http://www.sciencedirect.com/science/article/B6T03-43B8BRH-B/2/90a66f194977ab7f9d558cd84f4e66f8>

Interleukin-6 (IL-6) is increased in brain of aged mice. The purpose of this study was to determine if binding of nuclear factor [kappa]B (NF[kappa]B) to the IL-6 promoter is responsible for the age-related increase in brain IL-6. In an initial study, the effect of age on IL-6 in brain was verified as IL-6 protein was increased in brain of aged mice compared to adult and juvenile mice. Competitive RT-PCR showed that IL-6 mRNA concentration was at least 4-fold higher in aged brain compared to adult brain. Next, binding of the transcription factor NF[kappa]B to the IL-6 promoter in brains of 1-, 6-, and 24-month-old mice was determined. Electrophoretic mobility shift assay showed that NF[kappa]B activity was increased in aged brain compared to adult and juvenile brain. Moreover, glial cells cultured from aged mice showed more NF[kappa]B DNA-binding activity and more IL-6 mRNA and protein expression than glia from adults. However, incubating glia from aged mice in the presence of [kappa]B decoy inhibited these effects of age. The same was observed in vivo as intracerebroventricular injection of [kappa]B decoy in aged mice decreased NF[kappa]B activity and IL-6 mRNA and protein in brain. These results show that

the DNA-binding activity of NF[κ]B is increased in the brain of aged mice and that at least one consequence is increased expression of IL-6.

Zwijnenburg, P. J. G., T. van der Poll, et al. (2003). "Interleukin-18 gene-deficient mice show enhanced defense and reduced inflammation during pneumococcal meningitis." Journal of Neuroimmunology **138**(1-2): 31.

<http://www.sciencedirect.com/science/article/B6T03-487KRJ5-1/2/0f8e8c8a2389462364c7bb736263d52f>

To determine the role of endogenous interleukin-18 (IL-18) in pneumococcal meningitis, meningitis was induced in IL-18 gene-deficient (IL-18^{-/-}) and wild-type (WT) mice by intranasal inoculation of *Streptococcus pneumoniae* with hyaluronidase. Induction of meningitis resulted in an upregulation of both pro- and mature IL-18 in brain tissue in WT mice. IL-18^{-/-} and WT mice were equally susceptible to develop meningitis after intranasal infection, yet IL-18^{-/-} mice showed a prolonged survival and a suppressed inflammatory response, as reflected by a less profound inflammatory infiltrate around the meninges and lower concentrations of cytokines and chemokines in brain tissue. These findings suggest that endogenous IL-18 contributes to a detrimental inflammatory response during pneumococcal meningitis and that elimination of IL-18 may improve the outcome of this disease.

Journal of Neuroscience Methods (10)

Castro, R. M. R. P. S., M. C. Landemberger, et al. (2004). "High capacity and low cost detection of prion protein gene variant alleles by denaturing HPLC." Journal of Neuroscience Methods **139**(2): 263.

<http://www.sciencedirect.com/science/article/B6T04-4CP17PJ-2/2/145d98e2cbee1b579fbbad6cd7b3e1cf>

Mutations in the human prion protein gene (PRNP) are responsible for hereditary diseases called transmissible spongiform encephalopathies (TSE) and a polymorphic site at codon 129 determines sensitivity to infectious forms of these maladies. More recently, codon 129 has been related to cognition performance in the elderly, in Alzheimer disease (AD) and in Down syndrome. Furthermore, a rare polymorphism at codon 171 was described in 23% of patients with mesial temporal lobe epilepsy related to hippocampal sclerosis (MTLE-HS), the most common form of surgically remediable epileptic syndrome. Thus, a method that permits fast and efficient screening of PRNP mutations and polymorphisms in patients, in high risk populations, and in family members is desirable. In the present study, we established the conditions for analysis of the PRNP open reading frame using denaturing high-performance liquid chromatography (DHPLC), whereby unpurified PCR products were subjected to denaturing and reannealing steps leading to heteroduplex formation. We described specific profiles for the PRNP polymorphisms at codons 129 (M/V), 117 (A/A silent), 219 (E/K), 171 (N/S), and the octarepeat deletion using amplified DNA from 562 samples. The chromatograms for TSE-associated mutations at codons 102 (P/L), 183 (T/A), and 210 (V/I) were also determined. Specificity of the DHPLC profile for each PRNP variant allele was confirmed in 100% of the samples by direct and cloned DNA sequencing in addition to endonuclease digestion when applicable. Therefore, the present study shows that DHPLC is a rapid, highly accurate and efficient technique for the detection of PRNP genetic

variants.

Dilks, D. W., R. H. Ring, et al. (2003). "High-throughput confirmation of differential display PCR results using reverse Northern blotting." Journal of Neuroscience Methods **123**(1): 47.

<http://www.sciencedirect.com/science/article/B6T04-47RBBV9-4/2/54f9e0c8cc624e10309406ac6d9d3ffa>

Nylon filter arrays spotted with differential display PCR (DD-PCR) clones and hybridized with radiolabeled cRNA generated from the source RNA pool (reverse Northern blot) provide a high-throughput means to screen clones for artifacts. Reverse Northern blots also confirm differential gene expression in parallel and require modest quantities of the source RNA pool. We describe a strategy to screen multiple candidates from DD-PCR by high-throughput ligation and transformation, followed by reverse Northern blotting. Purification of re-amplified DD-PCR clones and fabrication of nylon arrays was facilitated by a batch-processing protocol using the widely available Biomek(R) laboratory robot and Bioworks(TM) scripts (available from the authors). A strategy to screen out DD-PCR product artifacts of an inappropriate size was also employed. Using these approaches, we identified several mRNAs that are differentially expressed in response to venlafaxine, fluoxetine or desipramine antidepressant treatment in rat C6 glioma cell lines and are candidates for full length clone isolation using 5'-RACE. Such an approach provides a rapid means to eliminate the high percentage of false positive clones from DD-PCR and enables independent confirmation of differential gene expression patterns generated by various experimental conditions.

Gioia, L., L. J. Vogt, et al. (1998). "PCR-based apolipoprotein E genotype analysis from archival fixed brain." Journal of Neuroscience Methods **80**(2): 209.

<http://www.sciencedirect.com/science/article/B6T04-3SVS087-C/2/f32dd60265dec99686d7204d8e0eb2f5>

A technique is described for determining the apolipoprotein E genotype (apo E; alleles [epsilon]2, [epsilon]3, or [epsilon]4) from tissues which have been fixed with 4-10% formaldehyde and archived. The procedure requires efficient extraction and exhaustive purification of DNA from the fixed tissue. Because the fixation process renders the DNA largely crosslinked and/or sheared (therefore unsuitable for traditional analysis), a nested polymerase chain reaction (PCR) is employed (using two apo E gene specific primer pairs) to specifically amplify the polymorphic region of the gene. The genotype was then determined using previously reported HhaI polymorphisms that occur as a direct result of the variant codons responsible for the three alleles. This protocol permitted the successful genotyping of 90% (34 out of 38) of the archived brain samples from Alzheimer's disease (AD) patients. These samples included such extremes as a sample that had been stored for 12 years in formalin. This procedure permits the retrospective analysis of samples that had been processed and stored well before the original characterization of apo E alleles as risk factors in AD. Finally, this approach is readily adapted to the analysis of any gene of interest, whether by restriction fragment length polymorphism or direct amplicon DNA sequencing. It is also a very robust assay for less stringent conditions such as DNA isolated from whole blood or frozen tissue.

Gutala, R. V. and P. H. Reddy (2004). "The use of real-time PCR analysis in a gene expression study of Alzheimer's disease post-mortem brains." Journal of Neuroscience Methods **132**(1): 101.

<http://www.sciencedirect.com/science/article/B6T04-4B42D2D-1/2/9b30501d8a2efcd11974171fbb66596f>

The measurement of gene expressions in brains with neurodegenerative diseases is a major area of brain research. The objective of our research was to determine whether quantitative real-time PCR could measure messenger RNA (mRNA) expression in brains with post-mortem intervals beyond 12 h. In the present paper, we examined the quality of RNA from brain specimens of both Alzheimer's disease (AD) patients (n=13) and non-demented normal control subjects (n=6). To determine an unregulated endogenous reference gene in AD, we measured mRNA expressions of the commonly used reference genes [beta]-actin, 18S rRNA, and GAPDH. In addition, we determined whether post-mortem interval, brain weight, or age at death influences mRNA expression. Our real-time PCR analysis results indicate that mRNA expression can be detected in all brain specimens for [beta]-actin, 18S rRNA, GAPDH, and also synaptophysin, a known marker for AD. Further, using real-time PCR analysis, we found that [beta]-actin and 18S rRNA are differentially expressed in the brain specimens of both AD and control subjects, while GAPDH is similarly expressed in AD and control brain specimens. These findings suggest that GAPDH can be used as an endogenous reference gene in the study of AD brains. A comparative gene expression analysis also suggests that synaptophysin is down-regulated in AD brain specimens compared to control brain specimens.

Lambert, J.-F., B. O. Benoit, et al. (2000). "Quick sex determination of mouse fetuses." Journal of Neuroscience Methods **95**(2): 127.

<http://www.sciencedirect.com/science/article/B6T04-3YJ9XNG-5/2/e8b8b4e03b0ffe1953a5ff20ad1450c3>

We designed a rapid, simple and accurate PCR method to determine sexual identity of mouse fetuses collected on embryonic day 15. A multiplex PCR amplification was used to detect male-specific sequence (Sry) in DNA extracted from fetal livers through SDS denaturation followed by high salt extraction and precipitation. This extraction method resulted in sufficiently purified DNA in <1 h and was suitable for PCR. The DNA obtained was amplified using a robot thermal cycler for 33 cycles. The reaction was performed in 50 [mu]l, using two sets of primers specific for Sry gene (chromosome Y) and IL3 gene (chromosome 11). Amplification duration was 1.5 h. The assessment of the results was done by electrophoresis in 3% agarose run at high voltage. The 402 bp band (Sry) obtained identifies the male fetuses and the 544 bp product (IL3) confirms the correct amplification of the template DNA. The entire procedure took <4 h. The specificity of the method was confirmed by fluorescent in situ hybridization using a specific male probe on cultured male and female neural stem cells. This method allowed the preparation and culture of pure male and female neural stem cells from fetal tissue.

Personett, D. A., M. Chouinard, et al. (1996). "Simplified RT/PCR quantitation of gene transcripts in cultured neuroblastoma (SN49) and microglial (BV-2) cells using capillary electrophoresis and laser-induced fluorescence." Journal of Neuroscience Methods **65**(1): 77.

<http://www.sciencedirect.com/science/article/B6T04-3VWPC7R-B/2/c39ab86ce993a3e02f31861567dc2d3d>

We developed a simplified protocol for sensitive quantitation of mRNA using polymerase chain reaction (PCR) amplification of cDNA made by reverse transcriptase (RT), as resolved with capillary electrophoresis (CE) and detected with laser-induced fluorescence (LIF). The conditions required for adequate accuracy of the simplified version of the RT/PCR quantitation, in which a

single concentration of external standard and amplification to within or near the plateau phase are used, were established for assay of mRNAs expressed at high, moderate, and low abundance. The mRNAs for the cytosolic enzyme, glyceraldehyde phosphate dehydrogenase (GAPDH) and the growth-associated protein GAP-43 in cultured SN49 neuroblastoma cells were used as target genes for high and moderate levels of expression, respectively. Using cultured mouse microglial cells (BV-2), we demonstrated the utility of this RT/PCR/CE/LIF protocol to quantitate a low-abundance mRNA, encoding a form of nitric oxide synthase (i-NOS) induced by treatment with endotoxin. The appearance of i-NOS mRNA after endotoxin treatment of BV-2 cells was confirmed by Northern blot analysis and in situ hybridization histochemistry, and functional enzyme activity was followed by release of nitric oxide (as nitrite) into the medium. The many advantages of the 'single-point' RT/PCR/CE/LIF protocol for quantitating mRNAs of interest include: simplified protocol, elimination of the use of radiotracers, high sensitivity and precision, and semi-automation of the quantitation phase of analysis.

Petraroli, R., G. Vaccari, et al. (2000). "A rapid and efficient method for the detection of point mutations of the human prion protein gene (PRNP) by direct sequencing." Journal of Neuroscience Methods **99**(1-2): 59.

<http://www.sciencedirect.com/science/article/B6T04-40X8CH9-7/2/3a5e383759be3eb3b01aa19745f9e76e>

Creutzfeldt-Jakob disease (CJD) and related disorders occur in sporadic, acquired and inherited forms. In sporadic, iatrogenic and new variant CJD the polymorphic codon 129 of the prion protein gene (PRNP) plays an important role for the susceptibility to the disease and for the clinical and neuropathological manifestations. All the inherited forms of CJD and related disorders are linked to point or insert mutations of PRNP. The analysis of PRNP is therefore important for a correct classification of these disorders and for the identification of novel mutations. The aim of the present study is to describe a fast and easy to perform method for the direct sequencing of the PCR amplified PRNP open reading frame, by using M13 tailed primers which allow a direct and rapid method of sequencing. The goodness of this method is demonstrated in the analysis of three sporadic CJD patients with different genotypes at codon 129 and three inherited cases bearing different point mutations of PRNP: the Pro102Leu mutation linked to Gerstmann-Straussler-Scheinker-syndrome, the Val210Ile mutation and a novel mutation at codon 211 (Gln211Glu) both associated to familial CJD.

Schimmelpfeng, J., K. F. Weibezahn, et al. (2004). "Quantification of NGF-dependent neuronal differentiation of PC-12 cells by means of neurofilament-L mRNA expression and neuronal outgrowth." Journal of Neuroscience Methods **139**(2): 299.

<http://www.sciencedirect.com/science/article/B6T04-4CS4RY0-1/2/01cd180a71535f64846e7c8af377ad8b>

We demonstrate that the degree of neuronal development of PC-12 cell differentiation can be quantified by the expression of neurofilament-L (NF-L) mRNA, when an optimal concentration of NGF (50 ng/ml) is used. During the first 7 days of NGF treatment, the relative amount of NF-L mRNA was found to increase continuously and to correlate with the outgrowth of neurites in a statistically significant way. Thus, mRNA expression is, under these conditions, a suitable means for reliably monitoring the differentiation of PC-12 cells as early as after 3 days of NGF treatment. The results obtained with 5 ng/ml NGF differ from those with 50 ng/ml: during the first 3 days of NGF treatment, neuronal outgrowth was less than with 50 ng/ml, although the NF-L mRNA levels did not depend significantly on NGF concentration. Beyond day 3, NF-L mRNA levels did not increase further at 5 ng/ml as opposed to 50 ng/ml NGF. These differences point to different

signal transduction processes involved in neuronal differentiation at high and low NGF concentration. Expression of NF-L protein in response to NGF treatment was also demonstrated. In summary, our results stress that stable and sustained differentiation of PC-12 cells can only be achieved with 50 ng/ml NGF.

Teupser, D., N. Heino, et al. (2002). "Rapid detection of the prion protein M129V polymorphism with the LightCycler." Journal of Neuroscience Methods **115**(1): 93.

<http://www.sciencedirect.com/science/article/B6T04-450HHKR-1/2/8b559299c7d239e59291a66e1e6b81c4>

The common single nucleotide polymorphism at codon 129 of the prion protein gene is a key determinant of the genetic susceptibility to Creutzfeldt-Jakob disease (CJD). Recently, a molecular classification of sporadic CJD based on the M129V genotype in conjunction with other determinants was proposed. In the present study, we describe the development and evaluation of a rapid fluorescent-based assay to detect this polymorphism using the LightCycler system. The two polymorphic alleles could be clearly distinguished by their melting points at 52.1 and 60.4 [deg]C, representing the 129V and 129M alleles, respectively. These results were confirmed by DNA sequencing. We evaluated our test in 400 patient samples and found no deviations from the expected melting patterns. The calculated allele frequency for the M-allele was 0.66. Thus, we have established a rapid, reliable fluorescent assay for high-throughput detection of the prion protein M129V polymorphism.

Torres, J. M., P. Sanchez, et al. (2004). "Quantitation of mRNA levels of steroid 5[alpha]-reductase isozymes in the rat brain by "one-step" RT-PCR and capillary electrophoresis." Journal of Neuroscience Methods **135**(1-2): 211.

<http://www.sciencedirect.com/science/article/B6T04-4BNMPTG-6/2/6a31b351d53580bdc82cec1e3659e1f4>

The enzyme 5[alpha]-reductase (5[alpha]-R) is present in many mammalian tissues, including the brain. The physiological importance of 5[alpha]-R in the brain derives from its capability to convert testosterone (T) to a more potent androgen, dihydrotestosterone (DHT), and to convert progesterone to its 5[alpha]-reduced derivative, precursors of allopregnanolone, potent allosteric modulator of the [gamma]-aminobutyric acid receptor (GABAA-R). 5[alpha]-R occurs as two isoforms, 5[alpha]-R type 1 (5[alpha]-R1) and 5[alpha]-R type 2 (5[alpha]-R2). We present an accurate, rapid, and modestly labor-intensive method to precisely quantitate 5[alpha]-R mRNA species in the cerebral cortex of the rat. This approach combines the high specificity of "one-step" reverse transcription-polymerase chain reaction (RT-PCR) with the sensitivity of laser-induced fluorescence capillary electrophoresis (LIF-CE). Both cDNA synthesis and PCR amplification are performed with the same enzyme and site-specific primers, improving the efficiency of cDNA synthesis. The specific target mRNA and a mimic DNA fragment, used as a competitive internal standard, were co-amplified in a single reaction in which the same primers are used. The method presented in this paper enables a more efficient quantitative determination of 5[alpha]-R mRNA isozymes, and may lead to a better understanding of the role of 5[alpha]-R isozymes in the physiology of the central nervous system.

Henry, C. H., A. P. Hudson, et al. (1999). "Identification of Chlamydia trachomatis in the human temporomandibular joint." Journal of Oral and Maxillofacial Surgery **57**(6): 683.

<http://www.sciencedirect.com/science/article/B6WKF-4CRR5T0-DM/2/8d77e1a217a1eb6cc10b2bb554627bdf>

Purpose: Reactive arthritis (ReA) as a consequence of triggering Chlamydia trachomatis infections has been extensively studied to better understand inflammatory arthritis. This study investigated whether the presence of C trachomatis can be shown in the TMJ of patients with internal derangement. **Patients and Methods:** Posterior bilaminar tissue removed from 31 patients (29 F, 2 M) during TMJ articular disc repositioning and posterior ligament repair was tested for the presence of C trachomatis. Cryosections were stained using a monoclonal antibody that identifies all chlamydial serovars. Highly specific polymerase chain reaction (PCR) assays independently targeting two genes of p p]C trachomatis also were performed; these assays also identify all serovars of this organism. **Results:** TMJ tissue from 6 of 30 patients (20%) showed the presence of C trachomatis in the posterior bilaminar tissue on immunostaining. PCR screening identified 12 of 31 patients (39%) as having C trachomatis DNA in tissue, including four of six positive by immunostaining. All chlamydia-positive patients were female, with an average age of 36.7 years (15 to 48 years). **Conclusions:** The presence of C trachomatis in the human TMJ has not been previously shown. The presence of this organism may serve as the pathogenetic mechanism for TMJ dysfunction, as demonstrated in other joints. Nonapparent chlamydial infection in females may also explain the marked prevalence of TMJ symptoms in women.

Honda, M. J., T. Yada, et al. (2004). "Cartilage formation by serial passaged cultured chondrocytes in a new scaffold: Hybrid 75:25 poly(L-lactide-[epsilon]-caprolactone) sponge." Journal of Oral and Maxillofacial Surgery **62**(12): 1510.

<http://www.sciencedirect.com/science/article/B6WKF-4DW8KWB-M/2/fa8b7dc99ba7e3d23ab244ac144b7910>

Purpose This study was designed to determine whether multiplied chondrocytes immersed in a new scaffold, 75:25 poly(L-lactide-[epsilon]-caprolactone) sponge coated with type I collagen (75-PLC scaffold), could be used to generate cartilage tissue in vivo and to evaluate the correlation between cartilage generation and the phenotype of the proliferated chondrocytes. **Materials and methods** Rat chondrocytes were suspended in 75-PLC scaffold at a density of 1 X 10⁷ cells/mL after proliferation in a monolayer for 1 (P1) to 4 passages (P4) and implanted in nude mice for 4 weeks. Cells were characterized by the expression of genes encoding type II collagen, aggrecan, and type I collagen by Northern hybridization, and consequently, the newly formed tissue was evaluated histologically. **Results** The expression of aggrecan messenger RNA gradually decreased with the passaged cultures; however, the expression of type I collagen messenger RNA increased with time. The cartilage formations in all specimens were found not only in P1 chondrocytes but also in P2 chondrocytes, although when P3 chondrocytes were grafted, approximately 50% of cartilage formation was still observed up to but not beyond P4. **Conclusion** It is suggested that cartilage tissue is generated with cultured chondrocytes up to P2 but not beyond P4. Northern blot analysis is useful for the assessment of whether the cells are capable of regeneration.

Asp, J., S. Inerot, et al. (2001). "Alterations in the regulatory pathway involving p16, pRb and cdk4 in human chondrosarcoma." Journal of Orthopaedic Research **19**(1): 149.

<http://www.sciencedirect.com/science/article/B6W7R-42M76BJ-S/2/6ed49f7dfc4b82d9560fd7a5bad1e968>

Dahlgren, L. A., H. O. Mohammed, et al. (2005). "Temporal expression of growth factors and matrix molecules in healing tendon lesions." Journal of Orthopaedic Research **23**(1): 84.

<http://www.sciencedirect.com/science/article/B6W7R-4CSYJG1-4/2/a23f6670fd8312bc5ded4fdb0daf3bc9>

Overuse tendon injuries are common among elite and recreational athletes. Tendon healing may be enhanced at the cellular level through the use of exogenous growth factors; however, little is known about the endogenous expression of growth factors in healing tendon. This study describes the temporal expression of insulin-like growth factor-I (IGF-I), transforming growth factor- β 1 (TGF- β 1), and collagen types I and III in healing tendon lesions. Collagenase-induced lesions were created in the tensile region of the flexor digitorum superficialis tendon of both forelimbs of 14 horses. Tendons were harvested from euthanatized horses 1, 2, 4, 8 or 24 weeks following injury. Gene expression was evaluated using Northern blot analysis (collagen types I and III), real time PCR (IGF-I and TGF- β 1), and in situ hybridization. Protein content was assayed by dye-binding assay (collagen types I and III), radioimmunoassay (IGF-I), ELISA (TGF- β 1), and immunohistochemistry. Samples were also processed for differential collagen typing, DNA and glycosaminoglycan content, and routine H&E staining. Microscopically, lesions progressed from an amorphous, acellular lesion soon after injury to scar tissue filled with collagen fibers and mature fibroblasts organized along lines of tension. Early lesions were characterized by immediate increases in expression of growth factors and collagen. Message levels for TGF- β 1 peaked early in the wound healing process (1 week), while IGF-I peaked later (4 weeks), as the regenerative phase of healing was progressing. In the first 2 weeks after lesion induction, tissue levels of IGF-I protein actually decreased approximately 40% compared to normal tendon. By 4 weeks, these levels had exceeded those of normal tendon and remained elevated through 8 weeks. Message expression for collagen types I and III increased by 1 week following injury and remained elevated throughout the course of the study. Collagen type I represented the major type of collagen in healing tendon at all time points of the study. Based on these results, IGF-I, administered exogenously during the first 2 weeks following injury, may provide a therapeutic advantage by bolstering low endogenous tissue levels and enhancing the metabolic response of individual tendon fibroblasts.

Devine, M. J., C. M. Mierisch, et al. (2002). "Transplanted bone marrow cells localize to fracture callus in a mouse model." Journal of Orthopaedic Research **20**(6): 1232.

<http://www.sciencedirect.com/science/article/B6W7R-45J8WMW-3/2/30e36d99e7e38b1bf1b1a8187f8214d>

Bone marrow contains many cellular elements that may contribute to fracture repair. We used a

pluripotential stromal cell in a mouse model to demonstrate the presence of transplanted cells in fracture hematoma and subsequently in maturing fracture callus. Cells were transduced with traceable genes (lac Z and neomycin resistance) and traced in vivo after intravenous injection into syngeneic mice. These transduced cells home to bone marrow, suggesting that they might be detected in fracture callus. Cells were injected intravenously into mice and stabilized femoral shaft fractures were induced. Control mice received intravenous lactated-Ringer's solution prior to fracture. Callus tissue and marrow were examined histologically from 1 to 10 weeks after fracture to detect transplanted cells. Transplanted cells were detected in fracture callus in areas, and at times, of most active bone formation. Control specimens showed minimal staining of the callus tissue. Levels of the traceable gene in fracture callus increased, reached a peak between 3 and 4 weeks after fracture, then diminished and disappeared by 10 weeks post-fracture as woven bone at the fracture site was replaced by lamellar bone with cells from the host mouse. The results show that pluripotent bone marrow cells home to the marrow after systemic injection and localize in fracture callus.

Mizuta, H., A. Sanyal, et al. (2002). "The spatiotemporal expression of TGF- β 1 and its receptors during periosteal chondrogenesis in vitro." Journal of Orthopaedic Research **20**(3): 562.

<http://www.sciencedirect.com/science/article/B6W7R-45GWRRP-C/2/2e31743a46de1dfd7c95079dfdefa69d>

Muramatsu, K. and A. T. Bishop (2002). "Cell repopulation in vascularized bone grafts." Journal of Orthopaedic Research **20**(4): 772.

<http://www.sciencedirect.com/science/article/B6W7R-44SJYB5-5/2/e0ba949f905fc6cd53c4369ac476c68e>

Following vascularized bone autografts, osteocyte viability is largely maintained. Viable cells within a graft may be surviving graft-derived cells, their progeny, or host-derived cells from circulation or surrounding bone. This study was conducted to define the process of cell repopulation, within vascularized bone grafts. Using inbred Lewis rats, 30 female vascularized tibial bones were transplanted to syngeneic male recipients and 45 male grafts were transplanted to female recipients. Twenty-five female recipients were immunosuppressed with FK506 to prevent rejection caused by Y-chromosome related antigens. The grafts were assessed up to 24 weeks post-transplant by radiography, histology and semi-quantitative polymerase chain reaction (PCR) using both Y-chromosome and autosome gene-specific primers. The female to male isograft transplants were useful to measure low levels of repopulation with host-derived cells, while male to female transplants more accurately quantified higher levels of cellular replacement. No host-derived cells were detected in the transplanted bone before six weeks. Thereafter, the ratio of host-derived cells gradually increased. By 24 weeks only 0.1-1.0% of graft-derived cells remained in the transplanted tibias. This study demonstrates that Y-chromosome-specific PCR is a useful tool to detect the cell lineage and cell repopulation following rat sex-mismatch vascularized bone grafting. Our results showed that donor cells in vascularized bone grafts were gradually repopulated with recipient cells. Correlation with histologic findings suggests that the periosteal hypertrophy observed by six weeks post-transplant results from graft-derived cells, while later remodeling is associated with host-derived cells.

Muramatsu, K., R. G. Valenzuela, et al. (2003). "Detection of chimerism following vascularized bone allotransplantation by polymerase chain reaction using a Y-chromosome specific primer." Journal

of Orthopaedic Research **21**(6): 1056.

<http://www.sciencedirect.com/science/article/B6W7R-48TKCBN-1/2/8afc05807dbc3bb578c4cb63f2666c4d>

Chimerism following allogeneic organ transplantation is a phenomenon known to occur and be associated with development of immunologic tolerance in allotransplantation. However, little is known about graft cell migration following vascularized bone allografting. In this study, chimerism was assessed following vascularized tibia transplantation from male DA or PVG donors to female PVG rat recipients using a semi-quantitative polymerase chain reaction for the Y-chromosome. FK-506 (Tacrolimus) was administered after transplantation for immunosuppression. All immunosuppressed PVG rat recipients of PVG bone grafts showed a high level of chimerism (1%) in the thymus, spleen, liver and cervical lymph nodes at 18 weeks post-transplant. Donor cells were also detected in the contralateral tibia and humerus. In non-immunosuppressed PVG rat recipients of DA bone grafts, donor cells were detected in the spleen in three of five rats within 2 weeks post-transplant. In these animals the bone grafts were severely rejected. In immunosuppressed PVG rat recipients of DA bone grafts, two of five, four of eight and eight of 10 rats showed low level chimerism (0.1%) in peripheral blood at 1, 12, and 18 weeks post-transplant. Six rats showed a high level of chimerism in the spleen and thymus. Histological studies revealed no rejection findings through 18 weeks post-transplant. Our results indicate that chimerism, or the presence of graft cells in host tissue, may occur in the face of acute rejection and be demonstrable following vascularized isograft and allograft living bone transplantation when chronic immunosuppression is maintained. Graft vascular patency during the short-term likely allows cellular migration, even in the face of acute rejection. Long-term survival and proliferation of graft marrow elements in host tissue may be possible with adequate immunosuppression.

Noth, U., A. M. Osyczka, et al. (2002). "Multilineage mesenchymal differentiation potential of human trabecular bone-derived cells." Journal of Orthopaedic Research **20**(5): 1060.

<http://www.sciencedirect.com/science/article/B6W7R-458P5XP-1/2/a2002819fec6975e9bdf5519d1dea600>

Journal of Pediatric Surgery (14)

Ara, T., T. Kusafuka, et al. (2000). "Determination of imbalance between MMP-2 and TIMP-2 in human neuroblastoma by reverse-transcription polymerase chain reaction and its correlation with tumor progression." Journal of Pediatric Surgery **35**(3): 432.

<http://www.sciencedirect.com/science/article/B6WKP-4C53P60-6/2/65523b87ca97365bd1e6884e14501f42>

Background/Purpose: The matrix metalloproteinases (MMPs) and their specific tissue inhibitors (TIMPs) have been implicated in tumor invasion and metastasis. Net matrix degradation and proteolysis depend on the critical local balance between MMPs/TIMP-2. We attempted to determine their expression balance and to evaluate its importance with tumor progression. **Methods:** Expressions of MMP-2, MMP-9, and TIMP-2 mRNAs was quantified by

reverse-transcription polymerase chain reaction (RT PCR) in tumor tissues from 25 neuroblastoma patients. Results: MMP-2, MMP-9, and TIMP-2 expression was observed in all the samples but with different trends. Increased expressions of MMP-2 mRNA was evident in advanced stages (Evans' stage III and IV; $P = .02$; unpaired ttest), and in patients who died of progressive disease ($P = .0001$). Whereas, the expressions of MMP-9 and TIMP-2 had no such significant association with clinical stages and prognosis. The ratio of MMP-2/TIMP-2 mRNAs was significantly higher in the advanced stages versus early stages (mean $\pm$ SD = 1.66 ± 0.65 and 1.11 ± 0.34 , respectively; $P = .02$) and in patients who died of progressive disease versus alive patients (mean = 2.13 ± 0.78 and 1.21 ± 0.36 , respectively; $P = .0006$). Conclusions: Coexpression of MMPs and TIMP-2 in neuroblastoma indicates the need to evaluate their expression balance. Significantly higher expression of MMP-2 mRNA and increased ratio of MMP-2 and TIMP-2 mRNAs in advanced stages or patients who have died of progressive disease suggests an association between elevated MMP-2 expression and poor prognosis. To establish the role of enzyme to inhibitor mRNA ratio as a reliable predictor, cohort studies with significant number of cases may be carried out.

Brizzolara, A., M. Torre, et al. (2004). "Histochemical study of Dom mouse: a model for Waardenburg-Hirschsprung's phenotype." *Journal of Pediatric Surgery* **39**(7): 1098.

<http://www.sciencedirect.com/science/article/B6WKP-4CN8TF3-P/2/e2725a66a6cedaf51afa3c3582e155f0>

Background/Purpose The spontaneous mouse mutant Dominant megacolon (Dom) represents the model of the Waardenburg-Hirschsprung's disease, a syndromic pathology, characterized by the association of pigmentation defects (PD), deafness, and Hirschsprung's disease (HD). The defect in Dom mouse is caused by a spontaneous mutation of the gene encoding the Sry-related transcription factor Sox10. This mutation affects several aspects of neural crest development leading to combined enteric innervation and pigmentation defects, both in mouse and human. The purpose of this report is to define, by enzymo-histochemical techniques routinely used for the diagnosis of human Hirschsprung's disease (AChE, LDH, NADPH-diaphorase), the innervative patterns of the affected gut. **Methods** Fifty-four siblings of Heterozygous Dom/+ mice underwent autopsy and were genotyped by direct sequencing of polymerase chain reaction (PCR) products for Sox10 mutations. The enteric nervous system of all the mice was studied by histochemical techniques indicated above. **Results** Genotyping showed that 43 mice were Dom/+ and 11 were Wild type +/+. Wild-type +/+ mice were used as control. The correspondence between genotype and at least 1 phenotypic aspect (PD or dysganglionosis) was present in 93% of cases (41 of 43). Among the Dom/+ mice, dysganglionosis was present in 79% of cases and PD in 90% of cases. Moreover, among Dom/+ mice, excluding those whose mantle was not evaluated as dead just after birth, PD and dysganglionosis (complete phenotype) were present in 68% of cases. **Conclusions** The histochemical methods that we used proved to be useful for identification of different aganglionic (AG), hypoganglionic (HG), and normoganglionic segments of Dom/+ mouse gut studied in longitudinal sections. Unlike humans, control mice (Wild type +/+) presented a rich component of AChE nerve fibers, whereas Dom/+ mice with dysganglionosis presented a decrease in AChE-positive nerve fibers. These data confirm the variable phenotypic penetrance in heterozygous mice. Because dysganglionosis in this animal model (Dom/+) was evident in 79% of cases (AG or HG), we concluded that Dom mice could represent important models for further experimental studies.

Cass, D. L., T. M. Quinn, et al. (1998). "Increased cell proliferation and decreased apoptosis characterize congenital cystic adenomatoid malformation of the lung." *Journal of Pediatric Surgery* **33**(7): 1043.

<http://www.sciencedirect.com/science/article/B6WKP-4CTD5NV-H/2/d8274c1e8cdb959a3547d2e14388ed30>

Background/Purpose: Congenital cystic adenomatoid malformations (CCAM) are lung lesions that demonstrate abnormalities of both mesenchymal and epithelial tissues. The pathogenesis of these tumors remains unknown. Because normal organogenesis requires a balance between cell proliferation and programmed cell death (apoptosis), the authors hypothesized that CCAM results from an increase in cell proliferation or a decrease in apoptosis within the developing lung, possibly mediated by keratinocyte growth factor (KGF). **Methods:** To examine cell cycle control in CCAM, we measured indices of cell proliferation and apoptosis in lesions requiring fetal (n = 4) or neonatal (n = 8) resection compared with those of normal fetal (14 to 28 week's gestation; N = 14) and neonatal (n = 3) human lung. Cell proliferation was analyzed by immunostaining for a proliferation marker (Ki-67). Apoptosis was examined using an in situ digoxigenin end-labeling technique to localize apoptotic bodies. The expression of KGF protein and KGF mRNA in CCAM and normal lung was examined using immunohistochemistry and semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR). **Results:** CCAM lesions in general showed a twofold increase in cell proliferation index (19.2% $\pm$ 1.4% v 9.6% $\pm$ 0.7%, $P < 0.05$). **Conclusions:** These results demonstrate that CCAM differs from normal lung by increased cell proliferation and decreased apoptosis. The increased proliferation does not appear to be mediated by the pneumocyte mitogen KGF. An examination of factors that control cell proliferation and apoptosis in CCAM may provide further insight into the pathogenesis of this tumor.

Kusafuka, T. and P. Puri (1997). "Altered mRNA expression of the neuronal nitric oxide synthase gene in Hirschsprung's disease." *Journal of Pediatric Surgery* **32**(7): 1054.

<http://www.sciencedirect.com/science/article/B6WKP-4CR8G4W-X/2/7e3c796839beca6b803d51f783f27a5c>

Nitric oxide has been described as an inhibitory neurotransmitter to mediate smooth muscle relaxation in the mammalian gastrointestinal tract. The enzyme neuronal nitric oxide synthase (NOS) catalyzes the formation of NO. The authors examined the expression of neuronal NOS gene at the mRNA level in intestinal specimens from seven patients who had Hirschsprung's disease (HD) using reverse transcription polymerase chain reaction (RT-PCR) technique. With 35 cycles of PCR reaction, substantial signals of neuronal NOS mRNA were observed in the ganglionic bowel in all seven patients, whereas in the aganglionic bowel, neuronal NOS signals were weak in three patients, and undetectable in four patients. By increasing the PCR cycle to 40 cycles, barely detectable signals were observed in two of the latter four patients. Semiquantitative analysis in the three patients who showed weak signals with 35 cycles of PCR indicated that neuronal NOS mRNA expression in aganglionic bowel was decreased at least 1/50 to 1/100 of the level of expressed in ganglionic bowel. Because absence or low level of expression of neuronal NOS mRNA may lead to impaired production of NO, our observations suggest that motility dysfunction in HD may be as a result of markedly decreased or no expression of the neuronal NOS gene at the mRNA level.

Kusafuka, T. and P. Puri (1997). "Altered RET gene mRNA expression in Hirschsprung's disease." *Journal of Pediatric Surgery* **32**(4): 600.

<http://www.sciencedirect.com/science/article/B6WKP-4CT1124-6R/2/331f56b7618ff14ad8823b948f30a7f2>

The RET proto-oncogene is a major cause of Hirschsprung's disease (HD) as demonstrated by the experimentally produced intestinal aganglionosis in mice with a null mutation of this gene and by the increased evidence of RET mutations in patients with HD. To evaluate the possible implication of the RET gene for the development of HD, we examined mRNA expression level of the RET gene in the bowel specimen of seven HD patients by using the reverse transcription-polymerase chain reaction technique. A significantly less intense signal for RET mRNA was found in the aganglionic bowel compared with the ganglionic bowel. In the hypoganglionic transitional zone, the RET mRNA level was higher than that of an aganglionic segment but lower than that observed in the ganglionic portion. In two patients where semiquantitative analysis was performed, the RET mRNA level in the aganglionic bowels was estimated to be approximately 1/500 of that in the ganglionic bowels. Because expression of RET mRNA plays an important role in establishing the enteric neuronal lineage, decreased RET mRNA expression in the aganglionic bowel may suggest maldevelopment of neural crest-derived cells in Hirschsprung's disease.

Liechty, K. W., T. M. Crombleholme, et al. (1999). "Elevated platelet-derived growth factor-B in congenital cystic adenomatoid malformations requiring fetal resection." Journal of Pediatric Surgery **34**(5): 805.

<http://www.sciencedirect.com/science/article/B6WKP-4CR8H6R-DD/2/50b4b8c92b19029c9cc8d57dfe86d657>

Background: During lung development, platelet-derived growth factor-BB (PDGF-BB) is maximal during the canalicular stage and decreases by the sacular stage. PDGF-BB stimulates lung growth by increasing cell proliferation. Fetal CCAMs have been shown to have an elevated proliferative index, but it is not known why some CCAMs rapidly enlarge in utero and cause fetal hydrops. The authors hypothesized that the high proliferative index and rapid enlargement of some fetal CCAMs may be caused by persistently elevated PDGF-BB production compared with normal fetal lung. **Methods:** To test this hypothesis, tissue was obtained at the time of resection from two fetal CCAMs (22 weeks), three full-term CCAMs, and three normal fetal lungs (21 to 22 weeks). PDGF-BB production by fetal CCAMs was compared with normal age-matched fetal lung using immunohistochemistry, reverse transcriptionase-polymerase chain reaction (RT-PCR), and Western blot analysis. **Results:** CCAMs resulting in fetal hydrops and requiring fetal resection had strong mesenchymal immunostaining for PDGF-BB next to epithelial lined cysts, increased PDGF-B gene expression by RT-PCR, and elevated PDGF-BB protein by Western blot, compared with normal age-matched fetal lung. Term CCAMs had minimal PDGF-BB staining, PDGF-B gene expression, and PDGF-BB protein production. **Conclusions:** CCAMs that grew rapidly and progressed to hydrops, requiring in utero resection, demonstrated increased mesenchymal PDGF-B gene expression and PDGF-BB protein production compared with age-matched normal fetal lung, which may, in part, be responsible for the autonomous growth and proliferation seen in hydropic fetal CCAMs.

Mason, J. E., P. J. Goodfellow, et al. (2000). "16q loss of heterozygosity and microsatellite instability in Wilms' tumor." Journal of Pediatric Surgery **35**(6): 891.

<http://www.sciencedirect.com/science/article/B6WKP-49SHG13-35/2/92a38ee43453a7ea3d166366a6fb99fa>

Background/Purpose: Wilms' tumor is the most common renal malignancy of childhood. Loss of heterozygosity (LOH) at 16q is seen in about 17% of cases and has been associated with a poor prognosis. To more precisely define the pattern of 16q deletion exhibited by Wilms' tumor, the authors performed a detailed LOH analysis of 96 specimens using polymorphic microsatellite repeat markers. The authors also evaluated the neoplasms for the presence of microsatellite

instability (MSI). Methods: A total of 96 DNA samples were studied using polymerase chain reaction-based LOH analyses amplifying polymorphic microsatellite repeat markers. Screening for MSI using 2 additional genetic markers also was carried out. Results: The authors found 16q LOH in 14 of the specimens evaluated. Comprehensive analysis of these LOH-positive specimens showed a region of loss spanning 16p11.2-q22.1 and a separate distal region of LOH at 16q23.2-24.2. The distal region of deletion is very small, estimated to be approximately 2.4 megabases. In addition to the observed LOH, 2 specimens were found to consistently exhibit MSI, which has not been reported previously in Wilms' tumor. Conclusions: The smallest consensus region of deletion in our analysis of Wilms' tumor 16q LOH measures 2.4 megabases at 16q23.2-q24.2. Additionally, MSI was present in a subset of tumor specimens suggesting that defects in DNA mismatch repair may contribute to the pathogenesis of Wilms' tumor. *J Pediatr Surg* 35:891-897. Copyright (c) 2000 by W.B. Saunders Company.

Oue, T. and P. Puri (1999). "Altered endothelin-3 and endothelin-B receptor mRNA expression in hirschsprung's disease." *Journal of Pediatric Surgery* **34**(8): 1257.

<http://www.sciencedirect.com/science/article/B6WKP-4CP6TFB-6X/2/d68d92700b59011a345ab718db75b8c3>

Background: Recently, the endothelin-3 (EDN3) and endothelin-B receptor (EDNRB) gene have been recognized as susceptibility genes for Hirschsprung's disease (HD). However, gene mutations have been observed only in limited cases, and the role of EDN3 in the pathogenesis and motility dysfunction in HD is not understood fully. To evaluate the possible implication of EDN3 and EDNRB for the development of HD, we examined the EDN3 and EDNRB mRNA level in bowel specimens of HD patients. Methods: Entire resected specimens of colon were obtained from 14 patients with HD. Eight age-matched control patients without gastroenteric disorders also were examined. mRNA was extracted from ganglionic and aganglionic segments of the HD specimens and normal colons. Reverse transcription-polymerase chain reaction (RT-PCR) was performed to evaluate the relative amount of EDN3 and EDNRB mRNA. Results: In normal colon, constant EDN3 and EDNRB mRNA expression was observed. In HD, EDN3 and EDNRB mRNA expression was observed. In HD, EDN3 and EDNRB mRNA levels were decreased both in ganglionic and aganglionic segment in 2 cases. In 6 cases, EDN3 mRNA expression was decreased in aganglionic segment and in another 2 cases, EDNRB mRNA expression was decreased in aganglionic segment. In the remaining 4 cases, EDN3 and EDNRB mRNA levels were similar to controls. Conclusion: The authors' findings indicate that loss of EDN3 and EDNRB function may be involved in the maldevelopment of neural crest-derived cells causing HD in many patients.

Piotrowska, A. P., V. Solari, et al. (2003). "Distribution of Ca²⁺-Activated k⁺ channels, SK2 and SK3, in the normal and Hirschsprung's disease bowel." *Journal of Pediatric Surgery* **38**(6): 978.

<http://www.sciencedirect.com/science/article/B6WKP-48NBHVV-1B/2/e813bf59396e4ba0573f0ded69edcf28>

Purpose: The aim of this study was to investigate the expression and distribution of SK2 and SK3 channels in the normal and Hirschsprung's disease (HD) bowel. Methods: Full-thickness colonic specimens were collected at pull-through operation from 10 patients with HD and from 6 patients during bladder augmentation. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis for SK2 and SK3 channels and double immunostaining using anti SK2/c-kit, SK3/c-kit, SK2/[alpha]-SMA, and SK2/PGP 9.5 antibodies was performed. Immunolocalization was detected using laser scanning microscopy. Results: RT-PCR analysis showed strong expression of SK2 and SK3 mRNA in the normal human bowel and significantly reduced SK3 expression in the

aganglionic bowel (P Conclusions: The results of this study provide the first evidence for the presence of SK2 and SK3 channels and for the immunocolocalization of SK3 channels in the ICCs in the normal human colon. Decreased expression SK3 channels in the aganglionic bowel may contribute to motility dysfunction in HD.

Quinn, T. M., K. G. Sylvester, et al. (1999). "TGF-[beta]2 is increased after fetal tracheal occlusion." Journal of Pediatric Surgery **34**(5): 701.

<http://www.sciencedirect.com/science/article/B6WKP-4CR8H6R-CS/2/611882acee24d34f5c15132498ef7902>

Background/Purpose: Fetal tracheal occlusion (TO) accelerates lung growth in normal and hypoplastic fetal lung. The mechanism of accelerated lung growth remains unknown but may be a result of growth factor induction. Previous studies of growth factors induced by tracheal ligation have characterized mRNA rather than protein expression. Although the transforming growth factor-[beta] (TGF-[beta]) family participates in normal lung morphogenesis, its role in lung growth after TO is unclear. The authors hypothesize that TGF-[beta] expression is increased with TO and may contribute to the accelerated lung growth seen after TO. **Methods:** Diaphragmatic hernia (DH) was created in 80-day-gestation sheep (n = 6; term, 145) by excising the left diaphragm. At 110 days, the trachea was occluded (n = 4) with a clip. DH controls (n = 2) were not occluded. Fetuses were killed at 139 days, and lung samples were snap frozen for tissue analysis. Non-DH control lungs were harvested from full-term animals (n = 2). TGF-[beta] mRNA was analyzed by semiquantitative reverse transcriptionase--polymerase chain reaction (RT-PCR). TGF-[beta] protein was assessed by Western blot analysis. **Results:** TGF-[beta]1 mRNA and protein were not increased with tracheal ligation compared with either non-DH or DH controls. TGF-[beta]2, however, was markedly increased, at both the mRNA and protein level, in ligated lungs compared with nonligated controls. **Conclusions:** TGF-[beta]2 protein, but not TGF-[beta]1, is increased in the hypoplastic lungs of fetal sheep after tracheal occlusion. Increased TGF-[beta]2 expression appears to result from increased or prolonged expression of mRNA transcripts. This is the first study to document a change in growth factor protein levels after TO. Increased TGF-[beta]2 expression may contribute to accelerated lung growth and decreased surfactant production observed after tracheal occlusion.

Safford, S. D., A. J. Freerman, et al. (2005). "Decreased E-cadherin expression correlates with higher stage of Wilms' tumors." Journal of Pediatric Surgery **40**(2): 341.

<http://www.sciencedirect.com/science/article/B6WKP-4FH06RR-B/2/c0d35520406291e8273e9e9ab76de649>

Purpose The aim of this study was to examine the association between E-cadherin expression and markers of Wilms' tumor aggression, including metastasis and recurrence. **Methods** Forty Wilms' tumor samples from the National Wilms' Tumor Study Group underwent immunohistochemical staining for E-cadherin. Tumor stage at diagnosis, recurrence, and loss of heterozygosity at 16q status was known for each of the tumor samples. E-Cadherin cell staining was defined as high (>33%) or low (Results Wilms' tumors presenting with metastatic (stage IV) disease demonstrated decreased levels of E-cadherin expression compared with localized tumors (stage I) (Fisher's Exact test, P Conclusions In this study, the authors have found an association between decreased E-cadherin expression and metastatic Wilms' tumor. Mutations identified may help identify a mechanism for downregulation. The functional significance of these mutations is supported by the conserved nature of the amino acids across multiple species. The authors believe these findings support the involvement of E-cadherin in the evolution of Wilms' tumor.

Solari, V. and P. Puri (2004). "Genetic polymorphisms of angiotensin system genes in congenital diaphragmatic hernia associated with persistent pulmonary hypertension." Journal of Pediatric Surgery **39**(3): 302.

<http://www.sciencedirect.com/science/article/B6WKP-4BT1J8H-B/2/5de3502302e1d8fa581162ff20e39c65>

Background/purposeThe renin-angiotensin system plays an important role in pulmonary artery remodelling. Several polymorphisms of genes encoding for components of the renin angiotensin system such as the angiotensin converting enzyme (ACE), the angiotensinogen (AGT) gene, and the angiotensin II type 1 receptor (AT1R) have been associated with the development of pulmonary hypertension. The aim of this study was to investigate the ACE I/D genotype, the M235 T polymorphism of the AGT gene and the A1166 C polymorphism of AT1R in the lungs of congenital diaphragmatic hernia (CDH) complicated by persistent pulmonary hypertension (PPH) in the newborn. **Methods**Genomic DNA was extracted from archival paraffin-embedded lung tissue from 13 newborns with CDH complicated by PPH and from 9 controls. Genotyping for the I/D-ACE, the M235 T-AGT, and the A1166 C-AT1R gene polymorphisms were determined by a polymerase chain reaction-based method with appropriate restriction digest when required. **Results**In controls, ACE genotype distribution of DD, ID, and II was 11%, 33%, and 55%, respectively, whereas in CDH it was 70%, 15%, and 15%, respectively. The ACE-DD genotype was significantly higher in CDH compared with controls (P < 0.05). **Conclusions**These data suggest that D allele of the ACE gene insertion/deletion polymorphism and angiotensinogen M235 T polymorphism may be associated with PPH in newborns with congenital diaphragmatic hernia.

Spilde, T. L., A. M. Bhatia, et al. (2004). "Aberrant fibroblast growth factor receptor 2 signalling in esophageal atresia with tracheoesophageal fistula." Journal of Pediatric Surgery **39**(4): 537.

<http://www.sciencedirect.com/science/article/B6WKP-4C2PRK5-6/2/3b85b36f672971d5520dfa80524afd88>

BackgroundAlthough the pathogenesis of esophageal atresia with tracheoesophageal fistula (EA/TEF) remains unknown, it has been shown that despite its esophageal appearance, the fistula tract originates from respiratory epithelium. The authors now hypothesize that defects in fibroblast growth factor (FGF) signaling contribute to the esophaguslike phenotype of the fistula tract. FGF2R is critical to normal lung morphogenesis and occurs in 2 isoforms (FGF2RIIIb and FGF2RIIIc), each with different ligand-binding specificity. To characterize FGF signaling in the developing EA/TEF, the authors analyzed levels of FGF2R splice variants in experimental EA/TEF. **Methods**The standard Adriamycin-induced EA/TEF model in rats was used. Individual foregut components from Adriamycin-treated and control embryos were processed for real-time, fluorescence-activated semiquantitative reverse transcriptase polymerase chain reaction on gestational days 12.5 and 13.5. **Results**Both fistula tract and Adriamycin-treated or normal esophagus showed significantly lower levels of FGF2RIIIb than either Adriamycin-treated lung buds (E12.5, P = .02; E13.5, P = .001). **Conclusions**Levels of FGF2R in the developing fistula tract resemble that of distal esophagus rather than developing lung. This defect in FGF2RIIIb signaling may account for the nonbranching, esophaguslike phenotype of the fistula, despite its respiratory origin.

Taira, Y., T. Oue, et al. (1999). "Increased tropoelastin and procollagen expression in the lung of nitrofen-induced diaphragmatic hernia in rats." Journal of Pediatric Surgery **34**(5): 715.

<http://www.sciencedirect.com/science/article/B6WKP-4CR8H6R-CW/2/97913541194d61856d52a8c7faf9c794>

Background/Purpose: Collagen and elastin, the predominant components of the lung connective tissue network, have been suggested to have an important influence on lung compliance and maximal expansion. Decrease in lung compliance and distensibility often is seen in human congenital diaphragmatic hernia (CDH) lung as well as in experimentally produced CDH lung. The aim of this study was to investigate mRNA levels of tropoelastin and [alpha]1 (I) procollagen, the precursors of elastin, and type I collagen, respectively, in CDH lung and to determine whether antenatal dexamethasone treatment has any effect on the production of these extracellular matrix proteins. **Methods:** CDH model was induced in pregnant rats after administration of 100 mg nitrofen on day 9.5 of gestation (term, 22 days). Dexamethasone (0.25 mg/kg) was given on day 18.5 and 19.5. Cesarean section was performed on day 21. The fetuses were divided into three groups: group I, normal controls; group II, nitrofen-induced CDH; and group III, nitrofen-induced CDH with antenatal dexamethasone treatment. Reverse transcription-polymerase chain reaction (RT-PCR) was performed to evaluate relative amounts of tropoelastin and [alpha]1 (I) procollagen mRNA. **Results:** Levels of both tropoelastin and [alpha]1 (I) procollagen mRNA were significantly increased in group II compared with group I (P < 0.05). **Conclusions:** The increased local synthesis of tropoelastin and type I procollagen in CDH lung may be responsible for the increased rigidity and decreased compliance observed in the CDH hypoplastic lung. Glucocorticoids have no effect on pulmonary tropoelastin and [alpha]1 (I) procollagen gene expression in CDH lungs.

Journal of Pharmaceutical and Biomedical Analysis (5)

Arakawa, H., A. Tsuji, et al. (1997). "Analysis of single-strand conformation polymorphisms by capillary electrophoresis with laser induced fluorescence detection." Journal of Pharmaceutical and Biomedical Analysis **15**(9-10): 1537.

<http://www.sciencedirect.com/science/article/B6TGX-3W0FSK8-4R/2/d8aa7b79ec12e5c82f780aabd6f9d66a>

Detection of point mutations in genomic DNA is important for diagnosis of inherited characteristics and genetic diseases. A point mutation in a specific region of DNA amplified by polymerase chain reaction (PCR) can be detected with single-strand conformation polymorphism (SSCP) analysis. Analysis of SSCP by laser-induced fluorescence capillary electrophoresis in entangled polymer solution (CE-LIF) has been developed in the present paper. K-ras genes including seven mutations were amplified with primer labeled with Texas Red at its 5' end. The labeled PCR products were dissociated to single strands by heating and separated with capillary gel electrophoresis and He-Ne laser-excited fluorescence detection. Our results suggest that all fragments having normal (Gly) and mutated (Ala, Arg, Cys, Ser, Val, Asp) sequences at codon 12 can be distinguished. Analysis of SSCPs with CE-LIF is well suited for clinical analysis of SSCPs because of its high sensitivity, resolution, reproducibility and speed.

Lauwers, S., Y. Vander Heyden, et al. (2002). "Screening and optimisation of an ELISA method for the quantitative detection of enterovirus specific RT-PCR products by means of a two-level experimental design." Journal of Pharmaceutical and Biomedical Analysis **29**(4): 659.

<http://www.sciencedirect.com/science/article/B6TGX-45JYJC6-F/2/0e1e018a4a32672b457a910209872898>

In a previous paper, optimal reaction conditions were determined for the RT-PCR part of a quantitative enterovirus specific RT-PCR ELISA method (J. Pharm. Biomed. Anal., 25 (2001) 131-142). In order to obtain a detection limit as low as possible, the ELISA part of the procedure was optimised as well. This was done by investigating the influence of seven factors at three levels in a multivariate approach. A reflected two-level screening design, derived from a Plackett-Burman design, was used. Optimal reaction conditions were established by calculation and by evaluation of the effects of the factors on the measured absorbance of the ELISA detection. Under these conditions, the linear range and detection limit of the test were determined and compared with the ELISA conditions before optimisation. The optimised RT-PCR ELISA will be used to study a possible longitudinal relationship between enteroviruses and the development of multiple sclerosis and juvenile diabetes.

Lauwers, S., Y. Vander Heyden, et al. (2001). "Screening of an enterovirus specific RT-PCR ELISA method for the quantification of enterovirus genomes in human body fluids by means of a three-level experimental design." Journal of Pharmaceutical and Biomedical Analysis **25**(1): 131.

<http://www.sciencedirect.com/science/article/B6TGX-42M7828-J/2/6637db87972d5e7573113fb04e43c931>

In order to obtain a detection limit as low as possible for a quantitative enterovirus specific RT-PCR ELISA assay, optimal reaction conditions, which give rise to the highest response, need to be determined. This was done by investigating the influence of 13 factors, selected from RT and PCR, in a multivariate approach by means of a well-balanced three-level screening design, derived from a three-level Plackett-Burman design. Optimal reaction conditions could be determined by calculation and evaluation of the effects of the different factors on the response, i.e. the measured absorbance of the ELISA detection. The method will be used to study a possible longitudinal relationship between enteroviruses and the development of multiple sclerosis and juvenile diabetes.

Verheyden, B., S. Lauwers, et al. (2003). "Quantitative RT-PCR ELISA to determine the amount and ratio of positive- and negative strand viral RNA synthesis and the effect of guanidine in poliovirus infected cells." Journal of Pharmaceutical and Biomedical Analysis **33**(2): 303.

<http://www.sciencedirect.com/science/article/B6TGX-492098W-3/2/ea0a9c5bb172d82c9c65c6cad3e056f7>

Quantitative reverse transcription-polymerase chain reaction enzyme linked immunosorbent assay (RT-PCR ELISA) is the method of choice to study positive- and negative strand viral RNA synthesis during poliovirus replication. In comparison with other methods used for this purpose, it fulfils all necessary requirements to accurately determine RNA of different polarity. It combines high specificity, high sensitivity, safety, speed, and the ability to perform quantitative analysis. The enterovirus specific RT-PCR ELISA method described in this work, was used to determine quantitatively the amount of de novo poliovirus positive- and negative strand RNA synthesis at different time-points in the viral replication cycle, both in presence and absence of the viral RNA synthesis inhibitor guanidine hydrochloride.

Verheyden, B., A. Thielemans, et al. (2003). "RNA extraction for quantitative enterovirus RT-PCR: comparison of three methods." Journal of Pharmaceutical and Biomedical Analysis **33**(4): 819.

<http://www.sciencedirect.com/science/article/B6TGX-4950CBP-5/2/687d80ab93c70773bbca05b0b890c902>

Quantification of virus-like RNA sequences in biological fluids, like serum and cerebrospinal fluid, requires an RNA extraction method that is both reproducible and fast. Three RNA extraction methods were tested on enteroviruses: (1) the acid guanidine thiocyanate-phenol/chloroform (AGPC) method; (2) a method based on differential precipitation of the RNA and (3) a 'bind-wash-elute' system based on silica-gel membrane binding. The latter two methods yielded a comparable detection limit as measured by RT-PCR ELISA. The detection limit for the AGPC method was 10 times higher. The relative standard deviation for the bind-wash-elute method (3%) was superior to that of the other methods tested (both 20%) and provides a reliable and fast method to extract (viral) RNA from biological fluids for quantification by RT-PCR.

Journal of Pharmacological and Toxicological Methods (2)

Cotreau, M. M., L. L. von Moltke, et al. (2000). "Methodologies to study the induction of rat hepatic and intestinal cytochrome P450 3A at the mRNA, protein, and catalytic activity level." Journal of Pharmacological and Toxicological Methods **43**(1): 41.

<http://www.sciencedirect.com/science/article/B6T8S-41PP0J6-6/2/6e9142ddaecb9a6a3ee1ba22bd48972b>

Studies were conducted to characterize assays for the isolation and quantitation of rat cytochrome P450 (CYP) 3A isoforms from hepatic and intestinal tissues. Isolated intestinal microsomes were analyzed for their alkaline phosphatase activity and CYP 3A immunoreactivity. The involvement of CYP 3A in the in vitro hydroxylation of midazolam (MDZ) was also evaluated using isoform specific chemical and antibody inhibitors. The effect of glycerol (a common constituent of the microsomal reconstitution buffer) concentration on in vitro MDZ hydroxylation was also investigated. Additionally, to verify that the intestinal preparation was adequate for use in studies investigating the induction of CYP3A at the mRNA, protein, and catalytic activity within a single animal, a separate induction study was carried out with the CYP 3A inducer dexamethasone (DEX). A reverse transcription-polymerase chain reaction (RT-PCR) assay and a quantitative Western blotting method were used to reliably detect differences in CYP 3A mRNA and immunoreactivity between DEX- and vehicle (VH)-treated tissues. The in vitro hydroxylation of MDZ evaluated CYP 3A catalytic activity and identified increases in CYP 3A activity caused by DEX in comparison to VH. Collectively, these described techniques provide an experimental model to study xenobiotic induction of rat hepatic and intestinal CYP 3A from the molecular to the catalytic level in individual rats without the need for pooling of tissue.

Davies, A., E. Whiting, et al. (1995). "The application of the human beta-globin gene locus control region and murine erythroleukemia cell system to the expression and pharmacological characterization of human endothelin receptor subtypes." Journal of Pharmacological and Toxicological Methods **33**(3): 153.

<http://www.sciencedirect.com/science/article/B6T8S-3YMWF3D-F/2/deda4e36ab68af617fb165a2284a3798>

The cDNAs encoding both A and B subtypes of the human endothelin receptor have been inserted into mammalian cell expression vectors that utilize the human globin gene, locus control region. These constructs have been introduced into murine erythroleukemia cells and inducible high level expression of the receptors has been achieved (~1.5-pM/mg membrane protein and ~13,500 binding sites/cell for both receptor subtypes). Cell lines expressing these receptors were obtained on a rapid time scale (3-4 weeks), facilitated by the need for the analysis of only small numbers of cell clones/receptor (~6). Competitive binding assays with endothelin-1 gave IC₅₀s of 130 +/- 30 pM for endothelin-A receptor and 160 +/- 30 pM for endothelin-B receptor. Similar studies with the different isoforms of endothelin, sarafatoxin-S6b and -S6c, BQ123 and BQ3020, all gave the expected selectivity profiles. The IC₅₀s for all compounds were in close agreement with those reported for native receptors. Thus, this expression system, which has several advantages over other described expression systems, is capable of rapidly providing large quantities of receptor for detailed pharmacological analyses or drug screening. In addition, the expressed receptors display the expected pharmacological profiles in the absence of any complicating, competing interactions from other subtypes or binding sites.

Journal of Photochemistry and Photobiology B: Biology (1)

Piette, J. (1992). "Molecular analysis of mutations induced by 4'-hydroxymethyl-4,5',8-trimethylpsoralen and UVA in the mouse HPRT gene." Journal of Photochemistry and Photobiology B: Biology **12**(1): 37.

<http://www.sciencedirect.com/science/article/B6TH0-44K8208-5/2/9b362dbc93a487920381ee71cb3d2d35>

The effects of the reaction photosensitized by 4'-hydroxymethyl-4,5',8-trimethylpsoralen (HMT) on a mouse lymphoma cell line have been examined. Using the hypoxanthine phosphoribosyltransferase (HPRT) locus as target gene, a mutagenic effect of the photoreaction can be detected concomitantly with a loss of cell viability. Isolation of HPRT deficient clones has permitted a molecular characterization of the mutational pattern induced by the photosensitization reaction mediated by HMT. Southern blotting analysis demonstrated that the HPRT deficiency could not be correlated with gene deletions larger than 300 bp. Using polymerase chain reaction on both DNA and cDNA, amplification products have been cloned into M13mp18 and sequenced. Base transversions targeted on thymine residues have been located in exon 2, 3, 8 and 9 together with spontaneous frameshift mutations occurring in a run of guanine residues in exon 3. HPRT deficiencies owing to mutations arising in the HPRT promoter region have also been observed. Dot and Northern blot analysis revealed that the photoreaction could lead to either a reduced level of gene transcription or to a complete absence of HPRT m-RNA. Using polymerase chain reaction (PCR) amplification and agarose gel electrophoresis, deletions in the HPRT promoter have been observed and correlated to deficient enzyme expression.

Journal of Psychiatric Research (1)

Heilig, M., O. Zachrisson, et al. (2004). "Decreased cerebrospinal fluid neuropeptide Y (NPY) in patients with treatment refractory unipolar major depression: preliminary evidence for association with preproNPY gene polymorphism." Journal of Psychiatric Research **38**(2): 113.

<http://www.sciencedirect.com/science/article/B6T8T-49H1HKV-4/2/3d6cb50b9a82fb94fd4a08e5228ee5bd>

Extensive animal studies suggest neuropeptide Y (NPY) to be involved in coping with a wide range of stressors, and that impaired central NPY signalling could be involved in the pathophysiology of anxiety and depression. Human studies of central NPY levels in depression have, however, been inconclusive. Here, we examined levels of NPY-like immunoreactivity (NPY-LI) in the cerebrospinal fluid (CSF) of medication-free subjects with treatment refractory unipolar depression. Patients were admitted to a research inpatient unit, examined under standardized conditions, and compared with a sample of volunteers in whom psychiatric morbidity was excluded. A robust suppression of NPY levels in patient CSF was found, while other putative CSF markers (monoamine metabolites, somatostatin) did not differ between the groups. We then explored whether this finding might be related to a recently described T1128C coding polymorphism which results in a Leu7-> Pro7 substitution of the signal peptide, and a previously not described T -399C polymorphism in the promoter region of the preproNPY gene. Preliminary evidence was found for an association of both markers with a diagnosis of depression, indicating the possibility of an underlying haplotype influencing the vulnerability for developing depressive illness. Our present findings are in line with an extensive animal literature, and further support the notion that impaired NPY function could contribute to depressive illness.

Journal of Reproductive Immunology (4)

de Luca Brunori, I., L. Battini, et al. (2003). "HLA-DR in couples associated with preeclampsia: background and updating by DNA sequencing." Journal of Reproductive Immunology **59**(2): 235.

<http://www.sciencedirect.com/science/article/B6T8W-48M7VYN-5/2/bb92e190d0abb5e883c4626b995548f3>

The placenta acts as an immunological barrier between the mother and the fetal "graft", allowing two antigenically different organisms to tolerate one another. In placentae from preeclamptic women, we have demonstrated, by an ultrastructural assessment and an immunohistochemical study, a placental barrier breakage leading to the mixing of maternal and fetal antigenically different blood. This condition could be responsible for the triggering of a maternal rejection reaction that we presume to be at the basis of the preeclamptic syndrome. Thus, we have investigated the Human Leukocyte class II DR antigens (HLA-DR), whose role in self and non-self recognition is well known, in women with preeclampsia, their partners and in control couples using the serological Terasaki technique. The results showed a statistically significant increase of HLA-DR homozygosity and a reduced antigenic variety in the preeclamptic women and their partners with respect to controls. In this update, we have examined the 2nd exon of the human gene, HLA-DRB1, on the short arm of the chromosome 6 using DNA sequence-based typing (S-BT) PCR in 56 preeclamptic couples and 64 control couples. The results have confirmed the significant excess of HLA-DR homozygosity in couples associated with preeclampsia versus controls. From our results, it emerges that HLA-DR homozygosity and the reduced antigenic

variety seem to be associated to a major risk for preeclampsia, which further appears to be a "couple's disease".

Muciaccia, B., A. Filippini, et al. (1998). "Testicular germ cells of HIV-seropositive asymptomatic men are infected by the virus." Journal of Reproductive Immunology **41**(1-2): 81.

<http://www.sciencedirect.com/science/article/B6T8W-3V5TKM2-7/2/3c7a90b4a25564e1b60dbe8ea00d858b>

In situ PCR hybridization studies in the testis of infected asymptomatic subjects detected the presence of HIV-1 proviral DNA in the nuclei of germ cells at all stages of differentiation suggesting that HIV-seropositive men produce infected spermatozoa that are released in the genital tract. In all subjects studied spermatogenesis was normal, the presence of provirus was not associated with germ cell damage and a very mild local immune response was observed. The HIV hybridization pattern observed in germ cells supports the hypothesis of a clonal infection. It is suggested the possibility of a direct infection of the germ cells by cell-free virus and that the testis might represent a site of early viral localization, well tolerated because of the immune privilege of this organ.

Pudney, J., H. Nguyen, et al. (1998). "Microscopic evidence against HIV-1 infection of germ cells or attachment to sperm." Journal of Reproductive Immunology **41**(1-2): 105.

<http://www.sciencedirect.com/science/article/B6T8W-3V5TKM2-9/2/7f7894481a17e8f3e332256650954531>

For a number of years we have intensively investigated the localization of HIV-1 in male genital tract tissues and secretions using a variety of microscopy techniques including immunocytochemistry, in situ hybridization, in situ PCR and electron microscopy. Our studies have failed to demonstrate an association between HIV-1 and either testicular germ cells or spermatozoa. In this article we present our results in the context of other related studies, and discuss the strengths and weaknesses of the techniques that have been used to address this important research question.

van den Elsen, P. J., S. J. P. Gobin, et al. (2001). "Transcriptional control of MHC genes in fetal trophoblast cells." Journal of Reproductive Immunology **52**(1-2): 129.

<http://www.sciencedirect.com/science/article/B6T8W-445B4KX-C/2/418e8b800184a4dca8115a505815001f>

Tight control of MHC expression is essential for the outcome of a successful pregnancy. The lack of MHC class II and class I mediated antigen presentation by fetal trophoblast cells is an important mechanism to evade maternal immune recognition. Interestingly, the deficient expression of MHC class II molecules (HLA-DR, -DQ and -DP) and of the classical MHC class I molecules HLA-A and HLA-B is also noted after IFN-[gamma] treatment in trophoblast-derived cell lines. Our studies show that in trophoblast cell lines the IFN-[gamma] induced transactivation of HLA-A and HLA-B promoters is repressed. Furthermore, it was found that trophoblast cells lacked IFN-[gamma] mediated induction of the class II transactivator (CIITA). This lack of CIITA expression in trophoblast cells is due to CIITA promoter hypermethylation. In addition to lack of CIITA expression, trophoblast cells also displayed a repressed expression of RFX5. Together,

these observations reveal a silencing of multiple activation pathways that are critical to the transcriptional control of MHC class II and class I antigen presentation functions by trophoblast cells.

Journal of Surgical Research (4)

Cheuk, B. L. Y. and S. W. K. Cheng (2004). "Differential expression of integrin [alpha]5[beta]1 in human abdominal aortic aneurysm and healthy aortic tissues and its significance in pathogenesis." Journal of Surgical Research **118**(2): 176.

<http://www.sciencedirect.com/science/article/B6WM6-4C7W8HT-9/2/a16aaf722abd4917999cc100bf268faf>

Background Abdominal aortic aneurysm (AAA) is a common aged disease of human aorta with increasing incidence. It is characterized by dramatic vascular remodeling via proteolysis and degradation of matrix proteins. Integrins are important cellular receptors for matrix proteins, which may have an association with pathological remodeling. The present study was undertaken to analyze the expression of integrin subunits in human aneurysmal aortas and with healthy aortic tissues as controls. **Materials and methods** The expression of integrin genes in AAA specimens and healthy human aortic tissues was detected by RT-PCR technique. The correlation of variation and distribution of smooth muscle cells (SMCs) and integrin protein expression in the corresponding tissues were studied immunohistochemically. **Results** The gene transcripts coding for integrin [alpha]4, [alpha]5, [alpha]V, [beta]1, [beta]3, [beta]5, and [beta]6 subunits were constitutively expressed in the normal aortas. Only gene expressions of integrin [alpha]5 and [beta]1 were significantly decreased by 81% and 85%, respectively, in AAA specimens (P < 0.05). **Conclusions** The marked decrease in integrin [alpha]5[beta]1 expressions was unique to aneurysmal aortic tissues and correlated to a decrease in density of SMCs, which are the major cells in maintaining the structure stability of normal aortas. As integrin [alpha]5[beta]1 specifically binds fibronectin and collagen, those results may suggest that the absence of integrin [alpha]5[beta]1 activity impair matrix protein attachment and alter the architecture in aortic media thereby lead to the deformity of aorta and aneurysm formation.

Concolino, P., V. Papa, et al. (2004). "The unsolved enigma of CDH1 down-regulation in hereditary diffuse gastric cancer." Journal of Surgical Research **121**(1): 50.

<http://www.sciencedirect.com/science/article/B6WM6-4CTCV63-F/2/7cd716cf028a4cbec7e0b82fc7203301>

Background Hereditary diffuse gastric cancer (HDGC) is a disease mediated by down-regulation of the tumor suppressor E-cadherin (CDH1). This disease is particularly dangerous because of the youth of the patients, and for clinical management, hampered by the submucosal spread of tumor invisible at endoscopy. Two mechanisms of CDH1 down-regulation have been described in HDGC: missense mutations in the CDH1 gene and gene silencing through promoter methylation. **Materials and methods** Seven patients affected by HDGC were enrolled. Tumor tissues were checked for CDH1 expression by immunohistochemistry (IHC). CDH1 DNA sequencing was performed for all its 16 exons from tumor and normal tissues of the same patients to detect somatic and germ-line mutations. Methylation promoter study was performed

using specific primers and PCR. Results IHC analysis confirmed CDH1 down-regulation in all patients. DNA sequencing revealed the presence of six missense mutations in five patients. Four mutations were at the EC-3 domain of CDH1, whereas the other two were found in the cytoplasmic region interacting with catenins. All six mutations were absent in normal tissue, thereby excluding its presence in germ-line cells. Four patients exhibited both DNA missense mutations and gene silencing through promoter methylation. In two patients we did not notice either DNA missense mutations or promoter methylation. Conclusion CDH1 somatic mutations and promoter methylation synergistically induce CDH1 down-regulation in HDGC patients, whereas germ-line mutations are relatively rare. However, other unknown mechanisms of CDH1 suppression are involved to explain CDH1 down-regulation in HDGC patients without CDH1 mutations and promoter methylation.

Knight, R. J., H. Liu, et al. (2003). "Cold ischemic injury, aortic allograft vasculopathy, and pro-inflammatory cytokine expression." Journal of Surgical Research **113**(2): 201.

<http://www.sciencedirect.com/science/article/B6WM6-49D6P0F-4/2/36f963684e96fb1e970ead996330283f>

Background The aim of this study was to understand the role of ischemic preservation injury and pro-inflammatory cytokine expression in the progression of allograft vasculopathy. Methods Using the rat aortic transplant model, grafts were stored at 4[deg]C for either 1 or 24 h. Graft vasculopathy was assessed at 4 and 8 weeks after transplantation. Intra-graft cytokine expression was measured at days 1, 3 and, 7 after transplantation. Results At 4 weeks, intimal hyperplasia of allografts was greater than isografts (P P P P Conclusions Prolonged ischemic preservation injury induced vascular disease in both isografts and allografts. The vessel wall reaction increased over time and was greater in allografts than isografts. The enhanced expression of T cell- and macrophage associated cytokines in allografts compared to isografts, suggested that early pro-inflammatory cytokine expression played an important role in progression of allograft vasculopathy.

Martin, R. C. G., Q. Lan, et al. (2005). "No apparent association between genetic polymorphisms (-102 C>T) and (-9 T>C) in the human manganese superoxide dismutase gene and gastric cancer1." Journal of Surgical Research **124**(1): 92.

<http://www.sciencedirect.com/science/article/B6WM6-4DS45W9-6/2/8fb09387391d5143bb21550c9a6a1f20>

Background Manganese superoxide dismutase (MnSOD) plays a critical role in the detoxification of mitochondrial reactive oxygen species, constituting a major cellular defense mechanism against agents that induce oxidative stress. A genetic polymorphism in the mitochondrial targeting sequence of this gene has been associated with increased cancer risk. This one base pair transition (-9 T>C) leads to a Val to Ala amino acid change in the mitochondrial targeting sequence. In addition, the MnSOD promoter contains an activator protein-2 (AP-2) binding site that modifies transcription of MnSOD. Mutations have been identified in the proximal region of the promoter in human tumor cell lines. One of these mutations (-102 C>T) has been shown to change the binding pattern of AP-2, leading to a reduction in transcriptional activity. The aim of our study was to investigate possible associations of the (-9 T>C) and (-102 C>T) polymorphisms with gastric cancer in a population-based case-control study conducted in Warsaw, Poland. Materials and methods DNA was obtained from a population based case-control study of stomach cancer conducted in Warsaw, Poland, between 1994 and 1996. The MnSOD -9 T>C genotype was determined by PCR-RFLP assay. The MnSOD -102 C>T genotype was determined using a TaqMan allele discrimination assay. Results The frequency of the -102 C>T

polymorphism was 41% (38/91) in gastric cancer cases and 38% (50/130) in the controls (odds ratio [OR] 1.1, 95% confidence interval [CI] 0.6-2.1). The frequency of the -9 T>C polymorphism was 44% (202/464) in cases and 56% (262/464) in controls (OR 1.1; 95% CI 0.9-1.37). The lack of association was observed in both non-smokers (OR 1.5; 95% CI 0.7-2.34) and smokers (OR 1.1; 95% CI 0.7-1.7). Furthermore, the association was not significant when smokers were segregated by extent of smoking history. Conclusion The association of the manganese superoxide dismutase polymorphisms at -102 C>T and the -9 T>C were not found to be associated with gastric cancer in a Polish case-control study.

Journal of the American College of Cardiology (7)

Lanfear, D. E., S. Marsh, et al. (2004). "Genotypes associated with myocardial infarction risk are more common in African Americans than in European Americans." Journal of the American College of Cardiology **44**(1): 165.

<http://www.sciencedirect.com/science/article/B6T18-4CRXMCG-14/2/22fa828d6747f2122439d18feb24e20b>

Objectives This study was designed to describe the frequencies of multiple myocardial infarction (MI) risk-associated genotypes among different racial groups. **Background** Racial disparities in the prevalence of cardiovascular disease (CVD) are well known. Recent large Japanese case-control studies identified connexin-37 (GJA-4), plasminogen activator inhibitor-1 (PAI-1), and stromelysin-1 (MMP-3) polymorphisms as risk factors for MI, but the prevalence of these genotypes among different racial groups in the U.S. needs to be determined. **Methods** Genomic deoxyribonucleic acid from 95 healthy African Americans (AA) and 95 healthy European Americans (EA) was used for genotyping. Deoxyribonucleic acid containing the region of interest was amplified using the polymerase chain reaction, followed by genotyping using pyrosequencing. **Results** All three MI-risk genotypes were observed in both populations and were in Hardy-Weinberg equilibrium. The frequencies of two of the three "risk-associated" genotypes were significantly higher in the AA population: GJA4 C1019T T/T: AA, 20%, EA, 7% ($p = 0.053$); MMP3 -1171delA A/A: AA, 78%, EA, 24% (p Conclusions We found higher frequencies of disease-associated genotypes in AA than in EA. Our results also show that more AA than EA carry multiple risk-associated genotypes. Future studies need to assess whether such genetic profiles predict adverse outcomes in U.S. populations and contribute to racial disparities in CVD burden.

Maekawa, Y., T. Anzai, et al. (2004). "Effect of granulocyte-macrophage colony-stimulating factor inducer on left ventricular remodeling after acute myocardial infarction." Journal of the American College of Cardiology **44**(7): 1510.

<http://www.sciencedirect.com/science/article/B6T18-4DFJDW5-Y/2/598ddd89fb0c0bdc2b6fd3745eb18b1>

Objectives We sought to determine the influence of granulocyte-macrophage colony-stimulating factor (GM-CSF) induction on post-myocardial infarction (MI) remodeling, especially in relation to the inflammatory response and myocardial fibrosis. **Background** Granulocyte-macrophage colony-stimulating factor modifies wound healing by promoting monocytopoiesis and infiltration of

monocytes and macrophages into injured tissue; however, the effect of GM-CSF induction on the infarct healing process and myocardial fibrosis is unclear. **Methods** A model of MI was produced in Wistar rats by ligation of the left coronary artery. The MI animals were randomized to receive GM-CSF inducer (romurtide 200 [μg/kg/day for 7 consecutive days) (MI/Ro) or saline (MI/C). **Results** Echocardiographic and hemodynamic studies on day 14 revealed increased left ventricular (LV) end-diastolic dimension, decreased fractional shortening, elevated LV end-diastolic pressure, and decreased LV maximum rate of isovolumic pressure development in MI/Ro compared with MI/C. Immunoblotting showed that expression of transforming growth factor (TGF)-[β]1 in the infarcted site on day 3 after MI was decreased in MI/Ro compared with MI/C. In the infarcted site, TGF-[β]1, collagen type I and type III messenger ribonucleic acid (mRNA) expression on day 3, and collagen content on day 7 were reduced in MI/Ro compared with MI/C, in association with marked infarct expansion. In MI/Ro, monocyte chemoattractant protein-1 mRNA level and the degree of infiltration of monocyte-derived macrophages (ED-1-positive) were greater in the infarcted site on day 7 than those in MI/C. **Conclusions** The GM-CSF induction by romurtide facilitated infarct expansion in association with the promotion of monocyte recruitment and inappropriate collagen synthesis in the infarcted region during the early phase of MI.

Ramet, M. E., M. Ramet, et al. (2003). "High-density lipoprotein increases the abundance of eNOS protein in human vascular endothelial cells by increasing its half-life." Journal of the American College of Cardiology **41**(12): 2288.

<http://www.sciencedirect.com/science/article/B6T18-48VWB6W-13/2/9e63bb568aa2fd42644186c27e8f057b>

Objectives Given the importance of endothelial nitric oxide synthase (eNOS) in regulating endothelium-dependent vasorelaxation, we investigated the effects of high-density lipoprotein in (HDL) on eNOS protein abundance in cultured human vascular endothelial cells. **Background** Endothelial dysfunction, characterized by decreased nitric oxide production, is one of the early features in the development of atherosclerosis. We have recently shown in vivo that niacin therapy increases plasma HDL concentration and improves endothelium-dependent vasorelaxation in patients with coronary artery disease. **Methods** Human vascular endothelial cells were cultured in the presence or absence of HDL or apolipoprotein (apo)A-I. The eNOS protein abundance was assessed by immunoblotting, and protein half-life was assessed by pulse-chase techniques. The eNOS messenger ribonucleic acid (mRNA) abundance was measured using real-time quantitative polymerase chain reaction. **Results** High density lipoprotein, or apoA-I alone, increased eNOS protein abundance by 3.5 ± 0.7 and 2.7 ± 0.5-fold, respectively (p < 0.05). **Conclusions** We demonstrate that HDL activates both extracellular signal-regulated kinase 1/2 (ERK1/2) and Akt, resulting in enhanced eNOS protein stability and subsequent accumulation of eNOS protein. This posttranslational regulation represents a previously unrecognized mechanism for regulating eNOS.

Rott, D., J. Zhu, et al. (2003). "Effects of MF-tricyclic, a selective cyclooxygenase-2 inhibitor, on atherosclerosis progression and susceptibility to cytomegalovirus replication in apolipoprotein-E knockout mice." Journal of the American College of Cardiology **41**(10): 1812.

<http://www.sciencedirect.com/science/article/B6T18-48MY548-Y/2/f54e71994f434526274490ddf9c9c9>

Objectives We examined whether selective cyclooxygenase-2 (COX-2) inhibition in apolipoprotein-E (apoE) deficient mice reduces cytomegalovirus (CMV) replication, and determined whether COX-2 anti-inflammatory activity leads to decreased atherosclerosis. **Background** Evidence suggests that CMV infection contributes to atherosclerosis

and that this occurs in part through inflammatory mechanisms. Cyclooxygenase-2 inhibitors are potent anti-inflammatory agents. They also inhibit CMV replication in vitro. **Methods** The apoE deficient mice were either treated or not treated with a selective COX-2 inhibitor, and either infected or not infected with CMV. Viral deoxyribonucleic acid load in salivary glands was determined by quantitative polymerase chain reaction. Atherosclerotic lesion analysis was performed by standard methods. **Results** In vivo COX-2 inhibition, unexpectedly increased viral load: in the CMV-infected animals viral load was 2.58 ± 1.0 in the nontreated group, 4.74 ± 1.38 in the group treated with 12 mg/kg/day MF-tricyclic, and 6.51 ± 1.64 in the group treated with 24 mg/kg/day MF-tricyclic (p TREND = 0.050). This increased viral load was paralleled by increased anti-CMV antibody titers. Most surprisingly, COX-2 inhibition significantly increased early atherosclerotic lesion area, independent of viral infection. **Conclusions** Our study demonstrates that selective inhibition of COX-2 in vivo increases viral load. The finding that inhibition of COX-2 increases atherosclerosis development in apoE deficient mice suggests, unexpectedly, that this enzyme exerts antiatherosclerosis activity, at least in this model.

Seko, Y., N. Kayagaki, et al. (2002). "Role of Fas/FasL pathway in the activation of infiltrating cells in murine acute myocarditis caused by Coxsackievirus B3." Journal of the American College of Cardiology **39**(8): 1399.

<http://www.sciencedirect.com/science/article/B6T18-45JG5R2-VI2/a50824b196aded16025e642bdf8f4628>

Objectives This study was designed to investigate the roles of Fas/FasL pathway in myocardial damage in murine acute myocarditis caused by Coxsackie virus B3 (CVB3). **Background** Cardiac myocyte apoptosis rarely occurs in murine acute myocarditis caused by CVB3. Fas/FasL belong to the tumor necrosis factor receptor/ligand superfamily of costimulatory molecules and are known to play a critical role in the induction of apoptosis, as well as in the cytotoxicity mediated by T-cells and natural killer cells. **Methods** We first analyzed the expression of Fas on cardiac myocytes in vivo and in vitro. Second, we examined the development of myocardial damage, in C3H/He mice treated with an anti-FasL monoclonal antibody (mAb), and in C3H/He-lpr/lpr mice and C3H/He-gld/gld mice infected with CVB3. Third, to investigate the effects of anti-FasL mAb treatment on the activation of the infiltrating cells, we examined the expression of interferon (IFN)-gamma and interleukin (IL)-2 as activation markers in the heart of mice by semiquantitative polymerase chain reaction. **Results** Fas was markedly induced on cardiac myocytes with acute myocarditis. Myocardial inflammation was decreased in mice treated with anti-Fas L mAb, C3H/He-lpr/lpr mice and C3H/He-gld/gld mice. Anti-FasL mAb-treatment also decreased the expression of IFN-gamma, IL-2, inducible nitric oxide synthase and CVB3 genomes in myocardial tissue. **Conclusions** Our findings strongly suggested that the Fas/FasL pathway played a critical role in the development of massive myocardial necrosis through activation of infiltrating cells, and raise the possibility of immunotherapy by blocking the Fas/FasL pathway to prevent myocardial damage and improve the prognosis of patients with viral myocarditis.

Snapir, A., P. Heinonen, et al. (2001). "An insertion/deletion polymorphism in the [alpha]2b-adrenergic receptor gene is a novel genetic risk factor for acute coronary events." Journal of the American College of Cardiology **37**(6): 1516.

<http://www.sciencedirect.com/science/article/B6T18-42VM8NN-4/2/d1a318e6e10b6f975142a43157efb60f>

OBJECTIVES Our aim was to study whether an insertion/deletion (I/D) polymorphism in the [alpha]2B-adrenoceptor gene is associated with the risk for cardiovascular diseases. **BACKGROUND** [alpha]2-adrenoceptors mediate contraction of vascular smooth muscle

and induce coronary vasoconstriction in humans. The α_2 -adrenoceptor subtype B mediates vasoconstriction in mice. A variant of the human α_2B -adrenoceptor gene that encodes a D of three residues in an intracellular acidic motif has been shown to confer decreased receptor desensitization. This receptor variant could, therefore, be involved in diseases associated with enhanced vasoconstriction.

METHODS This study was part of a prospective population-based study investigating risk factors for cardiovascular diseases in a cohort of middle-aged men from eastern Finland. Nine hundred twelve men aged 46 to 64 years were followed for an average time of 4.5 years.

RESULTS In this study population, 192 men (21%) had the D/D genotype; 256 (28%) had the I/I genotype, and 464 (51%) had a heterozygous genotype. In a Cox model adjusting for other coronary risk factors, men with the D/D genotype had 2.2 times (95% confidence interval: 1.1 to 4.4, $P = 0.02$) the risk to experience an acute coronary event ($n = 15$ for D/D, 10 for I/I and 12 for I/D) compared with men carrying either of the other two genotypes. The α_2B -adrenoceptor genotype was not associated with hypertension in this study population.

CONCLUSIONS The D/D genotype of the α_2B -adrenoceptor is a novel genetic risk factor for acute coronary events, but not for hypertension.

Yousufuddin, M., D. J. Cook, et al. (2004). "Angiotensin II receptors from peritransplantation through first-year post-transplantation and the risk of transplant coronary artery disease." Journal of the American College of Cardiology **43**(9): 1565.

<http://www.sciencedirect.com/science/article/B6T18-4C8G0WD-D/2/0a1a5579de6369ece47c3cb341d952e4>

Objectives We evaluated whether the angiotensin II (Ang II) receptors from perioperation through one-year post-transplantation predict the transplant coronary artery disease (TCAD) progression.

Background The role of Ang II receptors (type 1: AT1R; type 2: AT2R) in TCAD is uncertain.

Methods We investigated 28 heart donors and the corresponding recipients. The levels of AT1R and AT2R messenger ribonucleic acid (mRNA) were examined in lymphocytes from the donor spleen and in the donor heart at one-week and one-year posttransplantation to determine their association with the progression of TCAD, measured as changes in maximal intimal thickness (CMIT) and plaque volume (CPV) by intravascular ultrasound (IVUS) examinations.

Results The AT1R mRNA in lymphocytes from the donor spleen (CMIT: $R = 0.73$, $p = 0.001$); AT2R mRNA in the donor hearts at one-year post-transplantation (CMIT: $R = 0.3$, $p = 0.001$); AT1R mRNA in lymphocytes and in the donor hearts at one-year post-transplantation proved to be multivariate predictors of the progression of TCAD.

Conclusions These data suggest a role for Ang II receptors in the pathogenesis of TCAD and support a novel concept that TCAD may have its origin in the donor per se and may be modulated by the recipient's inherent biological factors.

Journal of the American College of Surgeons (2)

Abe, T., M. Oka, et al. (2001). "Interleukin-6 production in lung tissue after transthoracic esophagectomy." Journal of the American College of Surgeons **192**(3): 322.

<http://www.sciencedirect.com/science/article/B6T91-42G0MFJ-5/2/cb9d5e7f504a3a35eef7488b82d77b9c>

Background: The mechanisms of the reported high increase in interleukin-6 (IL-6) levels after

esophagectomy are unclear. We investigated the influence of an intrathoracic procedure, esophagectomy, on IL-6 production in lung tissue. Study Design: Fourteen paired lung tissue samples were obtained from patients before and after they underwent transthoracic esophagectomy for esophageal cancer. IL-6 levels in the lung were measured with enzyme-linked immunosorbent assay, and IL-6 mRNA expression was determined with real-time quantitative reverse transcription-polymerase chain reaction. Immunohistochemical staining was used to localize IL-6, and circulating levels were also measured. Results: IL-6 protein and mRNA were significantly increased in lung tissue after this intrathoracic procedure (p Conclusions: Transthoracic esophagectomy causes an increase in IL-6 production from airway epithelial cells, secondary to increased expression of IL-6 mRNA. Local response of lung tissue may be one source of increased serum IL-6 after this procedure.

Schneider, S., K. Uchida, et al. (2005). "Downregulation of TS, DPD, ERCC1, GST-Pi, EGFR, and HER2 gene expression after neoadjuvant three-modality treatment in patients with esophageal cancer." Journal of the American College of Surgeons **200**(3): 336.

<http://www.sciencedirect.com/science/article/B6T91-4FJT8T9-6/2/cba5a2216cfcc27129b32e6d18a0fc1b>

Background To find out if neoadjuvant therapy could alter tumor response determinants that might affect tumor sensitivity to the treatment, we investigated intratumoral expressions of genes associated with chemosensitivity, radiosensitivity, or both before and after radiochemotherapy. Study design Twenty-four patients with locally advanced, resectable esophageal cancer (cT2-4, Nx, M0) received neoadjuvant 5-FU/cisplatin/36 Gy treatment followed by transthoracic en bloc esophagectomy. Expression levels of thymidylate synthase, dihydropyrimidine dehydrogenase, excision repair cross-complementing gene 1, glutathione S-transferase Pi, epidermal growth factor receptor, and HER2 were measured in matched preradiochemotherapy endoscopic tumor biopsies and in postradiochemotherapy resection specimens. mRNA was isolated from formalin-fixed, paraffin-embedded, laser microdissected tumor tissues, and a quantitative fluorescent dye real-time reverse transcription polymerase chain reaction system was used for gene expression measurement. Results There was a significant reduction in the expression levels of thymidylate synthase (p Conclusions The expression levels of a set of genes that are possible determinants of 5-FU/cisplatin/radiation therapy antitumor activity are significantly downregulated by neoadjuvant radiochemotherapy in esophageal cancer.

Journal of the American Society for Mass Spectrometry (2)

Benson, L. M., A. P. Null, et al. (2003). "Advantages of thermococcus kodakaraensis (KOD) DNA Polymerase for PCR-mass spectrometry based analyses." Journal of the American Society for Mass Spectrometry **14**(6): 601.

<http://www.sciencedirect.com/science/article/B6TH2-48CX6M7-4/2/afe37edd9ff93709f981110b7b967ffc>

The advantages of the thermostable DNA polymerase from *Thermococcus kodakaraensis* (KOD) are demonstrated for PCR amplification with subsequent detection by mass spectrometry. Commonly used DNA polymerases for PCR amplification include those from *Thermus aquaticus*

(Taq) and *Pyrococcus furiosus* (Pfu). A 116 base-pair PCR product derived from a vWA locus was amplified by Taq, Pfu, or KOD DNA polymerase and compared by agarose gel electrophoresis and electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS). KOD DNA polymerase demonstrated a 2- to 3-fold increase in PCR product formation compared to Pfu or Taq, respectively, and generated blunt-ended PCR product which allows facile interpretation of the mass spectrum. Additionally, we demonstrate the advantage of using high magnetic fields to obtain unit resolution of the same 116 base pair (~72 kDa) PCR product at high m/z.

Frahm, J. L., D. C. Muddiman, et al. "Leveling response factors in the electrospray ionization process using a heated capillary interface." Journal of the American Society for Mass Spectrometry **In Press, Corrected Proof** <http://www.sciencedirect.com/science/article/B6TH2-4FNP2J5-3/2/0f6262ef8376dfc211464d005fae41a6>

Several investigators have observed a discrepancy in electrospray response of complementary strands from denatured DNA, which has been attributed to the difference in hydrophobicity between the two strands; the more hydrophobic species tend to have higher ion abundances. The implementation of a heated electrospray source has allowed us to "level" the electrospray response for two equimolar complementary strands with different hydrophobicities. As the temperature was increased, the ratio of ion abundances of the less hydrophobic noncoding strand to the more hydrophobic coding strand approached unity. Furthermore, the heated electrospray source was used to denature amplicons containing 7-deaza purines, which can be used to facilitate sequencing by mass spectrometry.

Journal of the Association for Laboratory Automation (2)

Bitner, R., S. Koller, et al. (2004). "Purifying genomic DNA from whole blood on automated, high-throughput, and moderate-throughput platforms." Journal of the Association for Laboratory Automation **9**(2): 64.

<http://www.sciencedirect.com/science/article/B75DF-4CWYXY8-7/2/68b585b938a4cd8655bedef2a9530e90>

We describe three new automated methods for purifying genomic DNA from whole blood. The MagneSil(R) Blood Genomic, Max Yield System uses MagneSil(R) paramagnetic particles (PMPs) in a 96-well format to purify the maximal amount of DNA from a 200- μ L blood sample. In contrast, the MagneSil(R) ONE, Fixed Yield Blood Genomic System uses MagneSil(R) Fixed Yield PMPs to purify a normalized amount of DNA from 60 μ L of blood in a 96-well format. These methods are implemented on the Beckman Coulter Biomek(R) FX automated workstation. The MagneSil(R) KF Genomic System uses MagneSil(R) PMPs to purify DNA from 1 to 15 samples of 200- μ L blood using the moderate-throughput Thermo Electron KingFisher(R) mL instrument. The MagneSil(R) Blood Genomic System typically yields > 4 μ g per 200 μ L of whole blood, depending on the white blood cell content. The MagneSil(R) ONE System is best suited where there is a requirement for purification of a narrow concentration range of DNA. This system purifies 1 μ g (+/-50%) of DNA from 60 μ L of blood. The MagneSil(R) KF System purifies 2 to 6 μ g of DNA from 200 μ L of blood. DNA purified using all of these methods is suitable for PCR, STR, READIT(R) SNP genotype analysis, and multiplexed PCR analysis.

Ugaz, V. M. and M. Krishnan (2004). "Novel convective flow based approaches for high-throughput PCR thermocycling." Journal of the Association for Laboratory Automation **9**(5): 318.

<http://www.sciencedirect.com/science/article/B75DF-4DGSX88-F/2/f94319647f53ba0f2942cc81a6f7de13>

A critical need exists for the development of next-generation genomic analysis instrumentation capable of offering significantly higher throughput at a lower cost than current technology. In this paper, we explore the potential of natural convection-based systems to address these issues by providing a thermocycling hardware platform capable of performing rapid polymerase chain reaction (PCR) amplification of DNA. These systems can be arrayed in a multi-well format that is simple to operate, is suitable for integration with high-throughput automated liquid handling systems, and can be easily and inexpensively mass-produced.

Journal of the Autonomic Nervous System (2)

Ivanov, T. R., J. Cay Jones, et al. (1998). "Characterization of a partial cDNA clone detected by imidazoline receptor-selective antisera." Journal of the Autonomic Nervous System **72**(2-3): 98.

<http://www.sciencedirect.com/science/article/B6T05-3V5D7XB-6/2/4470612ad752851cfe3896e509d4afae>

A cDNA clone has been isolated from a human hippocampal cDNA expression library by relying on the selectivity of two antisera that are specific for imidazoline binding proteins. A 1789 bp cDNA clone was sequenced and shown to contain a single open-reading frame that predicts a 66 kDa polypeptide, but it is truncated based on its lack of a stop codon and poly-A+ tail. Two regions of homology exist for the predicted amino acid sequence in common with chromogranin-A and B proteins, a zinc finger protein, and the ryanodine receptor. Northern blot analyses of poly-A+ mRNA from 36 human tissues indicated two differentially expressed transcripts of 6.0 and 9.5 kb. The 6.0 kb mRNA form was enriched in brain and endocrine tissues as compared to other tissues, but not in strict concordance with 11-imidazoline binding sites. The highest overall amounts of the combined transcripts were found in pituitary. In situ hybridization histochemistry revealed an enrichment of the message in neuronal cell bodies of the rat hippocampus and cerebellar cortex. This clone has some of the properties expected of an imidazoline receptor.

Tajti, J., R. Uddman, et al. (1999). "Messenger molecules and receptor mRNA in the human trigeminal ganglion." Journal of the Autonomic Nervous System **76**(2-3): 176.

<http://www.sciencedirect.com/science/article/B6T05-3WS61Y5-F/2/935e4aa9c2abdfef18cf12a5ef36f2a7>

The presence and distribution of neuromessenger molecules and receptor mRNA in human trigeminal ganglion was studied with immunocytochemical, in situ hybridisation and RT-PCR techniques. Immunofluorescence staining revealed that calcitonin gene-related peptide (CGRP) immunoreactive (-ir) neurons occurred in high numbers, constituting 36-40% of all nerve cell

bodies in the ganglion. Accordingly, in situ hybridisation demonstrated CGRP mRNA in a large portion of the trigeminal neurons. A small number of the nerve cell bodies showed substance P (SP)-ir, (18%), nitric oxide synthase (NOS)-ir (15%), and pituitary adenylate cyclase activating peptide (PACAP)-ir (20%). Double immunostaining revealed that only few CGRP-ir neurons also were NOS-ir (less than 5%). The C-terminal flanking peptide of neuropeptide Y, C-PON, was not visible in any of the nerve cell bodies studied. Agarose gel electrophoresis of the RT-PCR products from the ganglia demonstrated the presence of mRNA corresponding to CGRP1, NPY Y1 and Y2, and VIP1 receptors. These results suggest both sympathetic and parasympathetic influence on the activity in the trigeminal ganglion.

Journal of the European Academy of Dermatology and Venereology (1)

Ward, K. A., S. A. McKernan, et al. (1995). "Concurrent oral and genital infection with HPV DNA types 6 and 11 in a heterosexual male." Journal of the European Academy of Dermatology and Venereology **4**(2): 160.

<http://www.sciencedirect.com/science/article/B6T92-3YS87RM-12/2/32edbaf43861c3f336f15407ba83573f>

Objective Detection of HPV DNA in oral and genital lesions of a heterosexual male, 4 months after oral and vaginal intercourse with a woman with vulvar warts. Possible modes of acquisition of oral HPV infection in the male sexual partner are discussed. Setting Genitourinary Medicine clinic. Methods Polymerase chain reaction amplification of genomic DNA from oral and genital lesions. HPV DNA typing by dot blot hybridization. Results HPV DNA types 6 and 11 were identified in a polypoid tongue lesion and in a penile wart from the male sexual partner. Conclusions The acquisition of oral HPV infection in the male sexual partner may have resulted from genital-oral HPV transfer, either by direct contact with vulvar warts or by digital self-inoculation.

Journal of the Neurological Sciences (25)

Aarskog, N. K., S. Aadland, et al. (2001). "Molecular genetic analysis of Charcot-Marie-Tooth 1A duplication in Norwegian patients by quantitative photostimulated luminescence imaging." Journal of the Neurological Sciences **188**(1-2): 21.

<http://www.sciencedirect.com/science/article/B6T06-43P3TH0-5/2/00060d51d9f36eff1cd4e1c970bff0eb>

Around 70% of Charcot-Marie-Tooth 1 (CMT1) cases are caused by a dominantly inherited 1.5-Mb duplication at 17p11.2-12 (CMT1A). Using photostimulated luminescence (PSL) imaging of MspI Southern blots, hybridization signals of the probe pVAW409R3a in relation to cohybridized probe SF85a, were densitometrically quantified and an RFLP allele-band ratio determined. A total of 55 Norwegian CMT patients and 16 asymptomatic family members from 26 separate families,

clinically and neurophysiologically classified as CMT1 (n=46) and CMT2 (n=9), were studied. Thirty-two of 46 CMT1 cases (69.6%), all heterozygous but one homozygous for the pVAW409R3a MspI polymorphism, from 12 of 21 families (57.1%) were positive for the CMT1A duplication. In autosomal dominant familial cases (n=30), 26 of 30 cases (86.7%), all heterozygous, from six of seven families (85.7%) were positive for duplication. None of the CMT2 patients, asymptomatic family members or healthy controls were positive for duplication. The CMT1A frequency of duplication in Norwegian CMT1 patients is in general agreement with those reported in other European countries and the present results show that quantitative densitometric PSL imaging is a highly reliable test in diagnosing CMT1A duplication.

Aguilera, I., J.-R. Garcia-Lozano, et al. (2001). "Mitochondrial DNA point mutation in the COI gene in a patient with McArdle's disease." Journal of the Neurological Sciences **192**(1-2): 81.

<http://www.sciencedirect.com/science/article/B6T06-44BM9KY-C/2/e19f34509d3a0206c5a9d73ce0f28a3c>

We studied a 57-year-old female patient with clinical and biochemical evidences of McArdle's disease. Her muscle biopsy also revealed signs of mitochondrial proliferation, scattered RRF, and a deficit in complex I of the respiratory chain. Molecular genetic analysis showed that the patient was heterozygous for the most common mutation at codon 49 in the myophosphorylase gene. Mitochondrial DNA analysis of muscle tissue revealed an additional G-to-A transition at nucleotide position 7444 in the cytochrome c oxidase subunit I (COI) gene.

Andrews, W. D., A. Al-Chalabi, et al. (1997). "Lack of evidence for HTLV tax-rex DNA in motor neurone disease." Journal of the Neurological Sciences **153**(1): 86.

<http://www.sciencedirect.com/science/article/B6T06-3RSFG7M-H/2/b5b8e086e8c597b99f814412e4dddf1d>

It has recently been claimed (Ferrante et al., 1995. HTLV tax-rex DNA and antibodies in idiopathic amyotrophic lateral sclerosis. J. Neurol. Sci. 129 (Suppl.) 140-144) that human T-lymphotropic virus (HTLV) tax-rex sequences are detectable in the peripheral blood mononuclear cells (PBMCs) of 40% of patients with motor neurone disease (MND). In an attempt to confirm this we employed a highly sensitive 'nested' polymerase chain reaction (PCR) assay, capable of detecting single molecules of HTLV proviral DNA, to look for tax-rex sequences in the PBMCs of 43 patients with MND. We were unable to detect the presence of HTLV tax-rex in any of 43 MND patients tested, using three different PCR primer sets under both high and low stringency conditions. Using the same DNA samples we were able to detect the presence of the single-copy pyruvate dehydrogenase gene, thus demonstrating that the extracted DNA was indeed amplifiable by PCR. To further exclude the possibility that the extracted DNA samples contained unrecognised inhibitory factors we conducted spiking experiments with trace amounts (approximately 10 copies) of HTLV proviral DNA. Spiked samples yielded PCR products of the expected size. We are therefore unable to confirm the presence of HTLV tax-rex sequences in this disease.

Cooper, J. M., V. M. Mann, et al. (1992). "Analyses of mitochondrial respiratory chain function and mitochondrial DNA deletion in human skeletal muscle: Effect of ageing." Journal of the Neurological Sciences **113**(1): 91.

<http://www.sciencedirect.com/science/article/B6T06-485RNBW-JT/2/3ed21d2856150c8fce6e9fb8c3541b53>

The analysis of human skeletal muscle mitochondria revealed a progressive decline in mitochondrial respiratory chain function with age. The activities affected to the greatest extent were those of complexes I and IV which were decreased by 59% and 47% respectively between the ages of 20-30 years and 60-90 years of age. Quantitation of the 5 kb 'common' deletion of mtDNA using PCR revealed a progressive accumulation with age, from approximately 1 in 100 000 at 21 years to 1 in 10 000 at 56 years and 1 in 5000 at 78 years of age. The low absolute levels of this mutation are unlikely to contribute significantly to the observed mitochondrial dysfunction.

Del Bo, R., G. P. Comi, et al. (1997). "The apolipoprotein E [epsilon]4 allele causes a faster decline of cognitive performances in Down's syndrome subjects." Journal of the Neurological Sciences **145**(1): 87.

<http://www.sciencedirect.com/science/article/B6T06-3R8617F-2M/2/42d17e0ee4afc598e8ebfae532b04d31>

The apolipoprotein E gene (APOE), located on human chromosome 19, has three common alleles ([epsilon]2, [epsilon]3, [epsilon]4) which encode for the three main isoforms indicated as E2, E3 and E4 respectively. Several findings indicate [epsilon]4 allele as an important risk factor in both sporadic and familial late-onset Alzheimer's disease (AD). Pathological changes similar to AD are seen in almost all patients with Down's syndrome (DS) aged over 35 (senile plaques, neurofibrillary tangles and neuronal loss); a proportion of these may subsequently develop dementia. Aim of this study is to evaluate the possible pathological role of [epsilon]4 allele as risk factor for developing AD in a DS population. ApoE [epsilon]4 allele frequency is not significantly different in DS cases and controls. We found a statistically significant inverse correlation between full scale IQ values and age of patients in the subgroup of DS subjects selected for the presence of at least one [epsilon]4 allele, while no correlation was observed in DS subjects with other ApoE genotypes. A longitudinal analysis of cognitive performances (available in 38 patients) showed a faster rate of decline in intellectual ability in those subjects carrying at least one [epsilon]4 allele. Our data support the hypothesis that ApoE [epsilon]4 allele has a contributory role in accelerating the mental deterioration of AD-type in DS patients. (c) 1997 Elsevier Science B.V.

Duggan, D. J., M. Fanin, et al. (1996). "[alpha]-Sarcoglycan (adhalin) deficiency: complete deficiency patients are 5% of childhood-onset dystrophin-normal muscular dystrophy and most partial deficiency patients do not have gene mutations." Journal of the Neurological Sciences **140**(1-2): 30.

<http://www.sciencedirect.com/science/article/B6T06-3YTCC1P-4/2/38550f3840bd3842055d95e640353c40>

[alpha]-Sarcoglycan (adhalin), a 50-kDa component of the dystrophin-associated complex of proteins, participates in the stabilization of the myofiber plasma membrane in the membrane cytoskeleton. Deficiencies of [alpha]-sarcoglycan cause a subset of childhood-onset muscular dystrophy (SCARMD) cases. However, secondary deficiencies of [alpha]-sarcoglycan are common. To begin to establish the rates of false positives (secondary deficiencies), we used immunofluorescence to screen 30 Italian dystrophin-normal muscular dystrophy patient biopsies and identified 4 patients with partial [alpha]-sarcoglycan deficiency and 2 patients with complete deficiency. The entire [alpha]-sarcoglycan gene was screened for mutations using RT-PCR and

SSCP of messenger RNA isolated from muscle biopsies in each of the six patients. Aberrant SSCP conformers and novel mutations were found only in the two complete immunohistochemical deficient patients. One patient was homozygous for a R34H amino acid substitution, while the other was a compound heterozygote (R77C, D97G). These three missense mutations, with additional mutations we and others have previously described, are all localized in the extracellular domain of [alpha]-sarcoglycan, and most result in the loss or gain of a positively charged amino acid. These data have strong implications for structure/function maps of the [alpha]-sarcoglycan molecule. Our results suggest that most patients showing partial [alpha]-sarcoglycan deficiency exhibit this as a secondary consequence of genetically distinct disorders. In support of this, we show biochemical data indicating that secondary deficiency patients show decreased immunostaining with antibodies directed against [alpha]-sarcoglycan, while having nearly normal quantities of [alpha]-sarcoglycan protein on immunoblot. This data also suggests that approximately 5% of childhood-onset dystrophin-normal muscular dystrophy patients will show a primary [alpha]-sarcoglycan deficiency.

Fassati, A., A. Bordoni, et al. (1994). "Chronic progressive external ophthalmoplegia: A correlative study of quantitative molecular data and histochemical and biochemical profile." Journal of the Neurological Sciences **123**(1-2): 140.

<http://www.sciencedirect.com/science/article/B6T06-485RNVW-7/2/7033a3d584fd925e05dcddcde4595a13>

We studied muscle biopsies of 5 patients with Kearns-Sayre syndrome and 3 patients with chronic progressive external ophthalmoplegia all with the common deletion. Steady state levels of normal and deleted mitochondrial DNA (mtDNA) measured in each patient by quantitative PCR were correlated with histochemical and biochemical features. We found that (1) normal mtDNA levels were higher in many patients than in controls; (2) as levels of deleted mtDNA increased, so did levels of normal mtDNA; (3) cytochrome c oxidase (COX) activity and the percentage of COX negative fibers were both related to the levels of deleted mtDNA; and (4) as percentage of ragged red fibers increased, so did levels of total, deleted and normal mtDNA. The quantity of deleted mtDNA plays a key role in determining the severity of COX deficiency, which is responsible for the overaccumulation of mitochondria in muscle.

Fukazawa, T., I. Yabe, et al. (1999). "Association of vitamin D receptor gene polymorphism with multiple sclerosis in Japanese." Journal of the Neurological Sciences **166**(1): 47.

<http://www.sciencedirect.com/science/article/B6T06-3X10TCY-7/2/2c595165166f4fea2acc70b93dd916be>

1,25-Dihydroxyvitamin D3 (1,25(OH)2D3), the biologically active form of vitamin D, exerts an immunosuppressive effect and can completely prevent experimental autoimmune encephalomyelitis (EAE). 1,25(OH)2D3 exerts most of its actions only after it has bound to its specific nuclear receptors. To investigate the possible role of vitamin D receptor gene (VDRG) polymorphism in susceptibility to or disease-modulation of MS, we evaluated 77 Japanese patients with 'conventional' MS and 95 controls. A VDRG allelic polymorphism was assessed by Bsm1 endonuclease restriction after specific PCR amplification. Genotypic polymorphism was clearly defined as BB (absence of restriction site on both alleles), bb (presence of restriction site on both alleles), or Bb (heterozygous). We found overexpression of the b allele (92.9 vs. 84.2%; $P=0.0138$) and homozygote bb (85.7 vs. 71.6%; $P=0.0263$) in MS patients compared with controls. The results indicate for the first time an association of MS with VDRG polymorphism, which may be involved in pathogenesis of MS, or in the linkage disequilibrium of VDRG to another pathogenic gene loci. The role of VDR gene polymorphism should be further studied in

other populations, and the distribution of other polymorphism, such as Apa I, Taq I, should be also analyzed to confirm another susceptibility gene for MS and to obtain more adequate strategies for treatment of MS.

Harness, J., M. P. Pender, et al. (2001). "Cyclosporin A treatment modulates cytokine mRNA expression by inflammatory cells extracted from the spinal cord of rats with experimental autoimmune encephalomyelitis induced by inoculation with myelin basic protein." Journal of the Neurological Sciences **187**(1-2): 7.

<http://www.sciencedirect.com/science/article/B6T06-43CR9GC-2/2/1b7add39f08243a6a9732a5852225d8f>

In Lewis rats, treatment with high doses of cyclosporin A (CsA) suppresses clinical signs of experimental autoimmune encephalomyelitis (EAE), although disease occurs when treatment is ceased. Treatment with low doses of CsA causes EAE to take a chronic relapsing course. We have previously shown that CsA treatment causes a decline in the number of T cells and increased inflammatory cell apoptosis in the spinal cord. The present study was undertaken to assess whether CsA therapy also modulates cytokine mRNA expression by inflammatory cells in the spinal cord of rats with EAE, looking for changes that might contribute to the observed effects of CsA on the course of EAE. EAE was induced in Lewis rats by inoculation with myelin basic protein and adjuvants. At the peak of neurological signs, on day 14 after inoculation, rats were given a single intraperitoneal injection of saline, or CsA at a dose of 8, 16, 32 or 64 mg/kg. The next day, rats were sacrificed, the spinal cords removed, inflammatory cells were extracted from the cords, and mRNA isolated from these cells. Expression of cytokine mRNA was assessed by semi-quantitative reverse transcription polymerase chain reaction (PCR) and by quantitative real-time PCR. With both techniques, we found that CsA suppressed the expression of interferon- γ mRNA and interleukin-2 (IL-2) mRNA. With real-time PCR, we found that CsA caused significantly increased expression of transforming growth factor- β mRNA. With the different techniques, we observed no consistent pattern of alteration of expression of interleukin-10 or interleukin-4 mRNA. It is possible that these changes in cytokine mRNA expression contribute to the modulation of the clinical course of EAE that is produced by CsA treatment.

Hug, A., B. Storch-Hagenlocher, et al. (2004). "Single-cell PCR analysis of the immunoglobulin heavy-chain CDR3 region for the diagnosis of leptomeningeal involvement of B-cell malignancies using standard cerebrospinal fluid cytopspins." Journal of the Neurological Sciences **219**(1-2): 83.

<http://www.sciencedirect.com/science/article/B6T06-4BNW2TD-1/2/4438339b85de92037ec1fcd25c6c6f83>

The diagnosis of leptomeningeal B-cell malignancies is based on the identification of malignant B cells in the cerebrospinal fluid (CSF). We have established a polymerase chain reaction (PCR) approach to characterize the clonally diverse gene encoding the immunoglobulin heavy-chain (IgH) third complementarity determining region (CDR3) of single B cells. We demonstrate that single-cell PCR is readily applicable to individual cells derived from routine CSF cytopspins and is a powerful method to discriminate monoclonal neoplastic from polyclonal reactive B-cell responses. Single-cell PCR analysis, as a new tool for the diagnosis and monitoring of neoplastic meningitis associated with B-cell malignancies, is particularly important if cytology, immunocytochemistry, flow cytometry and automated gene scanning of CSF samples are unable to detect malignant monoclonal proliferation.

Kobayashi, T., H. Matsumine, et al. (2003). "Pseudo-autosomal dominant inheritance of PARK2: two families with parkin gene mutations." Journal of the Neurological Sciences **207**(1-2): 11.

<http://www.sciencedirect.com/science/article/B6T06-479TPP1-2/2/5cba44b49a15d7f339976294cf93b95e>

We report two families (Family S and Family N) with early-onset parkinsonism in two generations. The mode of inheritance appeared to be autosomal dominant, however, haplotype analysis suggested linkage to chromosome 6q25.2-27, the PARK2 locus, and all affected members were homozygotes in their haplotypes. In Family S, the affected father was married to unaffected mother, who carried one disease-linked haplotype at chromosome 6q25.2-27. In Family N, the unaffected mother carried one disease-linked haplotype. Quantitative PCR amplification analysis revealed exon 3 deletion in Family S and exon 5 deletion in Family N. The age of onset was from 18 to 22 years in Family S and 25 to 42 years in Family N. In both of their hometowns, most people lived in the same districts for many generations and consanguineous marriages had been common. Thus, the carrier state of the parkin gene might have been high in those communities, and marriage of a patient and a carrier is expected to result in autosomal dominant like inheritance. We conclude that PARK2 cannot be excluded even if the mode of inheritance appears as autosomal dominant, when the affected patients are young.

Lynas, C., T. J. Hill, et al. (1993). "Latent infection with the MS strain of herpes simplex virus type 2 in the mouse following intracerebral inoculation." Journal of the Neurological Sciences **120**(1): 107.

<http://www.sciencedirect.com/science/article/B6T06-485YBS7-4H/2/33c529bf1656ccc8d74e47195248a841>

Intracerebral inoculation of the MS strain of herpes simplex virus type 2 (HSV-2) into mice causes an acute encephalitis associated with multifocal demyelination and necrotizing retinitis. We have studied the distribution of latent virus in mice that had recovered from the acute encephalitis. Four weeks or longer after inoculation, HSV-2 could be recovered from the trigeminal ganglia of all mice examined by co-culture of explants in roller tubes. The virus could not be recovered from explants of retina or brain stem. HSV-2 latency associated transcript (LAT) was readily detected in the trigeminal ganglia by reverse transcriptase-PCR more than 4 months after inoculation. LAT was also demonstrated in the brain but this required nested PCR for consistent detection. Both LAT and ICP0 mRNA were detected in brain tissue during the acute encephalitis but, unlike LAT, ICP0 mRNA could not be amplified from the trigeminal ganglia or brain beyond 4 weeks after inoculation of the virus. In situ hybridisation with a double-stranded DNA probe to the ICP0/LAT overlap region of HSV-2 revealed signal in trigeminal ganglion neurons and occasional cells in the brain stem. These findings indicate that HSV-2 introduced by intracerebral inoculation becomes latent in the trigeminal ganglia and that transcription of LAT also persists within the brain.

McDonnell, G. V., C. W. Kirk, et al. (2000). "An evaluation of interleukin genes fails to identify clear susceptibility loci for multiple sclerosis." Journal of the Neurological Sciences **176**(1): 4.

<http://www.sciencedirect.com/science/article/B6T06-40J1D85-2/2/ffc60ab7a79e967f2264640a94460aa7>

Differential expression of interleukins may influence susceptibility to inflammatory diseases such as MS. IL-1a production is increased in MS patients during acute relapse, IL-2 receptor (IL-2R) secretion correlates with disease activity in several inflammatory disorders and is variable in MS.

Both IL-4 and IL-10 expression vary significantly with relapse/remission in MS and IL-9 is postulated to inhibit steroid-induced apoptosis. To examine the influence of interleukin (IL) genes on MS susceptibility and clinical course, gene association studies using separate polymorphic microsatellite markers for il-1[alpha], il-2, il-2r[beta], il-4 il-9 and il-10 were performed, incorporating 150-177 relapsing-remitting or secondary progressive MS (RR/SPMS) patients, 100-110 primary progressive (PPMS) patients and 152-210 controls. No significant differences existed in allele frequencies between either MS group and controls for any of the interleukin microsatellite markers studied, nor were statistically significant differences observed in PPMS vs. RR/SPMS for any marker. These data indicate that the IL-1[alpha], IL-2, IL-2R[beta], IL-4, IL-9 and IL-10 genes are unlikely to be susceptibility loci for MS in this population.

Melberg, A., J. Hetta, et al. (1995). "Autosomal dominant cerebellar ataxia deafness and narcolepsy." Journal of the Neurological Sciences **134**(1-2): 119.

<http://www.sciencedirect.com/science/article/B6T06-3Y45GTF-1P/2/9e2ed4a9dbd407008752363536cd6526>

A new autosomal dominant syndrome in a Swedish pedigree is described. Five patients were affected with cerebellar ataxia and sensorineural deafness. Four of these patients had symptoms of narcolepsy. Optic atrophy, other neurological abnormalities and psychiatric symptoms developed with increasing disease duration. Three patients had non-neurological disease in addition, including diabetes mellitus in two and hypertrophic cardiomyopathy in one. Autopsy with neuropathological examination was performed in one case. Molecular studies focused on the short arm of chromosome 6, including the HLA DR2 locus associated with narcolepsy and the (CAG)_n repeat at the spinocerebellar ataxia type 1 (SCA1) locus. Biochemical investigation of muscle biopsy of one case indicated mitochondrial dysfunction with selective decrease in ATP production for substrates that normally give the highest rates. The activity of glutamate dehydrogenase was reduced, indicating a low mitochondrial density. We postulate an autosomal dominant genetic factor responsible for this syndrome. Linkage was excluded to HLA DR2, and a normal sized SCA1 repeat was observed. We conclude that a locus predisposing to ataxia, deafness and narcolepsy exists outside this region of chromosome 6.

Niemann, C. U., T. O. B. Krag, et al. (2000). "Identification of genes that are differentially expressed in extraocular and limb muscle." Journal of the Neurological Sciences **179**(1-2): 76.

<http://www.sciencedirect.com/science/article/B6T06-41H3KMT-8/2/fd583026a595a4779dbdc02aa532615a>

The extraocular muscles (EOM) are anatomically and physiologically distinct from other striated muscles in mammals. Among other differences, they can be driven to generate individual twitch contractions at an extremely high frequency and are resistant to [Ca²⁺]-induced myonecrosis. While EOM are preferentially targeted in some neuromuscular diseases such as myasthenia gravis and congenital fibrosis of the extraocular muscles, they are enigmatically spared in Duchenne's muscular dystrophy, despite the widespread damage seen in all other skeletal muscle groups during the course of this disease. To address the molecular mechanisms that specify the EOM-phenotype, we characterized the transcriptional profile of genes expressed in rat EOM versus limb muscle using a differential display strategy. Ninety-five putative differentially expressed cDNA tags were cloned, from which fourteen were confirmed as being differentially expressed by RNA slot blot and Northern blot analysis. Ten of these cDNAs were homologous to known human or murine genes and ESTs, while four genes that were upregulated in EOM were novel, and have been named expressed in ocular muscle (eom) 1-4. The identification of these differentially expressed genes may provide mechanistic clues toward understanding the unique

patho-physiological phenotype of EOM.

Niino, M., T. Fukazawa, et al. (2000). "Vitamin D receptor gene polymorphism in multiple sclerosis and the association with HLA class II alleles." Journal of the Neurological Sciences **177**(1): 65.

<http://www.sciencedirect.com/science/article/B6T06-4123D0R-8/2/285f1c4bd70f82e21ed26ba3c02c2c08>

We have previously reported that the association between Bsm I polymorphism, one of the vitamin D receptor genes (VDRG) polymorphism, and multiple sclerosis (MS). In this report, we investigated the further possible role or relevance of VDRG in the pathogenesis of MS. Apa I polymorphism was detected by PCR-RFLP from the DNA of 77 conventional MS patients and 95 healthy controls. The study of the Bsm I and Apa I haplotypes was carried out by employing previously reported Bsm I data. The AA genotype and the [A] allele in the profiles were significantly more prevalent in MS patients than in controls ($P=0.0070$ and $P=0.0321$, respectively). In the [A] allele-positive MS patients, the positive rate of DPB1*0501 in HLA was significantly higher than that of the [A] allele-positive controls and that of the [A] allele-negative MS patients even when the corrected P value (Pcorr) was applied ($P_{\text{corr}}=0.0220$ and $P_{\text{corr}}=0.0077$, respectively). The frequency of DRB1*1501 was higher in the [A] allele-positive patients than in the [A] allele-positive controls and the [A] allele-negative patients ($P_{\text{uncorr}}=0.0431$ and $P_{\text{uncorr}}=0.0089$, respectively), but the P values did not reach statistical significance after P corrections. The rate of Bsm I and Apa I haplotypes was much higher in bA/bA-positive MS patients than in the controls ($P=0.0003$), and in the bA positive MS patients, the positive rate of DPB1*0501 was higher than that of the bA-positive controls and that of the bA-negative MS patients ($P_{\text{corr}}=0.0308$ and $P_{\text{corr}}=0.0033$, respectively). These results indicate that VDRG polymorphism may be associated with susceptibility to MS, and HLA alleles may correlate with risk for MS together with VDRG.

Niino, M., S. Kikuchi, et al. (2000). "Estrogen receptor gene polymorphism in Japanese patients with multiple sclerosis." Journal of the Neurological Sciences **179**(1-2): 70.

<http://www.sciencedirect.com/science/article/B6T06-41H3KMT-7/2/648b50c613b61e5a8fe7c092ca82ccce>

Estrogen has been reported to have immunosuppressive functions, and to inhibit the progression of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS). Since estrogen shows its biological effects via estrogen receptors (ER), we investigate the possible role of ER genes (ERG) in the pathogenesis of MS. PvuII and XbaI polymorphisms in ERG were detected by PCR-RFLP from the DNA of 79 conventional MS patients and 73 healthy controls. The [P] allele in the profiles in PvuII was significantly more prevalent in MS patients than in the controls (PXBal polymorphism, the onset age of MS patients with the Xx genotype was earlier than that of the xx genotype group (mean age $\pm$ S.D.; 22.60 ± 8.04 , and 27.49 ± 9.14 , respectively) (PPvuII polymorphism might be associated with susceptibility to MS, and XbaI polymorphism with onset age of MS. ERG polymorphism should be further studied in other populations to improve strategies for treatment of MS.

Nishimura, M., M. Nukina, et al. (1997). "Characterization of Campylobacter jejuni isolates from patients with Guillain-Barre syndrome." Journal of the Neurological Sciences **153**(1): 91.

<http://www.sciencedirect.com/science/article/B6T06-3RSFG7M-J/2/82ea6508ac9ff892a8ead3d3b6706506>

Campylobacter jejuni is a major pathogen preceding Guillain-Barre syndrome (GBS), and most *C. jejuni* isolates from GBS patients belong to Penner serotype 19 (heat-stable; HS-19). We analyzed sixteen independent clinical isolates from GBS patients, twelve of which belonged to HS-19, three to HS-2, and one to HS-4, using PCR-based RFLP analysis of a flagellin-A (*flaA*) gene. Two isolates from patients with Miller Fisher syndrome (MFS), and 27 from patients with uncomplicated enteritis were also examined. All HS-19 isolates, regardless of GBS, showed an identical pattern (Cj-1) by RFLP typing and were distinguishable from those of the other Penner serogroups. In contrast, HS-2 and HS-4 isolates were divided into several different RFLP groups, suggesting HS-19 strains are genetically distinctive among *C. jejuni* isolates. A DNA fingerprinting method also failed to detect any specific band pattern for GBS-related *C. jejuni* isolates. We examined relationships among anti-GM1 antibody titres in the sera of GBS patients, clinical forms of GBS, serotype of *C. jejuni*, and the presence of GM1-like structures in lipopolysaccharide (LPS) components from *C. jejuni* isolates by immunoblotting. HS-19 related GBS was significantly associated with elevated anti-GM1 antibody titers in the sera of the patients, but not associated with any clinical pattern of GBS. No significant correlations were found between anti-GM1 antibody and the pattern of disease, or between GBS-related *C. jejuni* strains and the presence of GM1-like structures. HS-19 strains seem to be unique among *C. jejuni* isolates, and HS-19-related GBS may provide an excellent model for clarification of the pathogenesis of GBS.

Sambuughin, N., K. Sivakumar, et al. (1998). "Autosomal dominant distal spinal muscular atrophy type V (dSMA-V) and Charcot-Marie-Tooth disease type 2D (CMT2D) segregate within a single large kindred and map to a refined region on chromosome 7p15." Journal of the Neurological Sciences **161**(1): 23.

<http://www.sciencedirect.com/science/article/B6T06-3V8RP1X-5/2/2bd80fb0c03d8751c1079b0740ffea5>

Two separate disorders, autosomal dominant distal spinal muscular atrophy type V (dSMA-V) characterized by marked bilateral weakness in the hands and atrophy of thenar eminence and the first interosseous muscle, and Charcot-Marie-Tooth disease type 2D (CMT2D) characterized by sensory deficits in addition to the upper limb weakness and wasting, have been independently linked to chromosome 7p. We identified a multigenerational Mongolian kindred with 17 members affected with either dSMA-V or CMT2D and mapped both syndromes to the same region on chromosome 7p15. A maximum two-point lod score of 4.74 at recombination fraction zero was obtained with marker D7S474. Tight linkage without recombination was also detected with markers D7S526 and D7S632. A multipoint lod score of 6.07 suggested that the gene is located between markers D7S526 and D7S474. A single conserved haplotype was associated with dSMA-V and CMT2D. Based on informative recombination events, the disease locus was placed between markers D7S516 and D7S1514 within the 7p15 band. Data obtained from this study suggest that a single gene is responsible for both syndromes, dSMA-V and CMT2D, and extend our knowledge of the candidate region.

Shiraiwa, N., A. Ishii, et al. (1993). "Content of mutant mitochondrial DNA and organ dysfunction in a patient with a MELAS subgroup of mitochondrial encephalomyopathies." Journal of the Neurological Sciences **120**(2): 174.

<http://www.sciencedirect.com/science/article/B6T06-485RJ8W-MC/2/38ada2a4cb28fd15715680811ba8edd0>

A point mutation of mitochondrial tRNA^{Leu}(UUR) gene is responsible for a MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) subgroup of mitochondrial encephalomyopathies. In most cases, the mutant mitochondrial DNA (mtDNA) coexists with normal mtDNA in a heteroplasmic manner. In order to quantify the content of mutant mtDNA, we developed a quantitative method of PCR. Using this method, the distribution of the mutant mtDNA was examined in 32 different tissues among 18 autopsied organs from a patient with MELAS, who had shown hypophyseal dysfunction. The percentage of the mutant mtDNA at nucleotide number 3243 in each tissue was ranged between 22% and 95%. The content of the mutant mtDNA was at the highest (95%) in the hypophysis and higher in the cerebral cortex than in the white matter. This study shows a possible correlation of tissue dysfunction with accumulation of the mutant mtDNA within the brain.

Wagnerova, J., L. Cervenakova, et al. (2002). "Cytokine regulation of E-selectin in rat CNS microvascular endothelial cells: differential response of CNS and non-CNS vessels." Journal of the Neurological Sciences **195**(1): 51.

<http://www.sciencedirect.com/science/article/B6T06-44VX674-1/2/685e416f20487d3ca4adf3e5190ffaa5>

We have compared the induced expression of E-selectin in primary cultures of rat brain microvascular endothelial cells (EC), pericytes and in non-CNS microvascular endothelium stimulated with the cytokines, IL-1[β] (20 ng/ml), and tumor necrosis factor (TNF)-[α] (75 ng/ml). Expression was studied at both the protein and mRNA levels. Fluorescence in-situ hybridization (FISH) was used to examine de novo synthesis of E-selectin mRNA. Laser cytometric analysis was used as a novel approach to the quantitation of FISH. In-situ hybridization was performed using two PCR-generated probes. The first probe (517 bp) spanned the lectin and epidermal growth factor (EGF)-like domain. The second probe (562 bp) spanned the CR3, 4, and 6 domains. E-selectin-specific mRNA was localized to the perinuclear regions of the EC. Both cytokines, IL-1[β] and TNF-[α] significantly increased E-selectin gene expression in CNS EC but not pericytes. IL-1[β] induced higher E-selectin mRNA levels than TNF-[α]. The maximum number of mRNA-positive cells was observed after stimulation for 4-6 h. Surface protein expression was sustained for up to 48 h following addition of cytokine. This was in contrast to the transient expression in non-CNS EC indicating that pure primary CNS EC display slightly different kinetics of E-selectin expression than non-CNS EC.

Yamada, T., A. Kondo, et al. (1995). "Apolipoprotein E mRNA in the brains of patients with Alzheimer's disease." Journal of the Neurological Sciences **129**(1): 56.

<http://www.sciencedirect.com/science/article/B6T06-3Y44T67-2F/2/7252236fd37878b433758be2d7ad9aa6>

To clarify the role of apolipoprotein E (apoE) in the pathophysiology of Alzheimer's disease (AD), we used an RT-PCR method to investigate apoE and glial fibrillary acidic protein (GFAP) mRNA expression in the brain. ApoE mRNA was significantly more abundant in AD (0.379 $\pm$ 0.191, mean $\pm$ SD) than in control (0.125 $\pm$ 0.073, $p < 0.05$) brain tissue, but in AD it was decreased in relation to the apoE-[ϵ]4 gene dosage. The GFAP mRNA content also was greater in AD (5.96 $\pm$ 2.94) than in control (3.80 $\pm$ 2.78) tissue, and in AD showed an increase relative to the apoE-[ϵ]4 gene dosage. AD patients who had long survival times showed high expression of apoE and low expression of GFAP. These results suggest that apoE suppresses the progression of AD, including gliosis, in the brain.

Yamashita, T., Y. Ando, et al. (2000). "A new diagnostic procedure to detect unknown transthyretin (TTR) mutations in familial amyloidotic polyneuropathy (FAP)." Journal of the Neurological Sciences **173**(2): 154.

<http://www.sciencedirect.com/science/article/B6T06-3YJ9XM5-D/2/35877d454693bebedbf5770d7ee0d13b>

Two patients with amyloidosis caused by transthyretin (TTR) were investigated by immunohistopathologic, mass spectrometric, and molecular genetic methods. After confirming the immunoreactivity of TTR in the amyloid deposits using anti-TTR polyclonal antibody, a new method: centrifugal concentration and electrospray ionization mass spectrometry (ESI-MS) was employed to detect the variant TTR in the serum. Only 50 [μl] of the serum and 30 [μl] of the anti-TTR antibody were needed for the analysis. After incubation with the antibody, the samples were passed through a 1000 kDa cut off centrifugal concentrator to retain the antibody, thereafter, the filtrate was analyzed by ESI-MS. Several forms of normal and variant TTR were detected in the serum samples: unconjugated TTR, cysteine and cysteine-glycine conjugated TTR. In the patients, a variant form of TTR was detected with a 26.0 Da higher molecular weight than that of normal TTR. Single-strand conformation polymorphism (SSCP) and direct sequence analysis confirmed the presence of a one-base substitution situated at the codon 50 from AGT (Ser) to ATT (Ile) in both patients, that corresponded to the increased molecular weight of 26.0. The present diagnostic procedure demonstrates the usefulness of both ESI-MS and SSCP to screen for TTR related amyloidosis rapidly. Moreover, the DNA samples obtained from the band showing abnormal electrophoretic migration pattern in SSCP, facilitate the direct sequence analysis to detect the unknown mutation, and the observed shift in molecular weight of the variant TTR in ESI-MS confirms the base substitution.

Yoshihara, T., F. Kanda, et al. (2001). "A novel missense mutation in the early growth response 2 gene associated with late-onset Charcot-Marie-Tooth disease type 1." Journal of the Neurological Sciences **184**(2): 149.

<http://www.sciencedirect.com/science/article/B6T06-42G0KYK-6/2/5505caba443481a5212146478e88258d>

A novel mutation (Arg381Cys) in the second zinc-finger domain of early growth response 2 (EGR2) was identified in a late-onset Charcot-Marie-Tooth disease type 1 (CMT1) patient. This patient had initial symptoms of numbness and weakness in the leg at age 59, and a median nerve motor conduction velocity of 27 m/s. A sural nerve biopsy showed a severe loss of myelinated fibers with numerous onion bulbs. This is the first report of the EGR2 mutation presenting a late onset of CMT1 phenotype. Its mutation was a different amino acid substitution at codon 381 (Arg381His) which demonstrated congenital hypomyelinating neuropathy or early-onset CMT1. This report suggests that the EGR2 mutation represents divergent phenotypes at codon 381, which may be a mutation hotspot.

Zhai, Q.-H., N. Futrell, et al. (1997). "Gene expression of IL-10 in relationship to TNF-[α], IL-1[β] and IL-2 in the rat brain following middle cerebral artery occlusion." Journal of the Neurological Sciences **152**(2): 119.

<http://www.sciencedirect.com/science/article/B6T06-3RH634P-1/2/eee9e17eb503cd360f95eb8a3ec0fce2>

To systematically elucidate the gene expression of inflammatory and immune modulators following middle cerebral artery occlusion (MCAO) in the rat, we studied interleukin-10 (IL-10) along with tumor necrosis factor alpha (TNF-[alpha]), interleukin-1 beta (IL-1[beta]) and interleukin-2 (IL-2). Gene expression of these cytokines was studied ipsilateral and contralateral to the MCAO, with mRNA expression levels evaluated 2, 4, 6, 8 and 12 h following permanent MCAO by reverse transcriptase polymerase chain reaction (RT-PCR). In the ischemic hemisphere TNF-[alpha] and IL-1[beta] mRNA increased at 2 h following MCAO and peaked at 6 h, with IL-10 mRNA detected only at 6 h. Contralaterally, both TNF-[alpha] and IL-1[beta] mRNAs were expressed with a similar pattern to that in the ischemic hemisphere, but at lower levels, with no contralateral IL-10 expression. There was no difference in IL-2 gene expression between control and experimental animals in either hemisphere. These results demonstrate that IL-10 and TNF-[alpha], IL-1[beta] gene expression is induced early following MCAO. The temporal profile of these cytokines is similar to that seen in sepsis, where TNF-[alpha] induces IL-10; subsequently IL-10 inhibits TNF-[alpha] expression. The similarity of the temporal profile of cytokine expression in sepsis and cerebral ischemia suggests that IL-10 should be studied as a potential inhibitor of TNF-[alpha] production in ischemic brain tissue. The factors inducing contralateral expression of the inflammatory cytokines, TNF-[alpha] and IL-1[beta], along with the potential clinical significance of this remote cytokine gene expression, merit further study.

Journal of the Society for Gynecologic Investigation (10)

Chen, Z., W. Huang, et al. (2004). "Serine racemase and -serine transport in human placenta and evidence for a transplacental gradient for -serine in humans." Journal of the Society for Gynecologic Investigation **11**(5): 294.

<http://www.sciencedirect.com/science/article/B6T93-4CPJM90-6/2/b0a11eb5a1e58a7206ddd100dcf98624>

ObjectiveTo investigate the possible role of human placenta in providing -serine to the developing fetus.
MethodsExpression of serine racemase in placenta was determined by reverse transcriptase polymerase chain reaction and northern analysis and confirmed by subsequent cloning. The transport of -serine by human ATB0 was characterized by expressing the cloned cDNA transiently in mammalian cells using the vaccinia virus expression system. -serine levels in maternal and fetal blood were measured by fluorescence high-performance liquid chromatography (HPLC) after derivatization of the amino acids with o-phthaldialdehyde and N-tertiary-butyloxycarbonyl--cysteine.
ResultsmRNA for serine racemase was detected in placenta. ATB0 was capable of -serine transport, and the transport process is obligatorily dependent on sodium (Na⁺) with a Na⁺:substrate stoichiometry of 1:1 and saturable with a Michaelis-Menten constant of 310 +/- 30 [mu]M. Furthermore, studies have shown that ATB0 is not expressed in the maternal-facing brush border membrane of human placental syncytiotrophoblast. The circulating concentration of -serine in maternal serum is 5.8 +/- 0.5 [mu]M, and the corresponding value in the fetal serum is 14.6 +/- 1.2 [mu]M, indicating a two- to three-fold higher concentration of -serine in the fetus than in the mother.
ConclusionWe speculate that -serine is synthesized in human placenta by the racemization of -serine and that ATB0, expressed on the basal membrane of the syncytiotrophoblast, mediates the efflux of -serine into the fetal circulation in exchange for other amino acids in fetal blood.

Cheung, C. Y. and R. A. Brace (1999). "Developmental expression of vascular endothelial growth factor and its receptors in ovine placenta and fetal membranes." Journal of the Society for Gynecologic Investigation **6**(4): 179.

<http://www.sciencedirect.com/science/article/B6T93-3X9RY8X-3/2/b26a90041d2507854d0895b91500ada8>

Objectives: Vascularity of the surface of the placenta in humans and of the placenta and fetal membranes in several species including sheep is an important determinant of intramembranous absorption of amniotic fluid. Our previous studies have shown that the total blood vessel surface area in ovine amnion and chorion increases with advancing gestation. Vascular endothelial growth factor (VEGF) is a potent angiogenic and permeability factor and is found to be expressed in the ovine placenta and fetal membranes. To investigate the role of VEGF in maintaining the absorptive function of the intramembranous microvessels, the present study was undertaken to determine the gestational change in gene expression of VEGF and its receptors, kinase insert domain-containing receptor (KDR) and fms-like tyrosine kinase (Flt-1), in ovine placenta, chorion, and amnion. **Methods:** Total RNA was extracted from placental cotyledon, chorion, and amnion of ovine fetuses at 60-140 days of gestation. The relative abundance of VEGF, KDR, and Flt-1 mRNA was determined by Northern blot analysis, and VEGF molecular forms expressed were identified by reverse transcriptase polymerase chain reaction. The gestational changes in mRNA levels of VEGF and its receptors were analyzed by regression analysis. **Results:** In ovine placenta, chorion, and amnion, VEGF mRNA levels increased significantly from 60 to 140 days. The major VEGF molecular form expressed in these tissues was VEGF₁₆₄, whereas VEGF₁₂₀, VEGF₁₄₄, and VEGF₁₈₈ were present at lower levels. In the placenta, KDR was the primary VEGF receptor expressed, although Flt-1 was also detected at very low levels. In the amnion and chorion, KDR was the only receptor expressed. A gestational-dependent change in VEGF receptor expression was not observed in the placenta and membranes. **Conclusions:** The increase in VEGF gene expression with advancing gestation in the amnion and chorion where KDR is expressed suggests that VEGF and its receptor are important determinants of vascularity and permeability, and thus exchange capacity, of the intramembranous pathway.

Chien, E. K., L. Sweet, et al. (2003). "Protease-activated receptor isoform expression in pregnant and nonpregnant rat myometrial tissue." Journal of the Society for Gynecologic Investigation **10**(8): 460.

<http://www.sciencedirect.com/science/article/B6T93-4B4NYRR-3/2/820a6f54409d49e7b1347ee364c8f544>

Objective Three protease-activated receptor (PAR1, 3, and 4) isoforms have been shown to be responsible for the cellular effects of thrombin; another PAR isoform (PAR2) is responsible for the cellular effects of trypsin. The present studies sought to test the hypothesis that one (or more) of these PAR isoforms is expressed in myometrial tissue, thereby accounting for the uterotonic effects of these novel agonists. **Methods** The rat PAR3 and 4 isoforms were cloned from a rat spleen cDNA library. PAR isoform mRNA expression was determined by using reverse-transcriptase polymerase chain reactions (PCR) in Sprague-Dawley rats. Confirmation of the identity of the amplified mRNA was done by sequence analysis. Relative quantification of the PAR1 and PAR2 isoforms was performed using a real-time quantitative reverse transcriptase PCR (RT-PCR) technique. PAR protein expression was confirmed by Western blots using polyclonal antibodies. **Results** The rat PAR3 and 4 homologues showed significant sequence homology to the mouse and human amino acid and nucleotide sequences. The RT-PCR studies confirmed PAR1-4 expression in myometrium from rats in estrus. PAR3 was expressed in uterus, spleen, kidney, liver, lung, brain, and heart. PAR4 was expressed in uterus, spleen, and lung. Messenger RNA for the PAR1 and 2 isoforms was expressed during the second half of gestation in myometrium from timed-pregnant rats. In contrast, mRNA for the PAR3 and 4 isoforms was not

detected in gestational myometrium. PAR protein expression appeared to match tissue mRNA expression patterns. Conclusion These RT-PCR studies confirmed ubiquitous expression of the PAR1 and PAR2 isoforms in myometrium and other rat tissues; in contrast, the PAR3 and PAR4 isoforms are expressed in a tissue-specific and gestationally related pattern.

Emmer, P. M., I. Joosten, et al. (2004). "Shift in expression of HLA-G mRNA spliceforms in pregnancies complicated by preeclampsia." Journal of the Society for Gynecologic Investigation **11**(4): 220.

<http://www.sciencedirect.com/science/article/B6T93-4C8FJYB-5/2/a9660fe2ca4d608703128845a53ee9bf>

Objective Despite emerging data on the in vitro modulatory effects of trophoblast-associated human leukocyte antigen G (HLA-G), its in vivo function needs to be determined. Immunohistochemical studies show a decrease in protein expression of trophoblast HLA-G in preeclampsia. Such a decrease in protein might be the consequence of a shift in HLA-G mRNA spliceform patterns. In an exploratory pilot study we determined trophoblast HLA-G mRNA spliceform distribution in preeclampsia. Methods Placental samples were collected immediately after cesarean delivery from pregnancies complicated by preeclampsia or the syndrome hemolysis, elevated liver enzymes, and low platelet count (HELLP) and uncomplicated normotensive pregnancies as controls. HLA-G mRNA spliceform distribution was analyzed using a semiquantitative reverse transcriptase polymerase chain reaction procedure. Results Analysis of HLA-G spliceform distribution showed a significant increase in frequency of the G5 form encoding for a soluble HLA-G molecule in preeclampsia. This increase in G5 form was not found in pregnancies complicated by HELLP. Conclusion The increased frequency in the expression of the HLA-G G5 spliceform may play a role in the pathophysiology of preeclampsia, in particular through a recently suggested effect of this soluble HLA-G molecule on remodeling of the spiral arteries.

Havrilesky, L. J., C. P. McMahon, et al. (2001). "Relationship between expression of coactivators and corepressors of hormone receptors and resistance of ovarian cancers to growth regulation by steroid hormones." Journal of the Society for Gynecologic Investigation **8**(2): 104.

<http://www.sciencedirect.com/science/article/B6T93-42XB4KM-8/2/765a065315245fd5e8cf50b3d38073e9>

Objective: To determine whether aberrant expression of hormone receptor corepressors or coactivators or defects in estrogen receptor-mediated transcription might underlie resistance of ovarian cancers to hormonal therapy. Methods: Northern analysis, Western analysis, and polymerase chain reaction were used to examine expression of estrogen receptor (ER), progesterone receptor (PR), the nuclear receptor corepressors N-CoR and SMRT, and the steroid receptor coactivator BRG-1 in ovarian cancer cell lines and primary cancers. The effect of BRG-1 transfection on ER-mediated transcription was examined. We also determined the effect of estrogen and the pure estrogen antagonist ICI 182,780 on cell cycle profile and expression of ER. Finally, we examined the ability of estrogen to upregulate expression of known estrogen-responsive genes. Results: Among primary ovarian cancers, 18 of 52 (35%) expressed N-CoR, and 37 of 52 (71%) expressed SMRT, but there was no correlation between expression of corepressors and hormone receptor status. All of the primary ovarian cancers and cell lines expressed BRG-1. Estrogen stimulation of two cell lines expressing ER (SKOV3, OVCA 432) elicited low levels of ER-mediated transcription that was not enhanced by BRG-1 transfection. ICI 182,780 did not induce cell cycle arrest in these cell lines, but there was evidence of downregulation of ER, indicating a ligand-receptor interaction. However, estrogen did not elicit increased transcription of estrogen-responsive genes (PR, myc, fos, pS2). Conclusion:

Inappropriate expression of the nuclear corepressors N-CoR and SMRT or the coactivator BRG-1 does not underlie the resistance of ovarian cancers to hormonal therapy. Further studies are needed to elucidate the mechanisms underlying the inability of ovarian cancers to undergo ER-mediated transcription if we hope to understand their resistance to hormonal therapy.

Jain, V., M. Longo, et al. (2000). "Expression of receptors for corticotropin-releasing factor in the vasculature of pregnant rats." Journal of the Society for Gynecologic Investigation 7(3): 153.

<http://www.sciencedirect.com/science/article/B6T93-40J1DY9-3/2/a9971c6dce8ef9a3f0fb9dafb5d82db9>

Objective:To identify and localize the receptor(s) responsible for modulating vascular effects of corticotropin-releasing factor (CRF) during pregnancy.**Methods:**Reverse transcriptase-polymerase chain reaction (RT-PCR), competitive RT-PCR, and Western blot analyses were used to study the expression of CRF receptors (CRFR1, CRFR2[alpha], and CRFR2[beta]) in the aorta and uterine vascular bed of nonpregnant and late (day 18) and term pregnant (day 22) Sprague-Dawley rats. Immunohistochemistry was done to localize the CRF receptor in the aortic wall. There were six rats in each study group.**Results:**Only CRFR2[beta] was identified in the aorta and uterine vascular bed by RT-PCR and Western blot analyses. The PCR product was sequenced to confirm its identity. Competitive RT-PCR and Western blot analyses showed that expression of CRFR2[beta] is not different in late pregnancy compared with the nonpregnant but is decreased at term. Immunohistochemistry showed high expression of CRFR2[beta] on the aortic endothelial surface but low expression in the smooth-muscle layer.**Conclusion:**Only CRFR2[beta] is expressed in vasculature of nonpregnant and pregnant rats and may mediate the vasorelaxant effect of CRF. This receptor is present predominantly in the vascular endothelium and to a lesser extent in the smooth muscle. The expression of CRF receptor in pregnant rat vasculature is down-regulated at term of gestation.

Nichols, J. E., S. E. Bulun, et al. (1995). "Effects of conditioned medium from different cultured cell types on aromatase expression in adipose stromal cells." Journal of the Society for Gynecologic Investigation 2(1): 45.

<http://www.sciencedirect.com/science/article/B6T93-3YCDS23-SM/2/b22b7fc3374307661301b11a046cfff>

To determine whether serum-free (SF) conditioned media (CM) from several human breast cancer cell lines and primary stromal cell cultures contain factor(s) that mimic the marked stimulatory effects of serum on aromatase activity and aromatase P450 (P450arom) gene expression in adipose stromal cells in culture (ASC) in the presence of dexamethasone (DEX). Adipose stromal cells, harvested from fresh adipose specimens, were grown to confluence, switched to SF media, and then incubated in the presence or absence of DEX with CM from T47-D breast cancer cells, pre-treated with or without 17[beta]-estradiol (E2), and with CM from stromal cell cultures. Aromatase activity of the ASC was determined by the [3H]water release assay. Total RNA was isolated, and reverse transcription-polymerase chain reactions were performed to determine the expression of various 5'-termini. T47-D CM stimulated aromatase activity in a concentration-dependent manner, similar to that of serum, in ASC incubated with DEX. Estrogen potentiated this in a dose-dependent fashion. The ASC CM and endometrial stromal cell CM also markedly induced aromatase activity in ASC. Heat inactivation destroyed the stimulating ability of CM. The majority of P450arom 5'-termini expressed by ASC incubated with CM plus DEX contained the promoter 1.4-specific sequence. Conditioned media from several breast cancer cell lines and primary stromal cell cultures can mimic the effects of serum in the presence of DEX to stimulate aromatase activity in ASC. These results suggest that undefined,

heat-labile and proteinaceous factors are present in CM that stimulate P450arom expression in a fashion similar to that of serum.

Schondorf, T., A. Dostal, et al. (2000). "Single mutations of the PTEN gene in recurrent ovarian carcinomas." Journal of the Society for Gynecologic Investigation 7(5): 313.

<http://www.sciencedirect.com/science/article/B6T93-41DHPR0-8/2/9995177120a8a93c691e3ff7cf9fa9d0>

Objective:To establish the role of phosphate and tensin homologue on chromosome 10 (PTEN) mutations in tumorigenesis of the ovary, we determined the mutational spectrum of the PTEN gene in surgical specimens of ovarian carcinomas. **Methods:**The study group consisted of 86 ovarian cancer specimens (18 fluids, 68 solid specimens), including 30 primary ovarian cancer specimens and 56 of relapsed ovarian cancer from women with a median age of 57.9 years and a range of 27-85 years. Each of the nine exons of the PTEN gene was amplified separately by polymerase chain reaction (PCR). Both strands of the PCR products were sequenced directly by standard cycle sequencing procedures and subsequent computer-aided alignments with the wild-type sequence. **Results:**In ascitic fluids of two women with recurrence of cancer, we observed mutations: one seven-base-pair insertion at codon 52 (GATGATG) and the other a base-pair substitution resulting in an amino acid change (T131I). We found no mutation in the primary ovarian cancers. **Conclusions:**Our data indicate that PTEN mutations have a subordinate role in tumorigenesis of the ovary.

Thaete, L. G., M. G. Neerhof, et al. (2003). "Infusion of exogenous platelet-activating factor produces intrauterine growth restriction in the rat." Journal of the Society for Gynecologic Investigation 10(3): 145.

<http://www.sciencedirect.com/science/article/B6T93-48BBJVM-5/2/ca59f0522fb73a70369bd0fbcc17d6e3>

Objective:To determine the impact of exogenous platelet-activating factor (PAF) on pregnancy outcome in the rat. **Methods:**Carbamyl-PAF (0.05, 0.5, or 5.0 [μg/kg per hour) or vehicle was infused intravenously for 7 days by osmotic pump into timed pregnant rats. Infusion was begun on day 14 of a 22-day gestation. Maternal mean arterial blood pressures were measured on days 1, 4, and 7 of the infusion. On gestational day 21 (PAF infusion day 7), fetal and placental weights and viability were evaluated at hysterotomy. Uterine and placental PAF receptor expression was analyzed by reverse transcription-polymerase chain reaction and agarose gel electrophoresis. Data were analyzed by analysis of variance, [chi]², or the Mann-Whitney U test as appropriate. **Results:**Fetal weights were dose-dependently lower than control, by 19% and 35%, respectively, at dosages of 0.5 and 5.0 [μg/kg per hour (P P P **Conclusions:**Exogenous PAF produces dose-dependent fetal growth restriction in the rat. Placental growth is particularly sensitive to PAF and, coupled with the dose-dependent decline in fetal growth, suggests a dose-dependent decline in function. An elevated level of PAF is detrimental to fetal growth and well-being in the rat.

Toivonen, S., E.-L. Romppanen, et al. (2004). "Low-activity haplotype of the microsomal epoxide hydrolase gene is protective against placental abruption." Journal of the Society for Gynecologic Investigation 11(8): 540.

<http://www.sciencedirect.com/science/article/B6T93-4DN176B-1/2/1731415704f3a20f3780599fadb340ef>

ObjectiveWe wanted to determine whether genetic variability in the gene encoding microsomal epoxide hydrolase (EPHX) contributes to individual differences in susceptibility to the occurrence of placental abruption.**Methods**The study involved 117 women with placental abruption and 115 healthy control pregnant women who were genotyped for two single nucleotide polymorphisms (SNPs), T-->C (Tyr113His) in exon 3 and A-->G (His139Arg) in exon 4, in the EPHX gene. Chi-square analysis was used to assess genotype and allele frequency differences between the women with placental abruption and the control group. In addition, single-point analysis was expanded to pair of loci haplotype analysis to examine the estimated haplotype frequencies of the two SNPs, of unknown phase, among the women with placental abruption and the control group. Estimated haplotype frequencies were assessed using the maximum-likelihood method, employing an expectation-maximization algorithm.**Results**Single-point allele and genotype distributions in exons 3 and 4 of the EPHX gene were not statistically different between the groups. However, in the haplotype estimation analysis we observed a significantly decreased frequency of haplotype C-A (His113-His139) among the placental abruption group compared with the control group ($P = .007$). The odds ratio for placental abruption associated with the low-activity haplotype C-A (His113-His139) was 0.552 (95% confidence interval, 0.358 to 0.851).**Conclusions**The use of two intragenic SNPs jointly in haplotype analysis of association demonstrated that the genetically determined low-activity haplotype C-A (His113-His139) was significantly less frequent in women with placental abruption.

Journal of Thoracic and Cardiovascular Surgery (1)

Absi, T. S., I. I. I. T. M. Sundt, et al. (2003). "Altered patterns of gene expression distinguishing ascending aortic aneurysms from abdominal aortic aneurysms: complementary DNA expression profiling in the molecular characterization of aortic disease." Journal of Thoracic and Cardiovascular Surgery **126**(2): 344.

<http://www.sciencedirect.com/science/article/B6WMF-4991FX6-12/2/f7aa9019d150c38afc83b882236fcc70>

ObjectivesThe purpose of this study was to profile altered patterns of gene expression that characterize degenerative ascending thoracic aortic aneurysms and to compare these patterns with those observed for infrarenal abdominal aortic aneurysms.**Methods**Full-thickness aortic wall tissues were obtained during surgical repair of degenerative thoracic aortic aneurysms and infrarenal abdominal aortic aneurysms ($n = 4$ each), with normal thoracic and abdominal aortas from organ transplant donors used as control preparations. Radiolabeled complementary DNA was prepared for each specimen and hybridized to complementary DNA microarrays, and differential levels of gene expression between aneurysmal and normal aortic tissues at each site were assessed by parametric statistics.**Results**Of 1185 genes examined, 112 (9.5%) were differentially expressed ($P < .05$) including yes-1 oncogene, mitogen-activated protein kinase 9, and intercellular adhesion molecule 1/CD54). Results for 9 genes were independently confirmed by quantitative reverse transcriptase-polymerase chain reaction.**Conclusions**Thoracic aortic aneurysms and infrarenal abdominal aortic aneurysms exhibit distinct patterns of gene expression relative to normal aorta from the same sites, with most alterations being unique to each disease. Degenerative aneurysms arising in different locations are thus characterized by a high degree of molecular heterogeneity, reflecting different pathophysiologic mechanisms.

Colonnello, J. S., K. A. Hance, et al. (2003). "Transient exposure to elastase induces mouse aortic wall smooth muscle cell production of MCP-1 and RANTES during development of experimental aortic aneurysm." Journal of Vascular Surgery **38**(1): 138.

<http://www.sciencedirect.com/science/article/B6WMJ-48XS59W-14/2/2b1b3074f6a24f00b5c77a892e255d37>

Purpose Abdominal aortic aneurysm (AAA) is associated with chronic transmural inflammation and destruction of the elastic media. The purpose of this study was to elucidate molecular mechanisms that might orchestrate leukocyte recruitment into the outer aortic wall by determining whether CC chemokines contribute to development of aneurysm degeneration in an elastase-induced mouse model of AAA. **Methods** Adult male C57BL/6J mice underwent transient elastase perfusion of the abdominal aorta to induce development of AAA. At various intervals after elastase perfusion (0, 4, 7, 14 days), real-time reverse transcription polymerase chain reaction and enzyme-linked immunosorbent assays were used to measure aortic wall expression of the CC ([beta]) chemokines, monocyte chemoattractant protein-1 (MCP-1) and regulated on activation, normal T-cell expressed and secreted (RANTES). Expression of these chemokines by cultured mouse aortic smooth muscle cells (AoSMC) was similarly assessed after transient (5 minutes) exposure to elastase solutions in vitro. **Results** Mouse aortic diameter (mean +/- SEM) increased to aneurysmal proportions by 14 days after elastase perfusion (from 0.51 +/- 0.03 mm to 1.34 +/- 0.32 mm; 163% increase; P < .001) of MCP-1 and RANTES, despite concomitant decrease in cell numbers. **Conclusions** Increased mouse aortic wall expression of MCP-1 and RANTES occurs early in development of elastase-induced AAA and before onset of the chronic inflammatory response. Moreover, elastase directly stimulates AoSMC chemokine production in vitro. Elastase-induced medial SMC production of CC chemokines may therefore provide an important link between enzymatic injury, leukocyte recruitment, and aneurysmal degeneration of the aortic wall.

Curci, J. A., D. Mao, et al. (2000). "Preoperative treatment with doxycycline reduces aortic wall expression and activation of matrix metalloproteinases in patients with abdominal aortic aneurysms." Journal of Vascular Surgery **31**(2): 325.

<http://www.sciencedirect.com/science/article/B6WMJ-4CYY107-2C/2/010445b60f9b2fc0693172a98c072886>

Purpose: Matrix metalloproteinases (MMPs) are considered to play a central role in the pathogenesis of abdominal aortic aneurysms (AAAs). Doxycycline (Dox) has direct MMP-inhibiting properties in vitro, and it effectively suppresses the development of elastase-induced AAAs in rodents. The purpose of this study was to determine if treatment with Dox suppresses MMPs within human aneurysm tissue and to elucidate the molecular mechanisms underlying this effect. **Methods:** Aneurysm tissues were obtained from 15 patients with an AAA, eight of whom had been treated with Dox before surgery (100 mg orally twice a day for 7 days). Protein extracts were examined by means of gelatin zymography and immunoblot analysis, and RNA was examined by means of reverse transcription-polymerase chain reaction (R-TPCR). The effects of Dox on MMP production were further examined in human THP1 mononuclear phagocytes in

vitro. Results: No detectable difference was found between groups by using substrate zymography as a means of assessing total AMP activity, but Dox treatment was associated with a slight (24.4%) reduction in the activated fraction of 72-kDa gelatinase (MMP-2; P P P Conclusion: In addition to its recognized effects as a direct MMP antagonist, Dox may influence connective tissue degradation within human aneurysm tissue by reducing monocyte/macrophage expression of MMP-9 mRNA and by suppressing the post-translational processing (activation) of proMMP-2. Through this complementary combination of mechanisms, treatment with Dox may be a particularly effective strategy for achieving MAP inhibition in patients with an AAA.

Journal of Virological Methods (213)

Aberham, C., C. Pendl, et al. (2001). "A quantitative, internally controlled real-time PCR Assay for the detection of parvovirus B19 DNA." Journal of Virological Methods **92**(2): 183.

<http://www.sciencedirect.com/science/article/B6T96-42D81PP-9/2/fde3d3e7f7f43cb0f896041e1ff48bdd>

Parvovirus B19 is an erythrovirus causing diverse clinical manifestations ranging from asymptomatic or mild, to more severe outcomes in, for example, immune-compromised patients. B19 is spread primarily via the respiratory route, but it can also be transmitted via blood and blood products. Viral loads in blood or plasma donations amount up to 1011 genome equivalents/ml. Therefore, screening of plasma for fractionation for the presence of B19 and removal of highly loaded donations is a way to limit considerably the input of B19 into production pools and to improve further the safety of plasma products. An assay for the quantitative detection of B19 DNA, based on real-time PCR using ABI Prism SDS7700 (TaqMan) is described here. This assay allows precise quantitation of viral loads over 7 orders of magnitude. An exogenous internal control (internal quality marker) is included in each individual sample to prevent false negative results. A linearized plasmid is used as an internal quality marker that contains the identical sequence of the B19 target sequence but with an altered probe hybridization site. This allows co-amplification of B19 and internal quality marker and co-detection of FAM (6-carboxyfluorescein) or VIC labeled probes respectively. The assay is validated according to current guidelines (of the International Conference on Harmonization, Paul Ehrlich Institute, and the Council of Europe) and is optimized for high throughput screening.

Agnes, F., J. M. Crance, et al. (1994). "Separate detection of the two complementary RNA strands of hepatitis A virus." Journal of Virological Methods **49**(3): 323.

<http://www.sciencedirect.com/science/article/B6T96-476TY1K-8/2/edb199dcce304e64fed3c2484ef8ad1f>

The minus strand of hepatitis A virus can be detected specifically by reverse transcription and polymerase chain reaction amplification in infected cell culture extracts. Several controls gave evidence that the amplified fragment actually used the minus strand as initial template. Non-thermostable reverse transcriptase was not efficient for this purpose because of self-priming of the positive-stranded viral RNA during the reverse transcription step. This problem was overcome by the use of the thermostable rTth DNA polymerase that also has reverse transcriptase activity in the presence of Mn²⁺.

Albrecht, B., N. D. Collins, et al. (1998). "Quantification of human T-cell lymphotropic virus type 1 proviral load by quantitative competitive polymerase chain reaction." Journal of Virological Methods **75**(2): 123.

<http://www.sciencedirect.com/science/article/B6T96-3V5F5S6-1/2/d33602ffb9310033bf5828a1d6013b4>

The polymerase chain reaction (PCR) has been established as a highly sensitive technique for detection of viral DNA or RNA. However, due to inherent limitations of PCR the amount of amplified product often does not correlate with the initial amount of template DNA. This is particularly true for PCR detection of viral infections that are characterized by low in vivo viral copy numbers in certain stages of the infection, such as human T-cell lymphotropic virus type 1 (HTLV-1) and simian T-cell lymphotropic virus type 1 (STLV-1). Therefore, we developed a quantitative competitive polymerase chain reaction (qcPCR) for detection of HTLV-1 and STLV-1 proviral DNA. The assay was optimized using an infectious HTLV-1 clone, ACH, HTLV-1 infected cell lines, MT-2.6 and HUT-102 and STLV-1 infected lines Kia and Matsu. Applicability of this system was demonstrated by determining HTLV-1 proviral load in peripheral blood mononuclear cells (PBMC) of human subjects with HTLV-1 associated diseases and an asymptomatic carrier as well as rabbits infected experimentally. This qcPCR method, the first designed specifically for HTLV-1 and STLV-1, will provide an important tool for pathogenesis studies of HTLV-1 and for evaluating the efficacy of antiviral drugs and vaccines against the viral infection using animal models.

Alcaraz, C., F. Rodriguez, et al. (1995). "Highly specific confirmatory Western blot test for African swine fever virus antibody detection using the recombinant virus protein p54." Journal of Virological Methods **52**(1-2): 111.

<http://www.sciencedirect.com/science/article/B6T96-3YXC151-W/2/4eb43f695bf6fc6097bd9155e7b760e0>

A Western blot technique using a recombinant protein has been developed to confirm positive results obtained in African swine fever (ASF)-specific antibody detection by ELISA. The new confirmatory Western blot is based on the use of protein p54, one of the most antigenic ASF virus structural proteins, expressed in *Escherichia coli* fused to the N-terminus of MS2 polymerase. The recombinant Western blot assay was highly specific and equally sensitive for ASF virus-infected pigs detection as the conventional Western blot, which uses virus-induced proteins ranging in molecular weight between 23 and 35 kDa. The novel Western blot assay provides a simpler interpretation of the test, eliminates the possibility of false-positive reactions produced by cellular compounds that contaminate the antigen employed in the conventional technique, and avoids the use of live virus in antigen production.

Argaw, T., A. Ritzhaupt, et al. (2002). "Development of a real time quantitative PCR assay for detection of porcine endogenous retrovirus." Journal of Virological Methods **106**(1): 97.

<http://www.sciencedirect.com/science/article/B6T96-46TBF6J-H/2/ab392041d9a6b7f599146874bab1b8f6>

Real time PCR technology was applied to the development of assays for detection and quantitation of porcine endogenous retrovirus (PERV) RNA and DNA sequences in tissues and

cells of human or animal origin. A plasmid construct encoding the PERV-pol gene or the in vitro transcribed RNA derived from the plasmid (cRNA) serves as a standard template for amplification of a 178 bp fragment. This study showed that the detection of this target sequence was linear over a range from 20 copies to 2 million copies of the plasmid and from 100 copies to 1 million copies of the cRNA. In addition, amplification of the target sequence was not inhibited by the presence of exogenous genomic DNA. These results demonstrate that a real time (TaqMan-based) PCR or RT-PCR assay can provide a sensitive, reproducible, and robust method for detecting and quantifying PERV DNA or RNA sequences in samples of human or guinea pig origin.

Ault, G. S., C. F. Ryschkewitsch, et al. (1994). "Type-specific amplification of viral DNA using touchdown and hot start PCR." Journal of Virological Methods **46**(2): 145.

<http://www.sciencedirect.com/science/article/B6T96-476F69S-BD/2/caa598ec4d1318cace2bb44a0c627ea5>

Two types of JC virus (JCV) are found in infected brain and kidney tissues. A highly reliable PCR assay to determine viral type in tissue is presented. This type-specific system is analogous to allele-specific PCR used to detect point mutations in cellular genes. Specific amplification of two fragments, using four pairs of type-specific primers, is based on a single nucleotide difference at the 3'-ends of the primers. A combination of three conditions in the PCR reaction was required for specificity: 'hot start', a ramped ('touchdown') cycle profile, and a slightly lowered molar concentration of the specific primers and dNTPs. Efficient yield of PCR product is not lost under these conditions, and even the least selective mismatches (C:A and T:G) provided specific amplification. Type-specific restriction enzyme sites within the amplified fragments confirm type designation.

Avellon, A., P. Perez, et al. (2001). "Rapid and sensitive diagnosis of human adenovirus infections by a generic polymerase chain reaction." Journal of Virological Methods **92**(2): 113.

<http://www.sciencedirect.com/science/article/B6T96-42D81PP-2/2/540b7580d8d9f11b8c1865872705eb3c>

A new adenovirus specific nested polymerase chain reaction (PCR) method is described. It was designed inside the hexon protein gene of the adenovirus genome, and was able to detect DNA of all 47 human adenovirus types in a wide range of clinical samples. A sensitive internal control system able to assure proper analytical conditions for the amplification of as few as 100 molecules of a heterologous DNA was included to avoid false negative results. Sensitivity was estimated at about 10 molecules per tube of a plasmid containing an insert of the first amplification product. The method was able to detect adenovirus infection in 31/43 conjunctival scrapings from patients with acute kerato conjunctivitis 10/40 nasopharyngeal aspirates from patients admitted to hospital with acute respiratory disease and 2/26 urine samples from patients with haemorrhagic cystitis with better sensitivity than cell culture or rapid diagnosis by antigen detection by immunofluorescence (IF) in the case of respiratory specimens. Only two of 17 stools positive for a group F adenovirus specific latex immunoassay were PCR negative. The internal control system avoided a false negative result on another two stool samples. In conclusion, the method described below was shown to be useful for rapid diagnosis of adenovirus infections with higher sensitivity than antigen detection by IF.

Baines, J. E., R. M. McGovern, et al. (2005). "Consensus-degenerate hybrid oligonucleotide primers (CODEHOP) for the detection of novel papillomaviruses and their application to esophageal and tonsillar carcinomas." Journal of Virological Methods **123**(1): 81.

<http://www.sciencedirect.com/science/article/B6T96-4DNB3BK-2/2/42b0ea14114390dc20621805c2b654de>

Human papillomaviruses (HPVs) have been identified in 99% of cervical tumors. Considerable speculation exists about the role of HPV in cancer at other sites, such as skin, esophagus, and lung. One barrier to the study of HPV in extragenital tumors may be the inability to detect novel HPV types. Our objective was to design broad-range primers for detecting papillomaviruses from any group in the HPV Sequence Database. Complete L1 protein sequences were aligned and 11 blocks of conserved protein sequences were identified. Primers were designed in the corresponding nucleotide regions of five blocks. One pair of primers, ME and MH, derived from blocks E and H, appeared promising when compared with established consensus primers. To improve the sensitivity for detection of multiple papillomavirus types, ME/MH primers were split into several primers with the 5' regions matched to individual papillomavirus supergroups. Compared with the commonly used MY09/11 primer set, our primer sets were broader in range and more sensitive against most papillomavirus types tested. Application of these primers to esophageal and tonsillar carcinomas identified no novel HPV types but confirmed a high level of sensitivity for detecting HPV from these clinical samples. These primers should facilitate the search for novel papillomaviruses.

Barbezange, C. and V. Jestin (2002). "Development of a RT-nested PCR test detecting pigeon Paramyxovirus-1 directly from organs of infected animals." Journal of Virological Methods **106**(2): 197.

<http://www.sciencedirect.com/science/article/B6T96-46WSX8S-2/2/5213504c066b45784a6c0b93533807ca>

A RT-nested PCR that amplifies part of the conserved nucleoprotein gene of avian Paramyxovirus type 1 is described. The technique allowed the detection of pigeon Paramyxovirus type 1 (pPMV-1) virus directly from a wide range of infected chicken and pigeon organs, and should be able to detect typical Newcastle disease viruses too. Compared with the reference method, the developed RT-nested PCR was found more sensitive, as it was able to detect virus genome in infected pigeon organs at late stage of infection, when virus isolation failed. Such a molecular technique represents an alternative method of diagnosis for research purposes on pPMV-1 variants, for example to study pathogenesis aspects of the infection or to assess the efficacy of vaccines.

Barlic-Maganja, D. and J. Grom (2001). "Highly sensitive one-tube RT-PCR and microplate hybridisation assay for the detection and for the discrimination of classical swine fever virus from other pestiviruses." Journal of Virological Methods **95**(1-2): 101.

<http://www.sciencedirect.com/science/article/B6T96-433NRB5-B/2/a24675b98a618b42aeb421941d4670b5>

Rapid, sensitive and specific laboratory diagnostic methods are necessary to confirm outbreaks of classical swine fever. The detection of classical swine fever virus (CSFV) and its discrimination from other pestiviruses can be achieved by virus isolation on cell culture, antigen detection, or molecular methods. To reduce the time and the number of steps in the diagnostic procedure a

sensitive and rapid detection method based on specific amplification of the pestiviral RNA by one-step reverse transcription-polymerase chain reaction (RT-PCR) followed by detection and differentiation of the amplification products by pestivirus-, bovine viral diarrhoea virus- (BVDV-) and CSFV-specific capture probe hybridisation and colorimetric assay in microwell plates (enzyme linked immunosorbent assay (ELISA)) was developed. Two different methods using two gene regions for pestivirus RT-PCR amplification were carried out. One pair of primers was selected from the 5'-UTR region and the second one from the gene region coding for Npro, C and E0 proteins. The designed oligonucleotide primers were used for several pestivirus reference strains as well as for some field isolates detection in cell culture supernatants and in clinical specimens. The specificity and sensitivity of both methods were compared using EZ rTth RNA PCR kit and ACCESS RT-PCR system for combined RT-PCR assay. The use of one-step RT-PCR eliminates the additional manipulations that are generally required for a two reaction system and limits the risk of carry-over contamination. Labelling of PCR products with digoxigenin (DIG) during the amplification reaction enables colorimetric assessment of hybridisation reactions. For solution hybridisation pestivirus-, BVDV- and CSFV-specific biotin-labelled capture probes were used. By serial dilutions of DIG-labelled PCR products the RT-PCR-ELISA was found to be 100-times more sensitive than the conventional agarose gel electrophoresis. Higher sensitivity of RT-PCR-ELISA detection using specific biotin-labelled probes offers the opportunity to eliminate strain specific nested PCR and to overcome the problems with contamination and false positive results.

Barlough, J., N. East, et al. (1994). "Double-nested polymerase chain reaction for detection of caprine arthritis-encephalitis virus proviral DNA in blood, milk, and tissues of infected goats." Journal of Virological Methods **50**(1-3): 101.

<http://www.sciencedirect.com/science/article/B6T96-47DKYF8-C/2/56659fd9129ad03f9351de6729c6c776>

A nested polymerase chain reaction (PCR) for detecting proviral DNA of caprine arthritis-encephalitis virus (CAEV) in biological samples was developed. Primers for both gag and pol sequences of the CAEV genome were included in a single tube for simultaneous amplification ('double' PCR), and the resulting bands were resolved visually in ethidium bromide-stained agarose gels. Internal gag and pol probes were used to verify the identity of the amplified products by non-radioactive Southern hybridization. Final confirmation of the identity of representative PCR bands was provided by DNA sequence analysis. A comparison between the PCR and an antibody ELISA (with recombinant CAEV p28 as target) using 141 caprine blood samples indicated very strong agreement between the two assays ($\kappa = 0.912$). Four of 7 goats with indeterminate ELISA results were PCR-positive as were 5 of 40 (12.5%) seronegative goats, most probably indicating delayed seroconversion. Eleven of 27 goats (41%) PCR-positive on blood had detectable CAEV proviral DNA in milk. Proviral DNA was also detected in lung, mesenteric lymph node, bone marrow, synovial membrane, and mammary gland of a seropositive, clinically affected goat, but not in equivalent tissues of a healthy seronegative goat.

Beaulieux, F., M. M. Berger, et al. (2003). "RNA extraction and RT-PCR procedures adapted for the detection of enterovirus sequences from frozen and paraffin-embedded formalin-fixed spinal cord samples." Journal of Virological Methods **107**(2): 115.

<http://www.sciencedirect.com/science/article/B6T96-47DTDTF-3/2/2adec925f3d102f950d4be0508ecb015>

Methods for detecting enterovirus RNA in both paraffin-embedded, formalin-fixed and frozen spinal cord sections from amyotrophic lateral sclerosis (ALS) patients were established. A

proteinase K digestion following the deparaffinization procedure was required for the fixed spinal cord sections, whereas only one step of crushing in phosphate buffered saline was necessary for the frozen samples prior to the extraction of the RNA. With an optimized reverse transcription and PCR procedure, enterovirus RNA could be detected from frozen and fixed archival spinal cord samples.

Bellau-Pujol, S., A. Vabret, et al. "Development of three multiplex RT-PCR assays for the detection of 12 respiratory RNA viruses." Journal of Virological Methods **In Press, Corrected Proof**
<http://www.sciencedirect.com/science/article/B6T96-4FJTNTM-2/2/0ba5a40b32fe1ff28df50d75b2fab22a>

Three multiplex hemi-nested RT-PCR assays were developed to detect simultaneously 12 RNA respiratory viruses: influenza viruses A, B and C, human respiratory syncytial virus (hRSV), human metapneumovirus (hMPV), parainfluenza virus types 1-4 (PIV-1, -2, -3 and -4), human coronavirus OC43 and 229E (HCoV) and rhinovirus (hRV). An internal amplification control was included in one of the RT-PCR assays. The RT-PCR multiplex 1 and the hemi-nested multiplex 1 detected 1 and 0.1 TCID₅₀ of RSV A, respectively, and 0.01 and 0.001 TCID₅₀ of influenza virus A/H3N2, respectively. Two hundred and three nasal aspirates from hospitalised children were retrospectively tested in comparison with two conventional methods: direct immunofluorescence assay and viral isolation technique. Almost all samples (89/91) that were positive by immunofluorescence assay and/or viral isolation technique were detected by the multiplex assay. This method also detected an additional 85 viruses and 33 co-infections. The overall sensitivity (98%), rapidity and enhanced efficiency of these multiplex hemi-nested RT-PCR assays suggest that they would be a significant improvement over conventional methods for the detection of a broad spectrum of respiratory viruses.

Belliot, G. M., R. L. Fankhauser, et al. (2001). "Characterization of 'Norwalk-like viruses' and astroviruses by liquid hybridization assay." Journal of Virological Methods **91**(2): 119.

<http://www.sciencedirect.com/science/article/B6T96-426XYG8-3/2/ee396f5d131df9dded538957d49b01ce>

'Norwalk-like viruses' (NLVs) and human astroviruses are causative agents of gastroenteritis in all age-groups. The typing of these agents is generally done by nucleotide sequencing, blot hybridization, or enzyme immunoassay. These techniques are expensive, time-consuming, and sometimes require scarce reagents, which limits the typing of NLVs and astroviruses to a few reference laboratories. This report describes a liquid hybridization assay that uses broadly reactive probes whose sequences are based on data from specimens in collections available at CDC and GenBank. Two astrovirus genogroup-specific probes were designed and tested successfully on 26 wild strains from all serotypes. Fourteen GII and 16 GI representative NLV strains were typed without cross-hybridization by using P1B- and P2A-specific probes, described previously, and new P2B- and P1A-specific probes. Analysis of the specificity of the probes, the effect of the mismatches during hybridization, and the sensitivity of hybridization assay demonstrates this method to be a rapid and simple technique for molecular typing of NLVs and preliminary characterization of astroviruses.

Bennett, J. M., S. Kaye, et al. (1999). "A quantitative PCR method for the assay of HIV-1 provirus load in peripheral blood mononuclear cells." Journal of Virological Methods **83**(1-2): 11.

<http://www.sciencedirect.com/science/article/B6T96-3XVPG88-2/2/2e45f7793c78e80a591462826d37427c>

The use of high activity antiretroviral therapies (HAART) to treat HIV-infected patients frequently results in the long-term suppression of plasma virus RNA loads below levels detectable by current assays. The measurement of provirus DNA load in peripheral blood mononuclear cells provides a means of continuing to monitor the efficacy of treatment and the decline in reservoirs of latent virus. A quantitative PCR assay was developed for HIV-1 provirus using a three-point internal calibrator system to give high reproducibility and accuracy at the low copy numbers of provirus seen in clinical samples. Provirus DNA copies are related to cell number in the samples using a fluorescent dye-binding assay for measurement of input DNA. The assay agreed closely with an end-point dilution PCR and gave accurate quantification of extracts from an HIV-1 infected continuous cell line containing known provirus copy numbers. The inclusion of a second primer set in the LTR region of the HIV-1 genome, optimised to non-clade-B virus strains improved the detection and quantification of samples from patients infected with genetically divergent virus strains. Application of the assay to clinical trial patients showed no relationship between changes in provirus DNA loads and plasma virus RNA and changes in provirus load over 24 weeks were small.

Bettinger, D., C. Mougin, et al. (1994). "Rapid detection of active cytomegalovirus infection by in situ polymerase chain reaction on MRC5 cells inoculated with blood specimens." Journal of Virological Methods **49**(1): 59.

<http://www.sciencedirect.com/science/article/B6T96-476F6X3-KS/2/4277fbf8076a971214cbc06ee1d9a158>

An in situ polymerase chain reaction was developed to amplify immediate early genes of human cytomegalovirus in cells cultured in a 96 well plate and infected with leukocytes. The technical parameters enabling optimal detection of the DNA sequences were defined. The key to this method is the fixation of cells, which facilitates the access of the PCR mixture into the cell nuclei and preserves cell morphology. Such a technique could have wide application for the detection and identification of other infectious viruses in cultured cells very early after inoculation of clinical samples.

Beuret, C. (2004). "Simultaneous detection of enteric viruses by multiplex real-time RT-PCR." Journal of Virological Methods **115**(1): 1.

<http://www.sciencedirect.com/science/article/B6T96-49V3JJG-1/2/1cf1f7ec80bbf36619d849b77af86662>

A multiplex real-time RT-PCR protocol for the simultaneous detection of noroviruses ("Norwalk-like viruses") of genogroups I and II, human astroviruses and enteroviruses is described. The protocol was developed and evaluated using the LightCycler(TM) and corresponding SYBR Green reagents. New primers were designed within conserved genome regions to optimize the detection range of virus subtypes of each genus. To enable the development of a multiplex PCR assay within one tube (capillary), similar mastermix- and cycling-conditions were respected for each individual primer system. Subsequent melting curve analysis allowed the determination of possible dual-contaminations of entero- and noro- or astroviruses by the formation of dual peaks. Special care was taken to minimize the loss of sensitivity, since the detection of small viral contaminations is a crucial parameter especially for food analysis. The multiplex assay was compared successfully to the single SYBR Green assay, and revealed to be at least 10 times

more sensitive than the one obtained with an endpoint PCR thermocycler protocol published previously.

Binder, T., W. Siegert, et al. (1999). "Identification of human cytomegalovirus variants by analysis of single strand conformation polymorphism and DNA sequencing of the envelope glycoprotein B gene region-distribution frequency in liver transplant recipients." Journal of Virological Methods **78**(1-2): 153.

<http://www.sciencedirect.com/science/article/B6T96-3W07P76-G/2/dc26e56fe1796ed67a375466047c1a9e>

Single strand conformation polymorphism analysis (SSCP) of PCR-amplified DNA and subsequent DNA sequencing of human cytomegalovirus (HCMV) glycoprotein B (gB) gene were applied to identify known HCMV strains and to detect new virus variants. 61 HCMV PCR positive patients were studied out of a cohort of 410 patients after liver transplantation (LTX). SSCP was able to distinguish between strains Davis, AD169, and Towne, and in addition could identify five new virus variants (Berlin B, C, E, F, and H). Their frequency, gB and gH types were determined. Simultaneous infections with two or three strains or variants, as well as a switch from one virus to another virus were observed during long-term follow-up. No correlation between the occurrence of certain virus strains or gB types and defined clinical manifestations of HCMV infection after LTX was drawn.

Black, E. M., J. P. Lowings, et al. (2002). "A rapid RT-PCR method to differentiate six established genotypes of rabies and rabies-related viruses using TaqMan(TM) technology." Journal of Virological Methods **105**(1): 25.

<http://www.sciencedirect.com/science/article/B6T96-45TTWC2-4/2/84fb96b7a1635f9735ea69436c30ec10>

A rapid and sensitive reverse transcriptase-polymerase chain reaction (RT-PCR) assay incorporating TaqMan(TM) probes has been developed that can distinguish among the six established rabies and rabies-related virus genotypes. TaqMan(TM) probes were designed and validated against 106 rabies and rabies-related virus isolates, one isolate of the Australian bat Lyssaviruses (genotype 7), and 18 other non-rabies viruses important in the veterinary field. The N gene was used as the target for the probes as it is well conserved and has been intensively used to genotype rabies isolates. Additionally, it was found to contain regions specific to each genotype conducive to probe design. The RT-PCR assay described amplifies a portion of the nucleoprotein gene of all 107 rabies and rabies-related viruses, but none of the other viruses tested. Inclusion of TaqMan(TM)-genotype-specific probes in the RT-PCR assay permits rapid identification of the virus present. By combining RT-PCR with TaqMan(TM) genotyping probes suspect rabies virus isolates can be identified in a single closed tube system that prevents potential PCR-product carry over contamination.

Bockstahler, L. E., P. G. Carney, et al. (1992). "Detection of Junin virus by the polymerase chain reaction." Journal of Virological Methods **39**(1-2): 231.

<http://www.sciencedirect.com/science/article/B6T96-476F6MB-HT/2/d5e47bbe329918cde17901be1436f7dd>

Argentine hemorrhagic fever is an often fatal human disease caused by Junin virus, an RNA-containing virus and member of the Arenavirus family. This virus was detected in vitro by the polymerase chain reaction (PCR) procedure. A pair of Junin virus-specific PCR DNA oligonucleotide primers and an oligonucleotide probe were designed from a known portion of the viral RNA sequence. RNA was isolated from Junin virus-infected monkey kidney cells and used to produce complementary DNA (cDNA) by reverse transcription. A DNA segment, 151 +/- 24 bp long, was amplified from this cDNA and characterized by agarose gel electrophoresis and Southern blot hybridization with the Junin virus-specific DNA probe. Sensitivity experiments showed that Junin virus could be detected with nanogram quantities of RNA isolated from virus-infected cells. The rapid and sensitive assay described here may contribute towards the development of a procedure for the early diagnosis of Argentine hemorrhagic fever.

Boerner, B., W. Weigelt, et al. (1999). "A sensitive and specific PCR/Southern blot assay for detection of bovine herpesvirus 4 in calves infected experimentally." *Journal of Virological Methods* **83**(1-2): 169.

<http://www.sciencedirect.com/science/article/B6T96-3XVPG88-M/2/7c0dfd4d4d7210d5a3602dbff0b6e9e>

A PCR/Southern blot assay for detection of bovine herpesvirus 4 (BHV-4) in the background of bovine cellular DNA was developed. A BHV-4 specific sequence within the gene coding for the glycoprotein B (gB) was selected for primer sequences to guarantee the specificity of the assay. With a detection limit of six molecules BHV-4 DNA in the background of 1 [mu]g of cellular DNA (equals about 150000 bovine cells) this PCR/Southern blot assay represents a highly sensitive method for detection of BHV-4 DNA. At low concentrations of BHV-4 genomes, this assay also allows to estimate the copy number of BHV-4: a distinction between fewer than 6, 6-59 and more than 60 BHV-4 genomes/100 [mu]l DNA suspension was possible. Tissue and blood samples of two calves, infected experimentally with BHV-4 were examined for the prevalence of BHV-4 DNA 130 days post infection. Ten days before taking samples, one of the calves was immuno-suppressed with dexamethasone. In both calves, BHV-4 DNA was detected in the leucocyte fraction of the blood, and beyond that in lower quantities in the spleen and the kidney of the immuno-suppressed calf. It is assumed that a latent BHV-4 infection was activated after application of dexamethasone and that the leucocyte fraction of the blood represents one site of latency of BHV-4 in cattle.

Boni, J. and J. Schupbach (1993). "Primer extension analysis provides a sensitive tool for the identification of PCR-amplified DNA from HIV-1." *Journal of Virological Methods* **42**(2-3): 309.

<http://www.sciencedirect.com/science/article/B6T96-476RM72-2J/2/16688fe329aa2ea32a615cf7e04e8d9a>

Primer extension analysis was evaluated as a means to identify PCR-amplified DNA from HIV-1. Solution hybridization with radioactive labeled oligonucleotides followed by an extension reaction with Klenow fragment of Escherichia coli DNA polymerase I and subsequent separation on denaturing polyacrylamide gels reveals single stranded DNA products of the predicted size. The specificity of the reaction is further demonstrated by specific endonuclease digestion. The analysis is more sensitive than Southern blotting and about equally sensitive as Slot blot analysis when double-stranded DNA probes are used for hybridization. With end-labeled oligonucleotide probes, primer extension analysis proved an order of magnitude more sensitive than membrane hybridization. The analysis also allows quantitation of amplified DNA from 1 pg to about 1 ng of DNA product. Under the conditions described for amplification, primer extension analysis is capable of detecting a single HIV-1 plasmid DNA molecule in the presence of 1 [mu]g of total

DNA. 3'-end mismatching of the oligonucleotide probe does not result in a significantly altered detection limit. Primer extension analysis can also be carried out with at least three different PCR-amplified DNAs in the same reaction tube.

Boom, R., C. Sol, et al. (2000). "Detection and quantitation of human cytomegalovirus DNA in faeces." Journal of Virological Methods **84**(1): 1.

<http://www.sciencedirect.com/science/article/B6T96-3Y21VDG-1/2/9c147c239ca178c36e64f171c29e57b8>

The development and performance of a robust and sensitive PCR assay are described for the detection and quantitation of human cytomegalovirus DNA in human faecal specimens. In this assay, CMV DNA was purified by an optimised DNA extraction protocol together with internal control DNA that monitored both DNA extraction efficiency and PCR efficiency. The lower detection limit of the assay was reached at about 100 CMV particles per ml of (25-50%) faecal suspension. CMV DNA could be quantitated in the range of about 300-100000 molecules per ml of faecal suspension. CMV DNA loads obtained in clinical faeces specimens suggest that the assay can be used to monitor the efficacy of antiviral treatment. Reconstruction experiments that monitored the efficiency of DNA extraction of a preliminary DNA extraction protocol, showed low DNA yields for 9% of the specimens (n=78). In all cases, low DNA extraction efficiency seemed to be due to a component present in faeces that prevented DNA binding to silica particles, presumably by competitive binding. Choosing the right ratio of silica particles to faeces specimen solved this problem. Similarly, reconstruction experiments showed that the strong PCR inhibition that was observed in 8% of the specimens could effectively be relieved by the inclusion of [alpha]-casein in the PCR mixtures.

Boonham, N., L. G. Perez, et al. (2004). "Development of a real-time RT-PCR assay for the detection of Potato spindle tuber viroid." Journal of Virological Methods **116**(2): 139.

<http://www.sciencedirect.com/science/article/B6T96-4B958S0-1/2/960177b13ab6d627eae729d0be24bf0>

Potato spindle tuber viroid (PSTVd) is a quarantine pathogen in the European Union and causes damaging diseases of solanaceous crops. Under the EU Plant Health directive 2000/29/EC, countries must have the ability to detect and identify accurately and rapidly the introduction of harmful organisms in plants or plant products; furthermore, if the quarantine pathogen is found, be able to survey extensively for it. In this respect, PSTVd poses an interesting technical problem, since its RNA does not code for any proteins and thus any diagnostic method must be based on the detection of the RNA and be suitable for scaling up to testing large sample numbers. With this in mind a one-tube real-time RT-PCR assay based on TaqMan(TM) chemistry was developed. Investigations were carried out into various aspects of the assay relevant to the efficient amplification of targets that have a significant amount of secondary structure such as viroids. Thus comparisons were made of reverse transcription temperature, concentration and type of reverse transcriptase, RNA denaturation, sample purity and single versus two-tube reaction format. The assay developed was shown to be able to detect a wide range of isolates of PSTVd and in comparison with a chemi-luminescent hybridisation system was shown to be 1000-fold more sensitive. A further significant advantage of this assay format compared with hybridisation is that it is suitable for scaling up to large sample numbers using robotic liquid handling systems.

Bootman, J. S. and P. A. Kitchin (1992). "An international collaborative study to assess a set of reference reagents for HIV-1 PCR." Journal of Virological Methods **37**(1): 23.

<http://www.sciencedirect.com/science/article/B6T96-476F5XR-52/2/e431ef2b94b8d76f1b0e7033094e40ba>

An international collaborative study was performed to evaluate a set of PCR reference reagents for HIV diagnosis. Twenty-six laboratories from 9 countries analysed a proficiency panel of 10 coded DNA samples using the PCR reference reagents and protocols. For comparison, these coded samples were then assessed using a laboratory's own 'in-house' reagents and methodologies. The objectives of the study were: (i) to assess inter-laboratory variation of PCR sensitivity, (ii) to evaluate the DNA 'carryover' problem and frequency of false negative results and (iii) to examine the utility of the complete set of reagents and templates to act as reference preparations for HIV PCR. Using the reference reagents, 46% of laboratories reported no false positive results in any of their assays of the negative controls. The remaining laboratories all reported a false positive result(s) in at least one assay. The overall false positive result rate for the study was 9.3%. In contrast, an overall false negative result rate of 7.4% was observed, with some laboratories recording negative results even for samples containing 10000 molecules of target DNA. The level of absolute sensitivity may be assessed accurately only from the 12 laboratories that obtained no false positive results. All 12 laboratories detected the sample containing 10 molecules of template DNA and 9 out of the 12 laboratories detected the sample containing 1 molecule. This is in close agreement with the theoretical detection rate based on a statistical probability model for the detection of a single molecule. These characterised reference reagents were at least as sensitive as any of the 'in-house' reagents and methodologies applied, including nested PCR. The complete set of characterised reference reagents is now available for quality control assessment of HIV-1 PCR from the MRC ADP.

Boriskin, Y. S., J. C. Booth, et al. (1993). "Rapid detection of mumps virus by the polymerase chain reaction." Journal of Virological Methods **42**(1): 23.

<http://www.sciencedirect.com/science/article/B6T96-47YGSVX-4/2/03c0453462ef9d8a8a4d7e2087948693>

A procedure for detecting mumps virus in under 48 h was developed using the PCR. The sensitivity of the PCR amplification reaction and of the detection of the PCR product was significantly improved by: (i) enriching for viral template RNAs by overnight culture of the virus in Vero cells and (ii) substitution of polyacrylamide gel analysis for agarose gel electrophoresis. The technique was capable of detecting 1-20 infectious units of virus or an equivalent of 1-10 pg of mumps virus-specific plasmid DNA.

Briddon, R. W., A. G. Prescott, et al. (1993). "Rapid production of full-length, infectious geminivirus clones by abutting primer PCR (AbP-PCR)." Journal of Virological Methods **43**(1): 7.

<http://www.sciencedirect.com/science/article/B6T96-476F666-8K/2/68fb7c8742f2e6189b54c24e8d228262>

The application of the polymerase chain reaction (PCR) method of DNA amplification for the isolation of full-length, infectious clones of geminiviruses is described. Non-overlapping, abutting 20-mer oligonucleotide primers were used to produce a linear product from the circular geminivirus genomic template. Clones of African cassava mosaic virus (ACMV) DNA A, obtained by this method, were infectious following mechanical inoculation (in the presence of ACMV DNA

B) onto *Nicotiana benthamiana*. Normal ACMV symptoms resulted and typical geminate viral particles were detected by electron microscopy. The use of PCR for the detection and production of full-length, infectious geminivirus clones is discussed.

Bruni, R., C. Argentini, et al. (1995). "A PCR-based strategy for rapid mapping of hepadnavirus integrated sequences in hepatocellular carcinomas." Journal of Virological Methods **52**(3): 347.

<http://www.sciencedirect.com/science/article/B6T96-3YXC14C-C/2/5aef364fd8afd7ef3fce0500c4187b0a>

A methodology based on polymerase chain reaction (PCR) and restriction analysis for rapid mapping of woodchuck hepatitis virus (WHV) integrations in hepatocellular carcinoma (HCC) tissues is described. Conventional PCR with viral primer pairs is not suitable for mapping WHV-integrated regions because the presence of minimum amounts of non-integrated (PCR amplifiable) WHV genome and replicative intermediates cannot be excluded. The first relevant part of the strategy is the identification of the cellular sequences flanking the WHV integration in order to select one (or more) integration-specific primer. The cellular flanking sequence can be rapidly obtained by means of inverse-PCR amplification of the viral/cellular junction and sequencing of the product. Mapping of the integrated regions is carried out by fixed flanking primer PCR (FFP-PCR) using the cellular primer as a 'fixed' primer in PCR in association with each of an available set of WHV primers. Amplification of episomal WHV sequences is thus avoided. PCR products can also undergo restriction analysis. PCR-positive viral primers and specific WHV restriction sites are assembled into a map, based on the size and restriction pattern of the PCR products. The results of WHV integration mapping in a woodchuck HCC are described.

Cameron-Wilson, C. L., P. Muir, et al. (2003). "Evaluation of a line probe assay for identification of hepatitis B virus precore variants in serum from chronic hepatitis B carriers." Journal of Virological Methods **114**(1): 97.

<http://www.sciencedirect.com/science/article/B6T96-49SWFWP-7/2/bde013aadafe321e685e3e429fb19b76>

A prototype line probe assay (LiPA) for identifying hepatitis B virus (HBV) precore variants (INNO-LiPA HBV precore) was evaluated using a panel of 50 sera from 46 patients with HBV infection. The assay detected sequence variations detected commonly in the precore promoter region and in amino acid codons 28 and 29 of the precore gene. There was strong agreement between INNO-LiPA HBV precore results and those of a codon 28 point mutation assay (PMA), with identical results obtained in 40 of 43 sera (93%) typeable by both assays (kappa coefficient ([kappa])=0.90). In addition, the precore codon 29 sequence identified by the INNO-LiPA HBV precore was confirmed by nucleotide sequencing in all seven samples analysed. However, the INNO-LiPA HBV precore identified precore promoter sequences much less efficiently. The prototype assay could identify codon 28/29 sequences from as little as 10 HBV genome equivalents in 10 [μl] serum, and in experiments using artificially prepared mixtures of variants could identify a minor component constituting 2.5% of the total viral DNA population. The INNO-LiPA HBV precore was also straightforward technically and rapid, and is therefore likely to be useful for epidemiological investigations into the prevalence, distribution and clinical significance of HBV precore variants.

Casas, I., A. Tenorio, et al. (1997). "Detection of enteroviral RNA and specific DNA of herpesviruses by multiplex genome amplification." Journal of Virological Methods **66**(1): 39.

<http://www.sciencedirect.com/science/article/B6T96-3RYCMYM-18/2/a8b89a2b6019a196e061ff4c236aae9a>

A reverse transcription (RT) multiplex polymerase chain reaction (PCR) assay was developed to allow rapid, sensitive and simultaneous detection of enteroviral RNA and herpesviral DNA specific sequences in a single tube. The method involves a reverse transcription step followed by a multiplex nested PCR in which the combination of primers amplifies cDNA from enteroviruses and specific herpesviruses DNA. Nested amplification utilises primers designed to anneal into the amplification product from the first reaction. Individual viruses were then detected and differentiated by the size of their PCR products determined using ethidium bromide stained agarose gels. To exclude false negatives due to sample inhibitors an internal amplification control, a cloned fragment of DNA from Pseudorabies virus (PRV DNA) was included in the reaction mixture. Detection levels between 0.01 and 0.001 TCID₅₀ of prototype strains of Polio and Coxsackie type B viruses and between 1 and 100 molecules of cloned-DNA of herpesviruses prototype strains were achieved. The RT multiplex PCR method proved capable of detecting enteroviral RNA or herpesviral DNA in cerebro spinal fluid (CSF) samples from patients with aetiologically well characterized encephalitis or aseptic meningitis.

Chatellard, P., R. Sahli, et al. (1998). "Single tube competitive PCR for quantitation of CMV DNA in the blood of HIV+ and solid organ transplant patients." Journal of Virological Methods **71**(2): 137.

<http://www.sciencedirect.com/science/article/B6T96-3VCVDKK-1/2/b46f708cdced2d2252040882fee76527>

Cytomegalovirus (CMV) infection is a major cause of morbidity and mortality in transplant and HIV-infected patients. However, CMV can also cause asymptomatic infection. An elevated blood viral load as assessed by various methods appears to be a predictor for symptomatic infections, and can be used to identify patients at the highest risk of developing CMV disease. We developed a single tube competitive quantitative PCR assay for CMV DNA, using as a competitor a plasmid carrying the target sequence for amplification with an internal deletion. The analysis of data from repeated extractions and amplifications of samples showed that the coefficient of variation of the assay was typically less than 20%. Clinical samples from 14 HIV-infected and 13 solid organ transplant patients were analyzed. Widely varying CMV DNA levels were found in leukocytes, with a positive correlation with the measure of infectivity in the leukocytes by quantitative culture on fibroblasts. The highest CMV DNA content in leukocytes was found in two patients with presumptive CMV disease. In HIV patients, the amount of DNA in leukocytes was much larger than in solid organ transplant recipients, when standardized for infectivity. Although based on a very limited number of patients, this observation probably points to a difference in the biology of CMV infection in these two categories of susceptible individuals. CMV DNA was also found in the plasma of some of the patients with a high CMV DNA leukocyte load. The present test should be useful for identifying patients at high risk of developing CMV disease, for monitoring therapeutic efficacy of antiviral regimens and to improve the understanding the pathogenesis of CMV infection.

Chimside, E. D. and W. J. M. Spaan (1990). "Reverse transcription and cDNA amplification by the polymerase chain reaction of equine arteritis virus (EAV)." Journal of Virological Methods **30**(2): 133.

<http://www.sciencedirect.com/science/article/B6T96-476KWJH-34/2/c882ab4e649025070a2e728af800f2c8>

A technique is described for the amplification and specific identification of equine arteritis virus (EAV) nucleotide sequences. The polymerase chain reaction (PCR) was evaluated initially by amplification of cloned virus specific cDNA sequences prior to amplification of single-stranded (ss) cDNA produced by reverse transcription (RT) of viral genomic RNA. Three separate primer pairs were used for RT/PCR of EAV genomic RNA, each pair producing only one band in agarose gels of the predicted size from the genomic nucleotide sequence. The viral origin of cDNA products was confirmed by hybridisation analysis with EAV-specific probes. RT/PCR analysis of clinical material indicates the methodology is sensitive enough to detect 600 pfu/ml EAV in seminal plasma.

Chin Yuan, C., W. Miley, et al. (2001). "A quantification of human cells using an ERV-3 real time PCR assay." Journal of Virological Methods **91**(2): 109.

<http://www.sciencedirect.com/science/article/B6T96-426XYG8-2/2/c6f338eb172e5c82ee0b71a1554d9fde>

A novel approach to quantifying human cells using a real time PCR assay was developed. The target sequence used in the assay is a 135 bp segment within the unique 1.7 kb Hind III / Pst I fragment of the ERV-3 envelope gene. ERV-3 is a full-length human endogenous retrovirus present in known copy number in all human cells. The detection range of ERV-3 by real time PCR is from 10⁶ to 10¹. The precision described, sensitivity and specificity of the assay indicate that the ERV-3 sequence is an accurate cell quantitation marker. The quantitative ERV-3 assay enables simple, fast, and reproducible detection and quantitation of the cell number. The assay can be used to determine the sample DNA conditions and also it can be used to adjust the quantitative DNA measurements of other target gene assays relative to the number of cell equivalents.

Contorni, M. and P. Leoncini (1993). "Typing of human papillomavirus DNAs by restriction endonuclease mapping of the PCR products." Journal of Virological Methods **41**(1): 29.

<http://www.sciencedirect.com/science/article/B6T96-476RMYJ-6C/2/8e2abca7118a98dc596152c434d950f0>

The polymerase chain reaction (PCR) for the diagnosis of human papillomavirus (HPV) infections, and in particular for the study of cervical HPV-associated lesions, is used widely. We identified a novel set of universal primers that are able to amplify a fragment spanning the E1 open reading frame (ORF) from different mucosotropic HPV types. A restriction endonuclease digestion of the amplified products is suggested for accurate typing. In particular, AluI digestion of the amplified fragments yields a distinctive fragment pattern for each 'high-risk' (16, 18, 31 and 33) HPV sequence, thus distinguishing them from 'low-risk' (6b and 11) HPV sequences.

Davidson, I. and M. Malkinson (1996). "A non-radioactive method for identifying enzyme-amplified products of the reticuloendotheliosis proviral env and LTR genes using psoralen-biotin labelled probes." Journal of Virological Methods **59**(1-2): 113.

<http://www.sciencedirect.com/science/article/B6T96-3VYV049->

V/2/53bd806cd3e82f6562a721458a51bdaf

A novel polymerase chain reaction (PCR) system based on the env gene of reticuloendotheliosis virus (REV) strain REV-A for the detection of proviral DNA is described. The designed PCR product of 807 bp was identified using an internal probe of 278 bp produced by nested PCR from REV-infected DNA CEF. The env-gene PCR was then compared with the previously described PCR for proviral REV-long terminal repeat and the PCR product served also as the probe. The probes were labelled with the psoralen-biotin system by photoactivation and the southern blot hybridization signal was detected colorimetrically. The advantages of using a non-radioactive means of probe labelling were demonstrated clearly in that study, as well as the effective labeling of probes with psoralen-biotin and the simple colorimetric method of detection. The env-gene PCR detected all eleven REV strains used in the study. These included three REV prototype strains and eight Israeli REV isolates. Both PCR systems had similar levels of sensitivity.

Desario, C., N. Decaro, et al. "Canine parvovirus infection: Which diagnostic test for virus?" Journal of Virological Methods **In Press, Corrected Proof**
<http://www.sciencedirect.com/science/article/B6T96-4FRHD09-1/2/6391ff1332d2ceb835611b30a558f7db>

Five laboratory tests for diagnosis of canine parvovirus type 2 (CPV-2) infection were employed to test 89 faecal samples collected from dogs with diarrhoea. The tests analysed were immunochromatography (IC), haemagglutination (HA), virus isolation (VI), conventional and real-time PCR. IC, HA, VI and conventional or real-time PCR were able, respectively, to detect CPV-2 antigen or nucleic acid in 41, 50, 54, 68 and 73 of the samples. The best correlation was found between conventional and real-time PCR, with an overall agreement of 94.38%. Sixty-eight samples that tested positive by HA, VI or conventional PCR were subjected to antigenic and/or genetic analyses of the CPV-2 strains by monoclonal antibody (MAb), restriction fragment-length polymorphism (RFLP) and/or sequence analyses. In sum, out of the 68 strains analysed, 26 were characterised as CPV-2a, 18 as CPV-2b and 24 as a CPV-2 Glu-426 mutant recently identified in Italy.

Dewhurst-Maridor, G., V. Simonet, et al. (2004). "Development of a quantitative TaqMan RT-PCR for respiratory syncytial virus." Journal of Virological Methods **120**(1): 41.

<http://www.sciencedirect.com/science/article/B6T96-4CHGDDP-1/2/3ff11d621d2f4d6514dcece3411c97ec>

Respiratory syncytial virus (RSV) is a ubiquitous RNA virus of the family Paramyxoviridae that may interfere with graft tolerance and with other interstitial lung diseases. The low viral titre observed in the immunodeficient transplanted patients requires a highly sensitive detection method. Although different tests already exist for the detection of RSV, reverse transcription-polymerase chain reaction (RT-PCR) has been shown to have the best sensitivity. In this study, a SYBR Green assay was established for the detection of RSV A and RSV B in a common screening test, and two quantitative TaqMan RT-PCRs were developed to quantify both RSV subgroups separately. Standard dilutions obtained from RSV cell infections were included in each test, and the assay was normalised using a housekeeping gene. RSV was found in 16% of the transplanted patients tested. The quantitative TaqMan assay is fast, reproducible, specific and very sensitive, and could facilitate considerably the detection of RSV virus. This would in-turn facilitate studies on the role of RSV in graft rejection.

A simple reverse transcription-polymerase chain reaction (RT-PCR) procedure for the detection of GB virus C (GBV-C) RNA in serum or plasma is described. In this method, total nucleic acid, extracted from a small volume of human plasma, is reverse transcribed using random hexamers. An aliquot of cDNA is then utilized in PCR employing GBV-C specific primers designed to highly conserved regions of the 5'nontranslated region (NTR). For additional sensitivity, a second round

of nested amplification is performed. Reactions are analyzed on an agarose gel and samples showing an ethidium bromide stained band of the appropriate size in the first and second amplification, or in the second amplification only, are designated to be positive. This protocol allows for the rapid and sensitive detection of GBV-C infection in human plasma or serum.

Erker, J. C., S. M. Desai, et al. (1999). "Rapid detection of Hepatitis E virus RNA by reverse transcription-polymerase chain reaction using universal oligonucleotide primers." Journal of Virological Methods **81**(1-2): 109.

<http://www.sciencedirect.com/science/article/B6T96-3XMPJNX-G/2/6e14405581559722cb6294105d75e4ab>

A rapid reverse transcription-polymerase chain reaction (RT-PCR) procedure for the detection of Hepatitis E virus (HEV) RNA in serum is described. Total nucleic acids are extracted from a small volume of human serum and reverse transcribed using random hexamers. An aliquot of cDNA is then utilized in nested PCR employing degenerate HEV consensus primers. These primers are designed to sequences conserved between the Burma, Mexico, and US HEV strains, generating amplicons within each of the three open reading frames. Reactions are analyzed by agarose gel electrophoresis and samples showing an ethidium bromide stained band of the appropriate size in the first and second amplification, or in the second amplification only, are designated as positive. This protocol allows for the rapid and sensitive detection of HEV infection in human serum.

Eun, A. J.-C., M.-L. Seoh, et al. (2000). "Simultaneous quantitation of two orchid viruses by the TaqMan(R) real-time RT-PCR." Journal of Virological Methods **87**(1-2): 151.

<http://www.sciencedirect.com/science/article/B6T96-40GJ2JM-J/2/4f08efce75bb16c36484b88e0b6ac30a>

Simultaneous quantitation of two orchid viruses, cymbidium mosaic potexvirus (CymMV) and odontoglossum ringspot tobamovirus (ORSV), were carried out using the TaqMan(R) real-time RT-PCR, a novel detection technique that combines RT-PCR with the power of fluorescent detection. Four TaqMan(R) probes were synthesized, targeting at the RNA-dependent RNA polymerase (RdRp) and coat protein (CP) genes of both viruses. The reporter dye FAM (6-carboxyfluorescein) was used to label the 5' terminus of probes specific to CymMV, while TET (tetrachloro-6-carboxyfluorescein) was used for the ORSV probes. TAMRA (6-carboxy-tetramethyl-rhodamine), which was attached at the 3' terminus of each probe, was used as the universal quencher. With increasing amounts of standard RNA templates, the respective threshold cycle (CT) values were determined and a linear relationship was established between these CT values and the logarithm of initial template amounts. The amounts of starting templates in mixed-infected *Oncidium* flowers and leaves were estimated from the standard curves. As little as 104 copies or 5 fg each of CymMV and ORSV could be detected simultaneously with either the RdRp or CP gene as the target. This system offers a sensitive, high throughput and rapid method for plant virus detection.

Evans, P. C., J. Gray, et al. (1998). "Optimisation of the polymerase chain reaction and dot-blot hybridisation for detecting cytomegalovirus DNA in urine: comparison with detection of early antigen fluorescent foci and culture." Journal of Virological Methods **73**(1): 41.

<http://www.sciencedirect.com/science/article/B6T96-3T2PCND-5/2/d3c00709a679180bccdcf47c6b8e4730>

Rapid, sensitive and specific assays are required for the diagnosis of CMV infection following transplantation. We describe our experience in developing assays for detecting CMV in urine. Conventional preparation of probes cloned after amplification in *E. coli* led to contamination with *E. coli* nucleic acids; these hybridised to *E. coli* DNA present in urine and produced false positive results. Two CMV probes (Hind III and gL) hybridised to human DNA despite high stringency; these probes were thus unsuitable for detecting viral nucleic acids in clinical samples. A PCR derived probe from the immediate early gene of CMV detected dot-blotted CMV DNA specifically. Optimal preparation of urine for detection of CMV DNA was as follows; four freeze/thaw cycles and ultracentrifugation before in vitro proteinase K/SDS treatment, phenol:chloroform extraction, heat denaturation and direct application onto a nylon membrane. However, dot-blot hybridisation was a poor test for CMV in urine; it had low sensitivity and specificity compared with virus isolation and DEAFF. Single round PCR of a 293bp region of CMV DNA was sensitive and specific to CMV targets. However, undiluted urine contained PCR inhibitors that could only be partly removed by using PEG precipitation. PCR of CMV DNA from urine was specific but was insensitive compared to conventional culture and DEAFF. A significant proportion of urine samples were toxic in conventional culture and DEAFF tests but, PCR of CMV DNA from urine is insensitive and despite its specificity is unlikely to be advantageous in clinical practice even when DEAFF or culture prove unreliable.

Fabre, F., C. Kervarrec, et al. (2003). "Improvement of Barley yellow dwarf virus-PAV detection in single aphids using a fluorescent real time RT-PCR." *Journal of Virological Methods* **110**(1): 51.

<http://www.sciencedirect.com/science/article/B6T96-48CNW0D-1/2/439cbec68940d7e63b490ad51961bc19>

One of the major factors determining the incidence of Barley yellow dwarf virus (BYDV) on autumn-sown cereals is the viruliferous state of immigrant winged aphids. This variable is assessed routinely using the enzyme-linked immunosorbant assay (ELISA). However, the threshold for virus detection by ELISA can lead to false negative results for aphids carrying less than 106 particles. Although molecular detection techniques enabling the detection of lower virus quantities in samples are available, the relatively laborious sample preparation and data analysis have restricted their use in routine applications. A gel-free real-time one-step reverse transcription polymerase chain reaction (RT-PCR) protocol is described for specific detection and quantitation of BYDV-PAV, the most widespread BYDV species in Western Europe. This new assay, based on TaqMan(R) technology, detects and quantifies from 102 to 108 BYDV-PAV RNA copies. This test is 10 and 103 times more sensitive than the standard RT-PCR and ELISA assays published previously for BYDV-PAV detection and significantly improves virus detection in single aphids. Extraction of nucleic acids from aphids using either phenol/chloroform or chelatin resin-based protocols allow the use of pooled samples or of a small part (up to 1/1600th) of a single aphid extract for efficient BYDV-PAV detection.

Falcone, E., E. D'Amore, et al. (1997). "Rapid diagnosis of avian infectious bronchitis virus by the polymerase chain reaction." *Journal of Virological Methods* **64**(2): 125.

<http://www.sciencedirect.com/science/article/B6T96-3RJ9BMF-2/2/8f8a7aebc8132e8848af2ec6d2901c8c>

A simple, sensitive and specific polymerase chain reaction (PCR) procedure was developed in

order to detect infectious bronchitis virus (IBV) directly in tissue samples. Viral RNA was extracted from allantoic fluids and cell cultures infected experimentally with different strains of IBV and from tissues of naturally infected birds. Viral RNA was then amplified and identified by a nested RT-PCR assay using two sets of primers flanking a well-conserved region of the nucleocapsid gene. The selected IBV nucleocapsid sequence was detected successfully by simple direct electrophoresis of amplified material.

Fang, G., G. Zhu, et al. (1998). "Minimizing DNA recombination during long RT-PCR." Journal of Virological Methods **76**(1-2): 139.

<http://www.sciencedirect.com/science/article/B6T96-3V5V5SV-H/2/7c71936eb89a8bdc4722309fe5e2d2b>

Recent developments have made it possible to reverse transcribe RNA and amplify cDNA molecules of >10 kb in length, including the HIV-1 genome. To use long reverse transcription combined with polymerase chain reaction (RT-PCR) to best advantage, it is necessary to determine the frequency of recombination during the combined procedure and then take steps to reduce it. We investigated the requirements for minimizing DNA recombination during long RT-PCR of HIV-1 by experimenting with three different aspects of the procedure: conditions for RT, conditions for PCR, and the molar ratios of different templates. We used two distinct HIV-1 strains as templates and strain-specific probes to detect recombination. The data showed that strategies aimed at completing DNA strand synthesis and the addition of proofreading function to the PCR were most effective in reducing recombination during the combined procedure. This study demonstrated that by adjusting reaction conditions, the recombination frequency during RT-PCR can be controlled and greatly reduced.

Feucht, H. H., B. Zollner, et al. (1995). "Comparison of conventional autoradiography with a new DNA enzyme immunoassay for the detection of hepatitis C virus-polymerase chain reaction amplification products." Journal of Virological Methods **55**(1): 105.

<http://www.sciencedirect.com/science/article/B6T96-3YYTF0W-19/2/ae45973e875d9693bcf16d31f7f60463>

The detection of HCV-PCR amplification products by DNA enzyme immunoassay (DEIA) was compared with conventional hybridization carried out with a ³²P-labelled oligonucleotide probe. The detection limit of both methods was shown to be between 100 pg and 1 ng of amplicon. All serum samples of 40 HCV-seropositive patients were positive after PCR in autoradiography, but only 38 with the DEIA technique (sensitivity 95%). There were no false-positive reactions by either method. The advantage of the DEIA method was the fast and non-radioactive detection of HCV amplicons. DEIA combines the specificity of the hybridization event with the speed of an ELISA procedure and is suitable for HCV-PCR.

Formiga-Cruz, M., A. Hundesa, et al. (2005). "Nested multiplex PCR assay for detection of human enteric viruses in shellfish and sewage." Journal of Virological Methods **125**(2): 111.

<http://www.sciencedirect.com/science/article/B6T96-4FFH1XG-2/2/3f8dd23d017b88125eccc9122d6e4fc>

Environmental samples and contaminated shellfish present frequently low concentrations of more

than one viral species. For this reason, a nested multiplex RT-PCR was developed for the detection of adenoviruses, enteroviruses and hepatitis A viruses in different environmental samples such as urban sewage and shellfish. This assay will save time and cost for detection of these enteric viruses with a smaller sample volume, which otherwise can be a limiting factor in routine analysis. The limit of detection was approximately 1 copy for adenovirus and 10 copies for enterovirus and hepatitis A virus per PCR reaction using titrated cell-cultured viruses as template material. In shellfish and environmental samples, this multiplex PCR was optimized to detect all three viruses simultaneously when the concentration of each virus was equal or lower than 1000 copies per PCR reaction. This is the level found predominantly in the environment and in shellfish when the numbers of fecal bacterial and phage indicators are low. The detection of human adenoviruses by PCR has been suggested as a molecular index of fecal contamination of human origin in the environment and food and the multiplex assay developed may be a tool for evaluating the presence of viral contamination in shellfish and water and to expand microbiological control to include viral markers.

Forslund, O., H. Ly, et al. (2003). "Improved detection of cutaneous human papillomavirus DNA by single tube nested 'hanging droplet' PCR." Journal of Virological Methods **110**(2): 129.

<http://www.sciencedirect.com/science/article/B6T96-48KMMH5-1/2/21b0818017e2050923d968ba9fd138bf>

A single tube nested 'hanging droplet' PCR was developed for detection of cutaneous human papillomavirus (HPV) DNA of the phylogenetic group B1. The nested PCR was compared with a single round PCR method by testing 56 fresh biopsies from Australian skin tumour patients. HPV DNA was detected in 64% (36/56) of the biopsies by nested PCR and in 30% (17/56) by single round PCR (PP=0.003], squamous cell carcinoma [43% (7/16) vs. 25% (4/16)] and in solar keratosis [93% (13/14) vs. 57% (8/14); P=0.038]. The nested PCR and the single round PCR system detected 26 and 11 different HPV types/putative types/subtypes, respectively. Multiple types were found in eight samples by the nested PCR and two samples by single round PCR. The nested HPV PCR is more sensitive and capable of amplifying a broad spectrum of HPV types from skin tumours, but further improvements are needed before all HPV infections in skin can be detected by a single assay.

Forsman, A., D. Uzameckis, et al. (2003). "Single-tube nested quantitative PCR: a rational and sensitive technique for detection of retroviral DNA. Application to RERV-H/HRV-5 and confirmation of its rabbit origin." Journal of Virological Methods **111**(1): 1.

<http://www.sciencedirect.com/science/article/B6T96-48NKTJ2-2/2/e7668d7aed4a8cd8be75c568f8c0011f>

It was reported earlier that a few patients suffering from non-Hodgkin's lymphoma had low amounts of DNA from the so-called fifth human exogenous retrovirus, HRV-5. A sensitive and rational method for large-scale screening for HRV-5 DNA was therefore developed. It is a single-tube nested quantitative PCR (stnQPCR), which uses two functionally isolated primer pairs and one probe target distinct from related endogenous retroviral sequences, yet encompassing known HRV-5 variation, allowing optimal use of sequence conservation. DNA from lymphoma, myeloma, and follicular dendritic cell lines was tested for HRV-5 positivity, as was DNA from whole blood of blood donors, non-Hodgkin's lymphoma and systemic lupus erythematosus patients, as well as DNA from lymph node biopsies of rheumatoid arthritis patients with lymphoma. One blood donor, one systemic lupus erythematosus patient, two previously known positive non-Hodgkin's lymphoma patients, and one rheumatoid arthritis lymphoma patient, came out positive. They had 24, 2, 148, 480 and 30 proviral copies per [mu]g of DNA from PBMC or lymphoma tissue,

respectively. During the completion of this work it was reported that HRV-5 is a rabbit endogenous retrovirus (RERV-H), and that HRV-5 positivity was due to presence of rabbit DNA. DNA from six RERV-H/HRV-5 positive samples was therefore retested. Three also contained rabbit mitochondrial DNA. A search for HRV-5 antibodies using synthetic peptides was negative in sera from three RERV-H/HRV-5 positive individuals, as well as in 144 other sera, according with a noninfectious origin of the RERV-H/HRV-5 DNA in human samples. A search for possible sources of rabbit DNA contamination was negative. Methods for prevention of PCR contamination were strictly adhered to. Three samples from RERV-H/HRV-5 positive individuals positive at the Uppsala laboratory were retested at one or two other laboratories, and all three were positive. Two other samples, which were positive in the Riga laboratory, were tested also in London and also found positive. One non-Hodgkin's lymphoma patient was RERV-H/HRV-5 positive in four consecutive samples, showing that positivity was a property of that patient. It is concluded that the stnQPCR developed to detect and quantify minute amounts of RERV-H/HRV-5 DNA is a principle which can be applied widely and HRV-5 is a RERV-H. Its presence in a few human blood samples could not be explained.

Forsyth, M. A., S. Parida, et al. (2003). "Rinderpest virus lineage differentiation using RT-PCR and SNAP-ELISA." Journal of Virological Methods **107**(1): 29.

<http://www.sciencedirect.com/science/article/B6T96-474DS2H-2/2/2d11968dd2eeb7be200157d2e733cbdb>

An RT-PCR/ELISA system has been developed that detects and differentiates Rinderpest virus (RPV) from the other closely related morbillivirus of ruminants, Peste des petits Ruminants virus (PPRV). In addition, using lineage specific probes, it is possible to determine whether the virus sample is wild-type or vaccine, and the likely origin of the outbreak if it is wild-type. It involves carrying out a RT-PCR with one digoxigenin (Dig)-labelled primer followed by a hybridisation step with a virus-specific, biotin-labelled, probe. The hybridisation step is carried out in an ELISA format on a streptavidin-coated plate. The DIG-labelled products are detected using a specific anti-DIG monoclonal antibody and an anti-mouse horseradish peroxidase conjugate. The hybridisation step replaces nucleotide sequencing or nested PCR for confirmation of the identity of DNA product. The assay is fast and easy to carry out and can give semi-quantitative estimates of the virus content of samples.

Foster, T. P., G. V. Rybachuk, et al. (1998). "Expression of the enhanced green fluorescent protein by herpes simplex virus type 1 (HSV-1) as an in vitro or in vivo marker for virus entry and replication." Journal of Virological Methods **75**(2): 151.

<http://www.sciencedirect.com/science/article/B6T96-3V5F5S6-3/2/cdcbe9ca99cc3181143189332fa1b1ef>

Glycoprotein K (gK) is involved in membrane fusion phenomena during infectious virus production and egress and is an important determinant for neurovirulence. To assess better the in vitro and in vivo roles of gK in virus replication, a recombinant virus was constructed expressing an engineered enhanced green fluorescent protein (EGFP) under the control of the human cytomegalovirus immediate early gene promoter (HCMV-IEP) inserted in place of the gK gene. The EGFP gene insertion was confirmed by diagnostic polymerase chain reaction (PCR), and the presence of the EGFP protein was detected by western immunoblot analysis using anti-GFP monoclonal antibody. Fluorescence microscopy revealed that virus infected cells emitted bright fluorescence when examined using filters for fluorescein. Fluorescence emission was detected as early as 4 h post-infection. Fluorescence intensity increased over time and was stable at late times after infection at which point viral plaques continued to emit bright green fluorescence. The

amount of fluorescence emitted by virus infected Vero cells was monitored by fluorescence cytometry using a FACS cytometer. At an MOI of 3, all infected cells emitted strong green fluorescence as quantified by cytometry at 48 h post-infection. The [Delta]gK-EGFP expressing recombinant virus will enable the determination of the role of gK in virus entry and egress as well as the role of gK in the molecular pathogenesis of herpes simplex virus type 1 (HSV-1).

Fuessel Haws, A. L., Q. He, et al. (2004). "Nested PCR with the PGMY09/11 and GP5+/6+ primer sets improves detection of HPV DNA in cervical samples." Journal of Virological Methods **122**(1): 87.

<http://www.sciencedirect.com/science/article/B6T96-4DDTMGF-1/2/ac059cf432fa0b44655c9edccf53a55e>

Based on epidemiological and research evidence, HPV has a causal role in cervical carcinogenesis. Several HPV detection methods exist to date; the most commonly used method for detection of genital HPVs consists of nested PCR using the MY09/11 and GP5+/6+ primer sets (MY/GP+). Recently, the PGMY09/11 primer set, a modified version of the MY09/11 primer set, was introduced for single PCR and was found to detect a wider range of HPV types. The next logical step was taken and the efficacy of nested PCR using the PGMY09/11 and GP5+/6+ primer sets (PGMY/GP+) to detect HPV in cervical samples was evaluated. In this comparative study, nested PCR using the novel PGMY/GP+ primer set combination was found to be more type sensitive than the nested PCR with the MY/GP+ primer sets, detecting a wider range of HPV types, low copy HPVs, and better characterizing samples infected with multiple strains of HPV. Standardization and use of the PGMY/GP+ PCR system could aid physicians in providing more efficient HPV screening and better treatment for patients.

Fujioka, S., H. Koide, et al. (1995). "Analysis of enterovirus genotypes using single-strand conformation polymorphisms of polymerase chain reaction products." Journal of Virological Methods **51**(2-3): 253.

<http://www.sciencedirect.com/science/article/B6T96-402KYYX-D/2/d910471a8c50421b04c3852185606e15>

Enterovirus genotypes were identified rapidly by reverse transcription-polymerase chain reaction (RT-PCR) followed by single-strand conformation polymorphism (SSCP) analysis. The primer pair was chosen from the highly conserved sequence at the 5' non-coding region of enterovirus genomes. RT-PCR amplified a 154 bp sequence in all samples from 14 serotypes of enteroviruses, including group A and B Coxsackie viruses, echoviruses and polioviruses. SSCP analysis of these products revealed different electrophoretic profiles. Thus, SSCP analysis will be useful for differentiating the genotypes of enteroviruses, and may be applicable for rapid diagnosis of enteroviral infection.

Gehring, H., K. Von der Helm, et al. (2003). "Development and evaluation of a phenotypic assay monitoring resistance formation to protease inhibitors in HIV-1-infected patients." Journal of Virological Methods **109**(2): 143.

<http://www.sciencedirect.com/science/article/B6T96-484VJJM-1/2/e41b6217f067fcd97d1cd3c98143f8f2>

A novel phenotypic assay, based on recombinant expression of the HIV-1-protease was

developed and evaluated; it monitors the formation of resistance to protease inhibitors. The HIV-1 protease-encoding region from the blood sample of patients was amplified, ligated into the expression vector pBD2, and recombinantly expressed in *Escherichia coli* TG1 cells. The resulting recombinant enzyme was purified by a newly developed one-step acid extraction protocol. The protease activity was determined in presence of five selected HIV protease inhibitors and the 50% inhibitory concentration (IC₅₀) to the respective protease inhibitors determined. The degree of resistance was expressed in terms of x-fold increase in IC₅₀ compared to the IC₅₀ value of an HIV-1 wild type protease preparation. The established test system showed a reproducible recombinant expression of each individual patients' HIV-1 protease population. Samples of nine clinically well characterised HIV-1-infected patients with varying degrees of resistance were analysed. There was a good correlation between clinical parameters and the results obtained by this phenotypic assay. For the majority of patients a blind genotypic analysis of the patients' protease domain revealed a fair correlation to the results of the phenotypic assay. In a minority of patients our phenotypic results diverged from the genotypic ones. This novel phenotypic assay can be carried out within 8-10 days, and offers a significant advantage in time to the current employed phenotypic tests.

Gibbs, A. and A. Mackenzie (1997). "A primer pair for amplifying part of the genome of all potyvirids by RT-PCR." *Journal of Virological Methods* **63**(1-2): 9.

<http://www.sciencedirect.com/science/article/B6T96-3RHM7T6-T/2/98d98dc0e4883bedac56c3fdd03157cf>

Sequence analysis was used to design a pair of degenerate oligonucleotide primers that amplified a 1.6-2.1 kbp fragment from the 3' end of the genome (virion protein gene and part of the NIb gene) of 17 species of the Potyviridae ('potyvirids'); 11 potyviruses, 2 bymoviruses, 2 macluraviruses, an ipomovirus and a rymovirus. The 'potyvirid primer 1' hybridizes to the 3' terminal poly-A region of the genome, and 'potyvirid primer 2' to the genomic region encoding the GNNSGQ-motif of the NIb protein. Database searches showed that the potyvirid 2 primer is specific for potyvirids. Associated analyses indicated that the published amino acid sequence of part of the wheat streak mosaic rymovirus NIb protein is probably incorrect in part.

Gibson, K. M., K. A. McLean, et al. (1991). "A simple and rapid method for detecting human immunodeficiency virus by PCR." *Journal of Virological Methods* **32**(2-3): 277.

<http://www.sciencedirect.com/science/article/B6T96-476KXFR-JG/2/fe551045ebfffc936ebbf04546eb4de>

A simple, sensitive and specific method using the polymerase chain reaction (PCR) for amplification of human immunodeficiency virus type 1 (HIV-1) is described. The method involves minimal manipulations. Peripheral blood mononuclear cells (PBMC) were prepared by a rapid Ficoll-Paque gradient method. Lymphocytes were lysed in PCR buffer containing Proteinase K and detergents, and subjected to amplification under stringent conditions, using two primer pairs. Amplified DNA sequences were hybridized with a 3'-end labelled probe, electrophoresed on agarose gels and visualised by ethidium bromide staining. Identification of amplified HIV-1 proviral DNA sequences was confirmed by autoradiography. HIV-1 sequences were amplified in all samples from 103 HIV-1 seropositive individuals, but not in 40 HIV-1 seronegative controls. The absence of contamination may be attributable in part to minimisation of manipulations before amplification.

Gough, K. C., W. Cockburn, et al. (1999). "Selection of phage-display peptides that bind to cucumber mosaic virus coat protein." Journal of Virological Methods **79**(2): 169.

<http://www.sciencedirect.com/science/article/B6T96-3WJDTS6-6/2/8d9b46546fa13509ce4d2304d087dacf>

Several discrete peptides that bind specifically to the coat protein of cucumber mosaic virus (CMV) were isolated from a diverse phage library displaying random nonapeptides on the major coat protein VIII. Enrichment was shown by polyclonal phage enzyme linked immunosorbent assay (ELISA) after three rounds of selection. Sequencing of the genes encoding 10 of these peptides revealed an absence of any conserved motifs, although nine of them contained a high proportion of proline residues. Some of the selected peptides were displayed at the N-terminus of thioredoxin and expressed in the cytoplasm of *Escherichia coli*. Both the phage-displayed and thioredoxin-fusion versions of the peptides could detect purified CMV and CMV present in crude leaf extracts from infected plants. By dot blot analysis, a thioredoxin-peptide fusion could readily detect as little as 5 ng of CMV. The peptides did not bind to other plant viruses. These peptides have been shown to be specific and highly sensitive tools in the detection of CMV and, as well as their diagnostic potential, they could form the basis for a novel disease resistance strategy.

Gouvea, V., S. J. Cohen, et al. (1997). "Identification of hepatitis E virus in clinical specimens: amplification of hydroxyapatite-purified virus RNA and restriction endonuclease analysis." Journal of Virological Methods **69**(1-2): 53.

<http://www.sciencedirect.com/science/article/B6T96-3S2BMBF-7/2/187546e9a6dc5b99c6e2ddc12dddb55e>

A multi-site nested reverse transcription and polymerase chain reaction (RT-PCR) followed by restriction endonuclease analysis (REA) was developed to identify hepatitis E virus (HEV) in clinical specimens. Four sets of primers were selected to amplify regions in the HEV genome supposed to encode the helicase, polymerase, and parts of the viral capsid protein. Digestion of the nested PCR products with *HinfI*, *HaeII*, *Avall*, *BglI*, *KpnI*, *SmaI*, or *EcoRI* generated readily recognizable profiles that confirm the HEV sequences and/or distinguish the unique Mexico genotype (our positive control) from all other isolates (Asian genotype). In addition, the hydroxyapatite (HA) adsorption method was compared to other adsorption and extraction methods widely used to purify viral RNA from clinical specimens for RT-PCR. All methods presented the same sensitivity of recovery of HEV RNA, but only the adsorption methods efficiently removed fecal enzymatic inhibitors. The HA method gave the best results and was the most economic in terms of time, cost, manipulations and reagents. The method was validated by screening a small number of serum and fecal specimens available from patients with acute non-A,B,C hepatitis in Nepal. HEV RNA was identified in half (5/11) of the fecal specimens obtained from patients with evidence of recent HEV infection, but in none of the 14 patients without a serological marker for hepatitis E.

Gouvea, V., N. Santos, et al. (1994). "Identification of Norwalk virus in artificially seeded shellfish and selected foods." Journal of Virological Methods **48**(2-3): 177.

<http://www.sciencedirect.com/science/article/B6T96-476F6FR-DM/2/5bb703002fee89b1144607dde0dbdb6>

A rotavirus dsRNA purification protocol was adapted to extract Norwalk ssRNA from artificially contaminated shellfish, and a sensitive reverse transcription-polymerase chain reaction assay for

Norwalk virus was devised to identify an estimated 20-200 genomic copies. The technique includes deproteinization with guanidinium isothiocyanate, adsorption of RNA to hydroxyapatite, and sequential precipitation with cetyltrimethylammonium bromide and ethanol. The protocol allows high recovery of viral RNA free of enzymatic inhibitors from oysters, clams, and a variety of food matrices. Norwalk virus sequences were copied and amplified by using primers selected from the polymerase gene. Digestion of the amplified products with restriction enzymes ensured the specificity of the test. This rapid and sensitive assay may significantly improve the prospect for the routine screening of the uncultivable Norwalk virus in food stuffs.

Grankvist, O., L. Walther, et al. (1996). "Nested PCR assays with novel primers yield greater sensitivity to Tanzanian HIV-1 samples than a commercial PCR detection kit." Journal of Virological Methods **62**(2): 131.

<http://www.sciencedirect.com/science/article/B6T96-3RHM7T6-H/2/64445824136310710ca8f94cbe9f017f>

To investigate the efficacy of the SK431/SK145 primer pair and two nested primer assays in amplifying African HIV-1 samples, a total of 35 Tanzanian PBMC samples were examined. These were assayed by two HIV-1 specific nested in-house PCR assays and a commercial HIV-1 PCR kit (GeneAmp™) using SK431/SK145 as the primer pair. One of the nested PCR assays has been evaluated previously (old assay), whereas the modified assay was constructed from the HIV-1 sequence alignment released in August 1993. The modified nested primer assay showed increased sensitivity in the gag and env regions compared to the old nested primer assay. However, both the old and the modified nested primer assays displayed higher sensitivity for the detection of Tanzanian HIV-1 proviruses than the GeneAmp™ assay. When two regions were used (gag and env) as targets for the amplification, the modified nested primer assay detected 97.1% (34/35) of the proteinase K lysed samples, compared to 68.6% (24/35) using the SK431/SK145 primer pair ($P < 0.01^{**}$). The results indicate that the SK431/SK145 primer pair may be less suitable when HIV-1 samples from Africa are analysed. The results also show that continuous modification of primer sequences can improve and maintain high sensitivity for the detection of highly divergent HIV-1 strains.

Gratzl, S., C. Moroni, et al. (1997). "Quantification of HIV-1 viral RNA and proviral DNA by isotopic competitive PCR." Journal of Virological Methods **66**(2): 269.

<http://www.sciencedirect.com/science/article/B6T96-3RYCMYM-8/2/c7a8302dd1ca779cfecce4db7bbba2c7>

A quantitative isotopic competitive PCR (icPCR) assay was established using ³²P-labeled primers targeting the HIV-1 gag gene followed by quantification using a phosphorimager. The detection limit varied from 3 to 10 molecules of DNA and 10 to 100 molecules of RNA per reaction. The icPCR quantification of HIV-1 DNA copies correlated well with the cell number of 8E5/LAV cells bearing a single provirus ($r^2 = 0.95$). Provirus quantification was applied to overnight infected donor PBMCs, thereby determining infectious virus titres in culture supernatants as a rapid alternative to limiting dilution culture. Parallel quantification of the HIV-1 RNA indicated the infectious virus fraction to be 0.3%. In 39 HIV-1-infected patients with clinical stages A ($n = 17$), B ($n = 15$), and C ($n = 7$), the HIV-1 RNA in the plasma was determined ranging from 100 to 90600 RNA copies/ml. The results of icPCR and a commercial assay (ROCHE Amplicor HIV-1 Monitor) correlated well ($r = 0.97$). In 13 additional patients, the plasma viral load per ml was compared with the proviral load per 10⁶ PBMC showing a viral excess of 10-1000-fold (mean of 85, $r = 0.7$, $P < 0.01$). It is concluded that icPCR is suitable for the measurement of proviral and viral load in experimental and clinical settings.

Gravitt, P. E., C. Peyton, et al. (2003). "Reproducibility of HPV 16 and HPV 18 viral load quantitation using TaqMan real-time PCR assays." Journal of Virological Methods **112**(1-2): 23.

<http://www.sciencedirect.com/science/article/B6T96-497RGSC-3/2/c55bd22df6d30913b5deec124ef5ed8f>

A reproducibility study was designed to assess within-assay, between-day, and interlaboratory variability of three real-time PCR assays targeting HPV 16, HPV 18, and the human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) pseudogenes. Fifteen HPV 16 and fifteen HPV 18 cervical swab samples were amplified in triplicate by GAPDH and HPV 16 and by GAPDH and HPV 18 assays, respectively. All samples were amplified undiluted and at a 1:10 dilution on 2 separate days in the same laboratory, and the same samples were amplified in a separate laboratory. HPV 16 and HPV 18 normalized viral load is reported as the number of HPV genomes per 20000 GAPDH copies. The analytic specificity of the HPV 16 and 18 assays was 100 and 97%, respectively. The intraclass correlation coefficients (ICC) were 0.99, 0.97, and 0.98 for HPV 16, HPV 18, and GAPDH, respectively, indicating that the variability due to experimental error was very low. Ten-fold differences in viral load could be readily discriminated across a six order of magnitude dynamic range (ca. 5-5 x 10⁶ copies). Power of discrimination was increased at higher target concentrations (>5000 copies). The correlation of normalized HPV 16 and 18 viral load was high between the two laboratories (Spearman rho ([rho])=0.96 and 0.87, respectively). These HPV 16 and HPV 18 quantitative PCR assays with GAPDH normalization are reproducibly quantitative over a broad linear dynamic range allowing for application in epidemiologic studies for measurement of viral load.

Greening, G. E., L. Woodfield, et al. (1999). "RT-PCR and chemiluminescent ELISA for detection of enteroviruses." Journal of Virological Methods **82**(2): 157.

<http://www.sciencedirect.com/science/article/B6T96-3XT0BN2-7/2/149fa3cfe9d254a79e6a213cd2cab2cd>

Reverse transcription followed by polymerase chain reaction amplification (RT-PCR) is now used commonly to detect the presence of enteric RNA viruses in environmental samples. A sensitive, non-isotopic microtitre plate hybridisation assay was developed and applied for detection of enteroviruses in environmental samples. Following reverse transcription, viral cDNA was labelled with digoxigenin (DIG)-dUTP during the PCR amplification step. The labelled PCR products were then hybridised with enterovirus-specific biotinylated oligonucleotide probe and captured in streptavidin-coated microtitre wells. Hybridised enteroviral PCR products were detected by an anti-digoxigenin peroxidase conjugate using either a colourimetric or a chemiluminescent substrate and automated measurement. Standard curves were established for poliovirus and other enteroviruses. The chemiluminescent assay was more sensitive than the colourimetric assay for detection of poliovirus, and was specific for enteroviruses. The chemiluminescent ELISA assay was used to confirm the presence of enteroviruses in environmental water samples.

Grimm, A. C. and G. Shay Fout (2002). "Development of a molecular method to identify hepatitis E virus in water." Journal of Virological Methods **101**(1-2): 175.

<http://www.sciencedirect.com/science/article/B6T96-44PVRKC-1/2/e7b6f2e02418d20b85f1e0d2d50bb872>

Hepatitis E virus (HEV) causes an infectious form of hepatitis associated with contaminated water. By analyzing the sequence of several HEV isolates, a reverse transcription-polymerase chain reaction method was developed and optimized that should be able to identify all of the known HEV strains. When tested under laboratory conditions, this method was able to detect low levels of five diverse HEV variants. In addition, internal controls were constructed so that any PCR inhibition could be detected. Finally, virus-spiked environmental water samples were analyzed successfully with these assays.

Hammond, R. W., J. M. Crosslin, et al. (1999). "Differentiation of closely related but biologically distinct cherry isolates of *Prunus necrotic ringspot virus* by polymerase chain reaction." Journal of Virological Methods **80**(2): 203.

<http://www.sciencedirect.com/science/article/B6T96-3X29D70-C/2/18edff2a89bc88f27b8881698af3c946>

Prunus necrotic ringspot ilarvirus (PNRSV) exists as a number of biologically distinct variants which differ in host specificity, serology, and pathology. Previous nucleotide sequence alignment and phylogenetic analysis of cloned reverse transcription-polymerase chain reaction (RT-PCR) products of several biologically distinct sweet cherry isolates revealed correlations between symptom type and the nucleotide and amino acid sequences of the 3a (putative movement protein) and 3b (coat protein) open reading frames. Based upon this analysis, RT-PCR assays have been developed that can identify isolates displaying different symptoms and serotypes. The incorporation of primers in a multiplex PCR protocol permits rapid detection and discrimination among the strains. The results of PCR amplification using type-specific primers that amplify a portion of the coat protein gene demonstrate that the primer-selection procedure developed for PNRSV constitutes a reliable method of viral strain discrimination in cherry for disease control and will also be useful for examining biological diversity within the PNRSV virus group.

Hauptli, D., L. Bruckner, et al. (1997). "Use of reverse transcriptase polymerase chain reaction for detection of vaccine contamination by avian leukosis virus." Journal of Virological Methods **66**(1): 71.

<http://www.sciencedirect.com/science/article/B6T96-3RYCMYM-1C/2/18f6ba294c42adbeeda2604566560edd>

A reverse transcriptase polymerase chain reaction (RT-PCR) for avian leukosis virus (ALV) was developed for the detection of contamination of vaccines produced in embryonated eggs and cell cultures derived from chicken. ALV is highly pathogenic and induces a wide spectrum of disease in infected animals. ALV can be divided into five subgroups (A-E). The envelope glycoprotein (env gp85) is the main antigen determinant and responsible for subgroup classification. Viral RNA of all subgroups (A-E) was isolated and amplified using three sets of primers. Subsequently, restriction endonuclease analysis confirmed the product identity and discriminated between subgroups. In specific pathogen free (SPF) eggs experimentally inoculated with ALV, viral RNA was found in allantoic fluids, as well as in vaccines spiked with different subgroups of ALV. No adventitious virus was detected in commercially available preparations. This system provides a rapid and specific *in vitro* method for the detection of ALV RNA as an extraneous agent and may be applied for quality control of avian vaccines.

Hawrami, K. and J. Breuer (1999). "Development of a fluoregenic polymerase chain reaction assay

(TaqMan) for the detection and quantitation of varicella zoster virus." Journal of Virological Methods **79**(1): 33.

<http://www.sciencedirect.com/science/article/B6T96-3W7XC6H-4/2/9bbe0ae1f4316bf4978918f709a3e76a>

A TaqMan based polymerase chain reaction (PCR) assay was developed for the detection and quantitation of varicella zoster virus (VZV). This method enables simple, reproducible, sensitive and specific detection and quantification of VZV. The TaqMan assay was able to detect four copies of VZV and did not cross-react with other herpesviruses DNA. The assay has several advantages over conventional PCR. First, in the TaqMan assay there is no need for gel electrophoresis and contact with hazardous chemicals. Second, the method is rapid allowing the analysis of 92 samples within minutes after completion of PCR. Finally, the incorporation of a specific probe into the PCR reaction enhances the sensitivity and specificity of the method compared with conventional PCR. The TaqMan system could, therefore, be a useful tool for the epidemiological and diagnostic investigation of VZV.

Hays, J. P. and S. H. Myint (1998). "PCR sequencing of the spike genes of geographically and chronologically distinct human coronaviruses 229E." Journal of Virological Methods **75**(2): 179.

<http://www.sciencedirect.com/science/article/B6T96-3V5F5S6-6/2/db84d5fc2a47343679b811d468d045f2>

A reverse transcription nested PCR (RT-PCR) sequencing methodology was developed and used to generate sequence data from the spike genes of three geographically and chronologically distinct human coronaviruses 229E. These three coronaviruses were isolated originally from the USA in the 1960s (human coronavirus 229E strain ATCC VR-74), the UK in the 1990s (human coronavirus 229E LRI 281) and Ghana (human coronavirus 229E A162). Upon translation and alignment with the published spike protein sequence of human coronavirus 229E 'LP' (isolated in the UK in the 1970s), it was found that variation within the translated protein sequences was rather limited. In particular, minimal variation was observed between the translated spike protein sequence of human coronaviruses 229E LP and ATCC VR-74 (1/1012 amino acid differences), whilst most variation was observed between the translated spike protein sequence of human coronaviruses 229E LP and A162 (47/1012 amino acid changes). Further, the translated spike protein sequence of human coronavirus 229E A162 showed three clusters of amino acid changes, situated within the 5' half of the translated spike protein sequence.

Heinemeyer, T., A. Klingenhoff, et al. (1997). "A sensitive method for the detection of murine C-type retroviruses." Journal of Virological Methods **63**(1-2): 155.

<http://www.sciencedirect.com/science/article/B6T96-3RHM7T6-1B/2/5f203004dd348500779dd345dc7a37fb>

A RT-PCR assay was developed for group-specific detection of murine C-type retroviruses using a nested set of degenerated primers. To distinguish exogenous viruses from related, but silent endogenous viruses, a DNase I pretreatment of supernatants is applied. This is followed by a heat inactivation/denaturation step. The PCR method is ultrasensitive, which enables the detection of 100 attogram of MoMuLV proviral DNA or up to 1-10 infectious mouse C-type retroviruses in 10 [mu]l supernatant of infected cells. The high specificity of the method allows the differentiation between mouse C-type retroviruses and related retroviruses of the A, B, and D type and C-type retroviruses found in other species. It serves as a valuable tool for the screening of

animal cell cultures for contaminations with mouse retroviruses, e.g. hybridomas or recombinant cell lines producing foreign proteins.

Hofmann, M. A. (2003). "Construction of an infectious chimeric classical swine fever virus containing the 5'UTR of bovine viral diarrhea virus, and its application as a universal internal positive control in real-time RT-PCR." Journal of Virological Methods **114**(1): 77.

<http://www.sciencedirect.com/science/article/B6T96-49SWFWP-9/2/ecf6aa0b9aa5ce4e7ebac259e29b85cc>

RT-PCR is used widely as a diagnostic method to detect and differentiate pestiviruses. The construction of two chimeric classical swine fever virus (CSFV) recombinants based on a marker virus constructed previously [J. Virol. 72 (1998) 5318-5322] is described. These viruses, termed vA187CAT_5UTRBVD and vA187CAT_IRESBVD, contain the entire 5' untranslated region (5'UTR) or the internal ribosome entry site (IRES) of bovine viral diarrhea virus (BVDV), respectively. Both chimeric viruses proved to be infectious in cell culture. Hence, the 5'UTR as well as the IRES element only of BVDV can substitute for the corresponding genome region of CSFV. Next, two sets of primers and corresponding dual-labeled TaqMan(R) probes were designed; one detecting specifically a conserved but CSFV-specific area within the 5'UTR of wild-type CSFV, the other one targeting the CAT gene inserted in vA187CAT_5UTRBVD. The two primer/probe sets were combined in a closed-tube multiplex one-step RT-PCR. To monitor the entire extraction and detection process limited amounts of vA187CAT_5UTRBVD were added directly to clinical samples before RNA extraction. The multiplex RT-PCR proved to be as sensitive as the single primer/probe set method, but allowed the validation of each sample tested individually, based on the detection of the CAT marker gene. vA187CAT_5UTRBVD was also used successfully for foot-and-mouth disease virus (FMDV) TaqMan(R) RT-PCR. Therefore, it is considered a universal internal positive control for RT-PCR assays to exclude loss of RNA during extraction, or failure of amplification due to inhibitory substances present in the sample.

Hofner, M. C., W. C. Carpenter, et al. (1993). "Detection of foot-and-mouth disease virus RNA in clinical samples and cell culture isolates by amplification of the capsid coding region." Journal of Virological Methods **42**(1): 53.

<http://www.sciencedirect.com/science/article/B6T96-47YGSVX-7/2/fb924bcb48df04e1e516d9f231b84406>

Foot-and-mouth disease is one of the most economically important virus diseases of livestock. Two important requirements for the control of this disease are rapid laboratory diagnosis and epidemiological investigation. The use of the polymerase chain reaction method (PCR) to amplify specific nucleic acid regions offers the unique possibility of combining swift viral detection with the production of genetic material suitable for sequencing and other methods of molecular epidemiological analysis. The sequencing of the region of foot-and-mouth disease virus (FMDV) genome encoding the capsid proteins of the virus (~ 2260 bps), provides valuable information that adds to the molecular characterization of an isolate. This paper describes the use of the PCR for the amplification of this region of the FMDV genome from bovine clinical samples and cell culture isolates. Suitable pairs of oligonucleotide primers were selected from the published sequence of FMDV type O1, Kaufbeuren. One primer set amplified 2091 bps of the capsid coding region of all seven serotypes of FMDV. The other primer set amplified 216 bp from this region of FMDV type O1, BFS 1860, in nucleic acid extracts from several clinical samples. Nucleic acid extracts from the picornaviruses, bovine enterovirus and swine vesicular disease virus, which affect the same animals, were not amplified. Direct sequencing was carried out on the amplified fragments and showed that the PCR products were >98% homologous to published FMDV

sequences.

Houng, H.-S. H., R. Chung-Ming Chen, et al. (2001). "Development of a fluorogenic RT-PCR system for quantitative identification of dengue virus serotypes 1-4 using conserved and serotype-specific 3' noncoding sequences." Journal of Virological Methods **95**(1-2): 19.

<http://www.sciencedirect.com/science/article/B6T96-433NRB5-3/2/fe9854a050a05acce7c4cbad7e48f6a1>

A fluorogenic reverse transcriptase-polymerase chain reaction (RT-PCR) system was developed for use as a rapid diagnostic test for determining dengue viremia. The dengue virus 3'-noncoding sequence was utilized to formulate serotype-specific RT-PCR assays for quantitative identification of the four different dengue virus serotypes. A generic RT primer set containing two dengue specific anti-sense primers (DV-L1 and DV-L2) could be used to transcribe extracted viral RNA of all four dengue virus types to complementary DNA (cDNA). The resultant dengue viral cDNA could be quantitatively identified at the serotype level by the 5'-3' exonuclease assay using four serotype-specific sense primers. The fluorogenic dengue type-specific RT-PCR can detect each of the four dengue types at similar low detection limits, i.e. 20-50 plaque forming units per milliliter of serum. Two panels with four dengue reference serotypes and 134 clinical samples were used to validate detection sensitivity and specificity of the dengue serotype RT-PCR assay, using virus isolation in cell culture as the criterion standard. By analyzing sera samples from Puerto Rico that were collected from 1999 through 2000, the assay demonstrated high level detection sensitivity and specificity of 92.8 and 92.4%, respectively, for all four dengue virus serotypes.

Houng, H.-S. H., D. Hritz, et al. (2000). "Quantitative detection of dengue 2 virus using fluorogenic RT-PCR based on 3'-noncoding sequence." Journal of Virological Methods **86**(1): 1.

<http://www.sciencedirect.com/science/article/B6T96-3YS34HX-1/2/0a5f4885616059f11995aef5f257f642>

A fluorescent DNA probe (DV2.P1) specific to the conserved distal 3'-noncoding region (nucleotides 10653-10678) of dengue 2 virus and a pair of flanking primers (DV2.L1 and DV2.U2) were designed to formulate a dengue 2-specific fluorogenic polymerase chain reaction (PCR). In addition, DV2.L1 was also used as a reverse transcription (RT) primer to generate superior cDNA from dengue viral RNA. Optimal assay conditions with zero background were established to detect low levels of dengue 2 virus from clinical specimens. The range of dengue virus detection in spiked human sera was determined to be from 10 to 10⁶ infectious virions per milliliter (plaque forming units determined using Vero cell line). Dengue 2 virus isolates from different geographic regions can be detected universally and identified by the fluorogenic RT-PCR assay. Moreover, the assay is specific for dengue 2 virus and does not recognize other related flaviviruses, including dengue serotypes 1, 3 and 4, Japanese encephalitis, St. Louis encephalitis, yellow fever, and Kunjin viruses. The assay also detected efficiently immunocomplexed dengue viruses. In practice, the fluorogenic RT-PCR assay detected readily viremia in sera collected from individuals ill with dengue fever. The rise and fall of dengue 2 virus concentrations in rhesus monkeys, reflecting viral proliferation and clearance, was also clearly illustrated by the assay.

Houng, H.-S. H., D. Norwood, et al. (2004). "Development and evaluation of an efficient 3'-noncoding region based SARS coronavirus (SARS-CoV) RT-PCR assay for detection of SARS-CoV

infections." Journal of Virological Methods **120**(1): 33.

<http://www.sciencedirect.com/science/article/B6T96-4CJVJR6-1/2/905fb4fa8e6218acd28373a8e9e90b64>

The severe acute respiratory syndrome (SARS) epidemic originating from China in 2002 was caused by a previously uncharacterized coronavirus that could be identified by specific RT-PCR amplification. Efforts to control future SARS outbreaks depend on the accurate and early identification of SARS-CoV infected patients. A real-time fluorogenic RT-PCR assay based on the 3'-noncoding region (3'-NCR) of SARS-CoV genome was developed as a quantitative SARS diagnostic tool. The ideal amplification efficiency of a sensitive SARS-CoV RT-PCR assay should yield an E value (PCR product concentration increase per amplification cycle) equal to 2.0. It was demonstrated that the 3'-NCR SARS-CoV based RT-PCR reactions could be formulated to reach excellent E values of 1.81, or 91% amplification efficacy. The SARS-CoV cDNA preparations derived from viral RNA extract and the cloned recombinant plasmid both exhibit the identical amplification characteristics, i.e. amplification efficacy using the same PCR formulation developed in this study. The viral genomic copy (or genomic equivalences, GE) per infectious unit (GE/pfu) of SARS-CoV used in this study was also established to be approximate 1200-1600:1. The assay's detection sensitivity could reach 0.005 pfu or 6-8 GE per assay. It was preliminarily demonstrated that the assay could efficiently detect SARS-CoV from clinical specimens of SARS probable and suspected patients identified in Taiwan. The 3'-NCR based SARS-CoV assay demonstrated 100% diagnostic specificity testing samples of patients with acute respiratory disease from a non-SARS epidemic region.

Hoyland, J. A., J. A. Dixon, et al. (2003). "A comparison of in situ hybridisation, reverse transcriptase-polymerase chain reaction (RT-PCR) and in situ-RT-PCR for the detection of canine distemper virus RNA in Paget's disease." Journal of Virological Methods **109**(2): 253.

<http://www.sciencedirect.com/science/article/B6T96-488G8YB-2/2/8749aa4932edd03dc0163ff7236499ce>

Previous evidence implicating Paramyxoviruses in the aetiopathology of Paget's disease of bone has proved controversial. Whilst several groups have demonstrated Paramyxoviruses using techniques such as in situ hybridisation (ISH), reverse transcriptase-polymerase chain reaction (RT-PCR), and in situ-RT-PCR (IS-RT-PCR), others have found no evidence of viruses using only RT-PCR. To investigate this latter finding, we have now compared detection of canine distemper virus by ISH, RT-PCR (three different methods) and IS-RT-PCR, in 10 patients with Paget's disease, and samples of non-diseased bone from four patients. Canine distemper virus was detectable in six of the samples by ISH, but only in five of the samples by RT-PCR, using one of the methods. Neither of the other RT-PCR methods detected canine distemper virus. IS-RT-PCR demonstrated canine distemper virus in all 10 samples. There was no evidence of virus in the control samples. We have shown that the ability to detect canine distemper virus in bone is dependent on the technique used. IS-RT-PCR clearly showed that canine distemper virus was present in 100% of Pagetic samples, whereas canine distemper virus was only found in 60% by ISH and in 50% using one particular RT-PCR method. These results provide conclusive evidence that canine distemper virus is present within Pagetic bone, and provide a possible explanation for the failure of some groups to detect Paramyxovirus sequences. These findings also have wider implications for other studies investigating viral expression.

Ijzerman, M. M., D. R. Dahling, et al. (1997). "A method to remove environmental inhibitors prior to the detection of waterborne enteric viruses by reverse transcription-polymerase chain reaction." Journal of Virological Methods **63**(1-2): 145.

<http://www.sciencedirect.com/science/article/B6T96-3RHM7T6-19/2/d082502d074d3579f7dfa4541d72c8a4>

A method was developed to remove environmental inhibitors from sample concentrates prior to detection of human enteric viruses using the reverse transcription-polymerase chain reaction (RT-PCR). Environmental inhibitors, concentrated along with viruses during water sample processing, are removed by the method through a series of steps that includes dialysis, solvent extraction, ultrafiltration and glass purification. The method was tested by spiking sodium phosphate with poliovirus type 1 with or without humic or fulvic acids and then measuring virus recovery by plaque assay and RT-PCR. Results of the study indicated that (i) 90% of the spiked virus could be recovered from samples at the end of the ultrafiltration step, (ii) virus was detected in the final eluate of samples containing as much as 0.5 mg of humic acid or 5.0 mg of fulvic acid, and (iii) as little as 0.06 plaque forming units (PFU) was detectable per RT-PCR reaction. These results indicate that the described purification method along with RT-PCR is a feasible approach for detecting waterborne human enteric viruses in the presence of interfering substances.

Inoshima, Y., A. Morooka, et al. (2000). "Detection and diagnosis of parapoxvirus by the polymerase chain reaction." Journal of Virological Methods **84**(2): 201.

<http://www.sciencedirect.com/science/article/B6T96-3YDG9HS-C/2/f99b5e663728bcc0f6b91033a27867b>

The genus Parapoxvirus includes four members, bovine papular stomatitis virus (BPSV), pseudocowpox virus (PCPV), orf virus (ORFV) and parapoxvirus of red deer in New Zealand (PVNZ). A set of primers for polymerase chain reaction (PCR) was designed to detect viral DNA from cells infected with each of the four parapoxviruses. The set of primers resulted in the amplification of appropriately sized products from cells infected with BPSV, PCPV, ORFV and PVNZ, respectively. The PCR method was applied for the detection of seven field isolates of parapoxvirus from cattle, sheep and free-ranging wild Japanese serows. The expected size of DNA was amplified from cells infected with each of the seven isolates. No specific PCR products were detected from vaccinia virus-, fowlpox virus- and mock-infected cells. Moreover, by a semi-nested PCR with an inner primer and Southern blot analysis, viral DNA was detected from lesions of clinically affected cattle, sheep and Japanese serows. These results suggested that the PCR method used in this study was specific for the detection of parapoxviruses and thus useful for diagnosis of parapoxvirus infections, especially in discrimination from diseases with similar clinical symptoms.

Ito, T., H. Ieki, et al. (2002). "Simultaneous detection of six citrus viroids and Apple stem grooving virus from citrus plants by multiplex reverse transcription polymerase chain reaction." Journal of Virological Methods **106**(2): 235.

<http://www.sciencedirect.com/science/article/B6T96-46WSX8S-1/2/58a09d09703fa70372a5ba9d5e86230c>

We developed a multiplex reverse transcription polymerase chain reaction (RT-PCR) to detect six citrus viroids: Citrus exocortis viroid (CEVd), Citrus bent leaf viroid (CBLVd), Hop stunt viroid (HSVd), Citrus viroid III (CVd-III), Citrus viroid IV (CVd-IV) and Citrus viroid OS (Cvd-OS) and Apple stem grooving virus (ASGV, synonym: Citrus tatter leaf virus (CTLV)) from citrus plants. The multiplex RT-PCR was also designed to distinguish Cvd-I-LSS (a distinct variant of CBLVd) from CBLVd. By the multiplex RT-PCR, one to eight fragments specific to the pathogens were simultaneously amplified from one sample and identified by their specific molecular sizes in 6%

polyacrylamide gel electrophoresis. The results of the multiplex RT-PCR were consistent with those of other diagnoses, such as uniplex RT-PCR, to detect each of the pathogens. The multiplex RT-PCR provides a simple and rapid method for detecting various viroids and ASGV in citrus plants, which will help diagnose many citrus plants at a time.

Jacquot, E., M. Tribodet, et al. (2005). "A single nucleotide polymorphism-based technique for specific characterization of YO and YN isolates of Potato virus Y (PVY)." Journal of Virological Methods **125**(1): 83.

<http://www.sciencedirect.com/science/article/B6T96-4FFH1XG-1/2/9cbcec844d2200fffb8c12c19f0afd84>

One of the most important properties used to classify Potato virus Y (PVY) isolates is their ability to induce (PVYN) or not (PVYO) veinal necrosis symptoms on the indicator host plant *Nicotiana tabacum* cv. Xanthi. As an alternative to biological assays, several serological and molecular detection tools have been developed for PVY detection and characterization and these have evolved as our knowledge of PVY has improved. However, the assays that have been previously published are all based on the use of neutral markers (antigenic determinants, sequence data, recombination sites or restriction enzyme cleavage sites), which are unlinked to the biological property being characterized (e.g. veinal necrosis). Using the recently identified molecular determinants of the tobacco leaf necrosis symptom induced by PVYN isolates, a one-step fluorescent [TaqMan[®]] RT-PCR assay, based on a single nucleotide polymorphism (SNP) linked to the necrosis property of PVY isolates, has been designed. This assay reliably detects and distinguishes PVYN and PVYO isolates. The method is simple (leaf soak extraction process, gel-free, no post-PCR manipulations), rapid (96 tests in less than 3 h from plants sampling to diagnostic results), sensitive (threshold in a range of 10⁴-10⁵ PVY copies), reliable (correctly assigns 42 PVY isolates in their respective group) and allows co-detection of mixed samples containing close to equivalent PVYN and PVYO quantities. All these characteristics suggest that the newly developed SNP assay could be used to reliably classify PVY isolates, as a substitute for biological assays performed on *N. tabacum* cv. Xanthi.

Jordens, J. Z., S. Lanham, et al. (2000). "Amplification with molecular beacon primers and reverse line blotting for the detection and typing of human papillomaviruses." Journal of Virological Methods **89**(1-2): 29.

<http://www.sciencedirect.com/science/article/B6T96-429RNYH-4/2/4deb02669f9ef56632dd6a8d9523d8e1>

A novel method for the detection and typing of human papillomavirus (HPV) was developed using molecular beacon primers. The method is based on the use of HPV-specific primers containing a hairpin loop structure in which fluorescent donor and quencher groups are held in close proximity such that fluorescence is quenched. Amplification of the target sequence results in the opening of the loop and the resulting fluorescence can be detected on a sequence detector system (SDS 7700 (Applied Biosystems), as used for TaqMan[®] assays. Fluorescent amplicons were identified on the SDS 7700 and then typed by a single hybridisation with specific probes immobilised in lines on a nylon membrane and detected on a fluorescent scanner. This novel beacon primer method compared well with conventional PCR for cervical scrape specimens. The combination of the beacon primer method and reverse line blotting should enable large-scale population studies of HPV infection.

Kanematsu, S., T. Hibi, et al. (1991). "Comparison of nonradioactive cDNA probes for detection of potato spindle tuber viroid by dot-blot hybridization assay." Journal of Virological Methods **35**(2): 189.

<http://www.sciencedirect.com/science/article/B6T96-476RM29-11/2/2ec189aaa939c16854589fee60f6800d>

Six nonradioactive cDNA probes were compared for their sensitivities for detecting potato spindle tuber viroid (PSTVd) by dot-blot hybridization assay. Three biotinylated PSTVd cDNA probes, labeled by photoactivation with photobiotin, by nick translation or by random priming with biotinylated deoxyribonucleotides, were all capable of detecting 20 pg of purified PSTVd by a colorimetric assay and 2-20 pg by a chemiluminescent assay. Digoxigeninlabeled probe was able to detect 200 pg of purified PSTVd. Two biotinylated probes prepared with polymerase chain reaction (PCR) incorporating biotinylated dUTP or dATP were the most sensitive: 0.2-2 pg of PSTVd was detectable by both assays. All six probes could detect PSTVd also in extracts of infected tomato leaves at a dilution of up to 1/250-1/1250. These nonradioactive probes are equal to radioactive probes in their sensitivity, and the biotinylated probes produced with PCR amplification are particularly suitable for practical diagnosis, as they are sensitive and rapidly prepared in large quantities.

Kato, H., E. Orito, et al. (2001). "Determination of hepatitis B virus genotype G by polymerase chain reaction with hemi-nested primers." Journal of Virological Methods **98**(2): 153.

<http://www.sciencedirect.com/science/article/B6T96-441N4XX-7/2/832c9c9efcf60538867ea86e644c8315>

Hepatitis B virus (HBV) has been classified into six genotypes designated A-F by sequence divergence in the entire genome exceeding 8%. Very recently, the seventh genotype was reported and named genotype G. HBV genotype G is distinct from genomes of the other six genotypes in that it possesses an insertion of 36 nucleotides in the core gene, and has been found so far in France and the United States. A method for determining HBV genotype G was developed by polymerase chain reaction (PCR) with primers deduced from the 36-nucleotide (nt) insertion in five isolates of HBV genotype G the sequences of which have been deposited in DNA databases. The validity of this method, for specifically detecting HBV genotype G, was verified on a panel consisting of 142 HBV isolates of six major genotypes and four of genotype G. A total of 540 sera containing HBV in Japan covering symptom free carriers and patients with a spectrum of chronic liver disease were tested by this method, but not a single HBV genotype G sample was found. A possible method for serological determination of hepatitis B surface antigen of genotype G is suggested, without amplification or sequencing nucleotides, which would expand epidemiological and clinical researches on HBV genotype G.

Kato, H., E. Orito, et al. (2003). "Frequent coinfection with hepatitis B virus strains of distinct genotypes detected by hybridization with type-specific probes immobilized on a solid-phase support." Journal of Virological Methods **110**(1): 29.

<http://www.sciencedirect.com/science/article/B6T96-48B0RKS-1/2/ea1b60574eee9bfdd94e8a79cb120c39>

A genotype-specific probes assay (GSPA) was developed for distinguishing the seven genotypes (A-G) of hepatitis B virus (HBV). Nucleotide (nt) sequences corresponding to preS1 region were amplified by PCR with a primer labeled with biotin, and delivered to eight wells on which complementary sequences specific to one or other genotype had been immobilized. Thereafter,

hybridization of HBV DNA sequences amplified from the test serum was detected by colorimetry. When 256 sera from HBV carriers in Bangladesh, Cameroon, Japan, South Africa, USA and Uzbekistan were subjected to GSPA, genotypes were concordant with those of ELISA with monoclonal antibodies to epitopes on preS2-region products in 242 (94.6%) of them; 8 sera (3.1%) were not genotypeable by either method. Cloning analysis confirmed the presence of two distinct HBV genotypes in the seven selected sera with coinfection. There were 7 (2.7%) sera with discordant genotyping results between GSPA and ELISA. When HBV DNA clones propagated from these sera were sequenced and analyzed phylogenetically, the genotypes determined by GSPA were verified. Coinfection with HBV strains of two distinct genotypes was identified by GSPA in 28 (10.9%) sera, while it was suggested by ELISA in only 2 (0.8%) sera. The GSPA method would be particularly useful for detecting the coinfection with distinct HBV genotypes of any clinical relevance, which seems to be more frequent than reported previously.

Kato, S., Y. Hiraishi, et al. (1998). "A plaque hybridization assay for quantifying and cloning infectious human immunodeficiency virus type 1 virions." *Journal of Virological Methods* **72**(1): 1.

<http://www.sciencedirect.com/science/article/B6T96-3SXDERR-1/2/f51fff8e04c825e5117efdc82ec50011>

A biological method was developed for quantifying and cloning of infectious virions of human immunodeficiency virus type 1 (HIV-1). Virus preparations were mixed with permissive cells for binding, and the cells were cast in an agarose gel. After incubation for 9 days viral particles released from infected cells propagating from each initially infected cell were transferred on nylon membrane and subjected to hybridization using a radioactive HIV-1 DNA probe. Infectious centers of HIV-1 were detected as hybridization spots on autoradiographs regardless of cytopathic effects or syncytium formation. Three different CD4⁺ cell lines (MT-4, MOLT-4 and U937) and peripheral blood mononuclear cells from healthy donors were used as recipient cells. Infectious virions were recovered from a portion of agarose gel corresponding to each hybridization spot. This assay is suitable for quantifying infectious HIV-1 virions with different cell tropisms and for investigating the relationship between the phenotype and genotype of HIV-1 at a clonal level.

Kay, P., K. Meehan, et al. (2002). "The use of nested polymerase chain reaction and restriction fragment length polymorphism for the detection and typing of mucosal human papillomaviruses in samples containing low copy numbers of viral DNA." *Journal of Virological Methods* **105**(1): 159.

<http://www.sciencedirect.com/science/article/B6T96-460DN3D-2/2/8d3a222d9b4ec8b3c8d97c518fd09b80>

Mucosal human papillomaviruses (HPVs) that infect the genital area have also been shown to infect the oral cavity. In this study a restriction fragment length polymorphism (RFLP) method was developed on a nested polymerase chain reaction (PCR) product to identify ten high risk HPV types 16, 18, 31, 33, 35, 45, 51, 52, 58 and 59 as well as the low risk HPV 11. HPV DNA was detected in 23/31 (74%) of buccal specimens using a sensitive nested PCR employing degenerate consensus primers (Williamson and Rybicki, 1991). Consensus PCR using the PGM09/11 primers was able to detect HPV in only 29% of the specimens that had tested positive using the nested HPV PCR primers. HPV 11 type specific primers detected HPV 11 DNA in only 66% of the specimens showing HPV 11 DNA by means of nested PCR and RFLP. A Genbank search revealed that the PCR primers could detect a wide range of mucosal HPV types including types HPV 70, 72 and 73 which have all been isolated from immunocompromised patients. Of the 23 buccal specimens that were positive for HPV DNA, 13 were single infections, five were dual infections and three were triple infections. The HPV types identified by RFLP were:

HPV 11 (18/23), HPV 18 (8/23), HPV 16 (3/23), and HPV 33 (1/23). HPV 13 (2/23) was identified by direct sequencing of the inner amplicon of the PCR product.

Kaye, S., C. Loveday, et al. (1991). "Storage and preservation of whole blood samples for use in detection of human immunodeficiency virus type-1 by the polymerase chain reaction." Journal of Virological Methods **35**(2): 217.

<http://www.sciencedirect.com/science/article/B6T96-476RM29-14/2/ca3b2230e241dea9b5bde9cab36a6b2b>

Methods used in the diagnosis of human immunodeficiency virus type-1 (HIV-1) infection by the polymerase chain reaction (PCR) usually require the separation of lymphocytes from a whole-blood sample within 24 hours of patient sampling. A method is described in which blood samples are mixed with a cryopreservative ('Glycigel'), stored frozen, and DNA suitable for use in an HIV PCR recovered. Samples can be stored at --20[deg]C for up to 3 months and still give positive results with all samples from infected patients; storage at --80[deg]C for at least 3 months shows no loss of titre. The method shows no loss of sensitivity compared to previously described sample preparation methods. Deglycerolised Glycigel supernatants were found to be suitable for conventional anti-HIV-1 serological studies and loss of sensitivity only represented the dilution effect due to sample preparation. Application of the method as a means of storing samples frozen at the point of sampling and transporting them to a central laboratory for processing is demonstrated using samples taken from HIV-1-infected mothers and their babies.

Khare, M., M. Sharland, et al. (2004). "Use of serial maternal urine cytomegalovirus PCR to detect primary CMV infection in seronegative pregnant women." Journal of Virological Methods **119**(1): 31.

<http://www.sciencedirect.com/science/article/B6T96-4C4X49B-1/2/481324f9b43b650ff596845f3a38616a>

The aim of the study was to determine if serial maternal urine polymerase chain reaction (PCR) tests can detect primary CMV infection during pregnancy. This was a prospective study conducted from 1 January 1999 to 31 December 1999 in an antenatal clinic setting of a teaching hospital. The study group included women who were CMV IgG negative and aged At first attendance, 1549 (42%) women were CMV IgG negative. Of the 696 eligible women, 609 (88%) participated in the urine PCR study. PCR was performed on 2263 urine samples (median of 4/pregnancy). Primary CMV infection was identified in one woman by urine PCR at 36 weeks (baby CMV negative). Cord blood samples were available from 152/609 infants (25%). Seroconversion was noted in only one woman. Replies to the questionnaire were received from 264/609 women (43%): 214 (81%) had little or no anxiety, and 220 (83%) felt reassured by their study participation. Serial urine PCR is a feasible method of detecting primary maternal CMV infection during pregnancy which has potential for evaluation in further studies.

Kim, Y., S. M. Gharaibeh, et al. (2002). "Comparison and verification of quantitative competitive reverse transcription polymerase chain reaction (QC-RT-PCR) and real time RT-PCR for avian leukosis virus subgroup J." Journal of Virological Methods **102**(1-2): 1.

<http://www.sciencedirect.com/science/article/B6T96-456T3KB-1/2/6e9c9b6a0052a9435c1a9243adcf070c>

Avian leukosis virus subgroup J (ALV-J) infections cause significant economic losses because of increased mortality, tumor production, decreased production, and cost for eradication. Current quantification methods for ALV-J expressed by TCID₅₀ are difficult to determine because of the lack of cytopathic effect in cell cultures and non-specificity of currently available antigen-capture ELISA tests. In this study, a one-tube fluorescent probe based real time RT-PCR method was developed for quantification of ALV-J and compared with available quantification methods. Cell lysates with different TCID₅₀s determined by cell culture and antigen capture ELISA (ag-ELISA) were used for one-tube real time RT-PCR using fluorogenic probe and quantitative competitive RT-PCR (QC-RT-PCR). The results of QC-RT-PCR and real time RT-PCR were highly correlated to the TCID₅₀s determined by conventional culture methods. They were also very specific, sensitive, easy to perform, reproducible, and rapid compared with conventional methods. These RT-PCR based quantification methods of ALV-J viral RNA will be useful for virological and pathogenesis studies.

Klein, S. A., S. Karsten, et al. (2003). "Comparison of TaqMan(TM) real-time PCR and p24 Elisa for quantification of in vitro HIV-1 replication." *Journal of Virological Methods* **107**(2): 169.

<http://www.sciencedirect.com/science/article/B6T96-47DTDTF-9/2/b94c148adc8c912c06fe39684545b9df>

In this study, TaqMan(TM) PCR was used to assess viral replication of HIV-1 infected cells in vitro. This PCR technique was compared with p24 ELISA as a standard method to monitor HIV-1 replication in cell culture. Hut78 T-lymphoblastoid cells were infected with different titres of HIV-1IIIB (MOI 0.05-0.0005). The course of HIV-1 replication was monitored by determination of p24 concentrations by ELISA in cell culture supernatants and by quantitation of HIV-1 gag RNA by TaqMan(TM) RT-PCR. Additionally, the number of HIV-1 proviral copies was assessed by TaqMan(TM) PCR. Monitoring of HIV-1 replication by p24 ELISA and TaqMan(TM) RT-PCR revealed comparable kinetics of infection. Both methods provided similar data on the exponential increase and on plateauing of HIV-1 replication. Furthermore, both methods were equally sensitive. However, a 7 log linearity of TaqMan(TM) HIV-1 gag PCR was demonstrated without dilution of the specimen, in contrast to p24 ELISA, where because of its narrow range of detectable p24 concentrations, sample dilution was necessary. Although determination of the number of proviral copies by TaqMan(TM) PCR does not measure HIV-1 replication, the kinetics of proviral copy number following in vitro inoculation of cells with HIV-1 was nearly the same as the kinetics of HIV-1 RNA copy numbers. In conclusion, TaqMan(TM) real-time RT-PCR was demonstrated as a reliable and sensitive tool to quantify and monitor HIV-1 replication in cell culture. It is suggested, therefore, that this technique be an alternative method to monitor HIV-1 replication in vitro.

Koch, N., N. Yahi, et al. (1999). "Genetic polymorphism near HIV-1 reverse transcriptase resistance-associated codons is a major obstacle for the line probe assay as an alternative method to sequence analysis." *Journal of Virological Methods* **80**(1): 25.

<http://www.sciencedirect.com/science/article/B6T96-3WM5CT3-4/2/92f54634868a5eb138d5389ecd3ae41d>

The performance of the line probe assay (LIPA) for the detection of mutations conferring resistance to nucleoside inhibitors of HIV-1 reverse transcriptase was evaluated in comparison with sequence analysis. The tests were undertaken on plasma samples from 63 patients (61 receiving combination therapy and 2 without treatment at the time of inclusion). In 27 cases (43%) which included codons 41, 69, 70, 74, 184 and 215, the sequence of the RT gene was distinct from the hybridization probes used in LIPA. Correspondingly, LIPA gave uninterpretable results in

15, 30 and 41% of cases for codons 184, 215 and 41, respectively. Overall, the concordance between LIPA and sequence analysis varied from 52% (codons 41 and 215) to 85% (codon 70). These data show that the polymorphism of the nucleotide sequence near resistance-associated codons is a major shortcoming of LIPA.

Koch, W. C. (1995). "A synthetic parvovirus B19 capsid protein can replace viral antigen in antibody-capture enzyme immunoassays." Journal of Virological Methods **55**(1): 67.

<http://www.sciencedirect.com/science/article/B6T96-3YYTF0W-16/2/706a803b814c4e78c220b3b597852ab7>

To establish a renewable source of parvovirus B19 antigens for diagnostic tests, gene sequences for the viral capsid proteins, VP1 and VP2, were cloned into baculovirus expression vectors and the recombinant viruses used to infect Sf9 insect cells. Cell lysates examined by immunoblotting demonstrated reactive proteins corresponding to the expected sizes of native VP1 (83 kDa) and VP2 (58 kDa). The VP2 protein was produced efficiently in quantity and self-assembled into empty capsids as shown by density equilibration in a CsCl step gradient. The VP2 protein was purified and used as an antigen in antibody-capture enzyme immunoassays for the detection of B19 IgG and IgM antibodies. Compared to a standard antibody-capture EIA based on whole viral antigen, the VP2-EIA gave a sensitivity of 100% and specificity of 97% in detection of B19 IgM in 138 patients suspected of B19 infection. No IgM-positive specimens were missed. IgG detection yielded a sensitivity of 100% and specificity of 96% in the same population. Recombinant VP2 capsid proteins expressed in baculovirus-infected insect cells can substitute for serum-derived B19 virus in standard antibody-capture EIA for the detection of B19 IgG and IgM with comparable results.

Koskiniemi, M., L. Mannonen, et al. (1997). "Luminometric microplate hybridization for detection of varicella-zoster virus PCR product from cerebrospinal fluid." Journal of Virological Methods **63**(1-2): 71.

<http://www.sciencedirect.com/science/article/B6T96-3RHM7T6-11/2/27d2c8daff233d32eae2eab70a91d62>

We modified and optimized a new microplate hybridization assay to detect the varicella-zoster virus (VZV) PCR product, and studied cerebrospinal fluid (CSF) samples of 287 patients with meningitis, encephalitis or other neurological diseases or symptoms. Specific antibodies to VZV and reference antigens were determined by enzyme immunoassay from serum and CSF, they were then compared with clinical findings and with the results obtained by VZV-PCR using different detection methods for VZV-specific amplified DNA. VZV DNA was found in the CSF of 25 patients using the microplate hybridization assay and chemiluminescence detection for amplified DNA. All 25 CSF samples were also positive in Southern blotting. Among the patients, 10 had chickenpox, 4 had shingles, and 11 had no rash at all. The detection rate of VZV-specific DNA by microplate hybridization was 30% higher than that obtained by conventional agarose gel electrophoresis. In most patients the diagnosis was confirmed by demonstrating specific intrathecal antibody production to VZV but not to other viruses. These results indicate the presence of VZV in the central nervous system (CNS) in many patients with chickenpox or shingles, and even in patients without a rash. The microplate hybridization assay based on chemiluminescence detection improves considerably the detection rate of the VZV-PCR product compared to agarose gel electrophoresis and will add to the list of recognized VZV infections in the CNS. It is especially useful in cases where there is no cutaneous manifestation.

Kumar, R., N. Vandegraaff, et al. (2002). "Evaluation of PCR-based methods for the quantitation of integrated HIV-1 DNA." Journal of Virological Methods **105**(2): 233.

<http://www.sciencedirect.com/science/article/B6T96-4619PYS-2/2/50ec536b8842a95e00e4b09d1a2a1017>

Integration of HIV-1 DNA is essential both for productive viral replication and for viral persistence in patients. Methods to measure specifically proviral HIV DNA are required for investigating the mechanisms of HIV integration, for screening novel integrase inhibitors in cell culture and for monitoring levels of persistent integrated viral DNA in patients. In this report, the linker primer polymerase chain reaction (LP-PCR) and Alu-PCR methods for the quantitation of integrated HIV-1 DNA have been modified and evaluated. Each of the two modified assays allowed the quantitative detection of 4 copies of integrated HIV DNA in presence of 2×10^5 cell-equivalents of human chromosomal DNA. The results show that proper DNA isolation procedures and the inclusion of appropriate controls in these assays are important for the accurate quantitation of integrated HIV DNA. With further improvements, it should be possible to use these methods as diagnostic tools to monitor closely the efficacy of antiretroviral therapy.

Kuno, G. (1998). "Universal diagnostic RT-PCR protocol for arboviruses." Journal of Virological Methods **72**(1): 27.

<http://www.sciencedirect.com/science/article/B6T96-3SXDERR-4/2/86a293289d7bd9826c94221c46474c83>

A selected number of PCR protocols were evaluated to determine if they could serve as a universal protocol for detecting and identifying all arboviruses. In this study, four parameters that affect the efficacy of RT-PCR (RNA extraction method, choice of reverse transcriptase, choice of DNA polymerase and thermocycling program) were evaluated in combination. The most optimal combination of those parameters employed use of silica gel membrane spin column, RAV-2 reverse transcriptase, Tth DNA polymerase, and a simple modification of a published thermocycling program. By this modified protocol, viral RNA could be amplified satisfactorily with more than 50 pairs of primers designed for diagnosis of arboviruses representing five families. The sensitivity and specificity obtained by this universal protocol were comparable to those obtained by the original protocol for each primer pair tested; and for some primers, improved sensitivity was observed. It was also found that a simple modification of a suggested protocol of a commercial RT-PCR kit could produce nearly identical results and serve as another universal protocol. With the use of a universal diagnostic reverse transcriptase-polymerase chain reaction (RT-PCR) protocol, simultaneous screening of clinical or biological specimens against a large number of RNA viruses belonging to many families can be performed more efficiently for etiologic determination in the situations complicated by the difficulty of differential diagnosis. Furthermore, such a universal protocol facilitates reducing the cost of PCR-based diagnostic operation and standardizing the qualities of PCR-based diagnosis within an institution or among collaborating institutions. A logical strategy is to conduct diagnosis in two stages by using broadly group-reactive primers in the first stage to narrow the range of possible etiologic agents and using virus-specific primers in the second stage for identification. Before such a strategy is employed, however, more group-reactive primers for a large number of arboviruses, for which no such primers currently exist, must be made available. Furthermore, the best pair or pairs of primers need to be selected for each virus for the second stage of the strategy.

Laassri, M., V. Chizhikov, et al. (2003). "Detection and discrimination of orthopoxviruses using microarrays of immobilized oligonucleotides." Journal of Virological Methods **112**(1-2): 67.

<http://www.sciencedirect.com/science/article/B6T96-496G002-1/2/007ea0d0150c0536163d989adc6b4e14>

Variola virus (VARV), causing smallpox, is a potential biological weapon. Methods to detect VARV rapidly and to differentiate it from other viruses causing similar clinical syndromes are needed urgently. We have developed a new microarray-based method that detects simultaneously and discriminates four orthopoxvirus (OPV) species pathogenic for humans (variola, monkeypox, cowpox, and vaccinia viruses) and distinguishes them from chickenpox virus (varicella-zoster virus or VZV). The OPV gene C23L/B29R, encoding the CC-chemokine binding protein, was sequenced for 41 strains of seven species of orthopox viruses obtained from different geographical regions. Those C23L/B29R sequences and the ORF 62 sequences from 13 strains of VZV (selected from GenBank) were used to design oligonucleotide probes that were immobilized on an aldehyde-coated glass surface (a total of 57 probes). The microchip contained several unique 13-21 bases long oligonucleotide probes specific to each virus species to ensure redundancy and robustness of the assay. A region approximately 1100 bases long was amplified from samples of viral DNA and fluorescently labeled with Cy5-modified dNTPs, and single-stranded DNA was prepared by strand separation. Hybridization was carried out under plastic coverslips, resulting in a fluorescent pattern that was quantified using a confocal laser scanner. 49 known and blinded samples of OPV DNA, representing different OPV species, and two VZV strains were tested. The oligonucleotide microarray hybridization technique identified reliably and correctly all samples. This new procedure takes only 3 h, and it can be used for parallel testing of multiple samples.

Leary, T. P., J. C. Erker, et al. (1999). "Optimized PCR assay for the detection of TT virus." Journal of Virological Methods **82**(2): 109.

<http://www.sciencedirect.com/science/article/B6T96-3XT0BN2-1/2/218fc9f1a094d1bcbb614bf4c889a922>

A polymerase chain reaction (PCR)-based procedure for the detection of TT virus DNA is described. In this method, total nucleic acid extracted from a small volume of serum or plasma is utilized as a template in PCR employing TT virus specific primers designed to highly conserved regions of the virus genome. Additional sensitivity is obtained by carrying out a second round of amplification. Reactions are analyzed by agarose gel electrophoresis, and samples having an ethidium bromide stainable fragment of the appropriate size in the first and/or second amplification are designated as positive. This protocol allows for the rapid and sensitive detection of TT virus in human plasma or serum.

Leary, T. P., J. C. Erker, et al. (2002). "Detection of reovirus by reverse transcription-polymerase chain reaction using primers corresponding to conserved regions of the viral L1 genome segment." Journal of Virological Methods **104**(2): 161.

<http://www.sciencedirect.com/science/article/B6T96-45NPJB7-2/2/6c1eba241a205166b4ff2f88b41d233b>

A rapid, reverse transcription-polymerase chain reaction (RT-PCR) procedure for the detection of reovirus RNA in cell culture is described. Total nucleic acids are extracted from a small volume of cell culture supernatant and reverse transcribed using random hexamers. An aliquot of cDNA is

then utilized in nested PCR. The PCR primers correspond to sequences conserved between prototype reovirus strains type 1 Lang, type 2 Jones, and type 3 Dearing, as well as those of several reovirus field-isolate strains. Reactions are analyzed by agarose gel electrophoresis, and samples showing a band of the appropriate size in the first and second amplification, or in the second amplification alone, are designated as positive. This protocol allows for the rapid and sensitive detection of reovirus in cell culture. The RT-PCR methods described below can easily be adapted to the amplification of reovirus from other media, including preserved tissues, clinical specimens, and water.

Lednicky, J. A. and J. S. Butel (1997). "A coupled PCR and restriction digest method for the detection and analysis of the SV40 regulatory region in infected-cell lysates and clinical samples." Journal of Virological Methods **64**(1): 1.

<http://www.sciencedirect.com/science/article/B6T96-3RHM7T6-1T/2/5e1cc0d23751272b6e2e2c9f33c73422>

The polymerase chain reaction (PCR) is an increasingly popular analytical tool for the detection of virus sequences in laboratory preparations as well as in human clinical samples. In studies involving papovaviruses SV40, BK virus (BKV), and JC virus (JCV), one of the primary targets for analysis is the viral regulatory region, as that section of the papovavirus genome is distinct. A primary concern with PCR-based studies is whether amplified DNA sequences may be derived from laboratory contaminants. Recognizing that common sources of PCR contamination are the positive control templates, we devised a facile method to distinguish between real and false-positive PCR-amplified SV40 regulatory region DNAs. SV40 constructs that had been engineered to contain different combinations of 72-basepair (bp) enhancer elements and 21-bp repeats, as well as two introduced unique restriction enzyme sites, were used as positive control templates for PCR amplification. Cleavage of PCR-amplified DNA identifies products from the engineered control plasmids. The procedure is rapid, simple and cost-effective. We also report that primer sets predicted to be specific for the SV40 regulatory region can be used to amplify BKV and JCV regulatory region sequences under conditions of reduced stringency.

Lee, K., Y.-G. Kim, et al. (2003). "Shuttle PCR-based cloning of the infectious adeno-associated virus type 5 genome." Journal of Virological Methods **111**(2): 75.

<http://www.sciencedirect.com/science/article/B6T96-4909KV7-1/2/d0e897df2d49beb9c542361f3efdddae>

Adeno-associated virus type 5 (AAV5), which is distinct from the other serotypes of AAV, has attracted considerable interest as a premier gene delivery vector. As do the other serotypes, AAV5 contains its 4.7 kb-sized, single-stranded genome flanked with inverted terminal repeats (ITRs) in a hairpin conformation, which serves frequently as pause and arrest sites for DNA polymerases during PCR. To amplify the full-length of the AAV5 genome in single step, we established a shuttled, long and accurate PCR (LA-PCR) procedure in the present study. Furthermore, helper oligonucleotides, which hybridize with the palindromic sequence elements in ITR, were designed and employed in PCR to prevent the formation of hairpin structures by highly GC-rich ITRs. Consequently, a 4.7 kb-sized PCR product was amplified successfully, and cloned into a pBluescript(R) II KS(+) plasmid. Six plasmids, harboring the full-length AAV5 genome, rescued wild type AAV5 viruses on transfection to HeLa and HEK 293 cells, which were co-infected with helper adenoviruses. Western and Southern blot analyses supported further the fact that the pAAV5 plasmids harbored the full-length AAV5 genome. The PCR method described in this study is applicable for the cloning of genomes containing variable palindromic structures, in addition to AAV genomes of other serotypes.

Legeay, O., S. Bounaix, et al. (1997). "Development of a RT-PCR test coupled with a microplate colorimetric assay for the detection of a swine Arterivirus (PRRSV) in boar semen." Journal of Virological Methods **68**(1): 65.

<http://www.sciencedirect.com/science/article/B6T96-3RYCMYM-X/2/6615b6b3f64d2a785fcc20c09bffd5>

Transmission of porcine reproductive and respiratory syndrome virus (PRRSV) through boar semen has been demonstrated, stressing the need for a reliable semen PRRSV detection test. A diagnostic assay was developed based on amplification of the PRRSV RNA by reverse transcription and polymerase chain reaction (RT-PCR) followed by detection of the amplification products by hybridization and colorimetric assay in microwell plates. A highly reproducible and efficient method of viral RNA isolation from semen samples was set up. A combined RT-PCR procedure was performed, incorporating the use of uracil-N-glycosylase (UNG) in combination with dUTP instead of dTTP to prevent false positive results due to carry-over contamination. An RNA internal control was added during the RNA isolation procedure to detect false negative results. The colorimetric detection in microwell plates of amplification products from either PRRSV or IC RNA, gave specific and objective results and was automated. A cut-off value of 1000 RNA copies or 10 TCID₅₀ of PRRSV per ml of semen samples could be detected with this assay. Semen samples collected from experimentally-infected boars were tested with this assay and showed PRRSV excretion early after infection and for an extended period.

Levy, L., I.-M. Lee, et al. (1994). "Simple and rapid preparation of infected plant tissue extracts for PCR amplification of virus, viroid, and MLO nucleic acids." Journal of Virological Methods **49**(3): 295.

<http://www.sciencedirect.com/science/article/B6T96-476TY1K-5/2/7de6b4c05bc1b67103801eec2e37183f>

A rapid, simple method for preparing plant tissues infected with viruses, viroids, or MLOs using a commercial product known as Gene ReleaserTM is described. The Gene ReleaserTM polymeric matrix method produced plant extracts suitable for PCR amplification without the use of organic solvents, ethanol precipitation, or additional nucleic acid purification techniques. Modification of maceration methods and/or extraction buffers resulted in the PCR amplification of potato spindle tuber, apple scar skin, and dapple apple viroids, as well as, genomic segments of plum pox potyvirus, grapevine virus B, grapevine leafroll-associated virus III, and elm yellows MLO. These pathogens were amplified from tissue of woody and herbaceous hosts such as peach, apricot, apple, grapevine, elm, periwinkle and potato. The application of this product for use with intractable tissue avoids lengthy and laborious extraction procedures. In our hands, about 20 samples could be prepared for PCR or RT-PCR in 1-2 h versus 1-3 days.

Lewis, J. G., G.-J. Chang, et al. (1992). "Direct sequencing of large flavivirus PCR products for analysis of genome variation and molecular epidemiological investigations." Journal of Virological Methods **38**(1): 11.

<http://www.sciencedirect.com/science/article/B6T96-476RMMH-49/2/1fa0ee383a7171f49574c6fceb90f585>

The polymerase chain reaction (PCR) was used to amplify viral DNA from selected regions of dengue genomic RNA by using appropriate 'consensus' primers. DNA amplicons containing the

structural genes from all 4 dengue serotypes were prepared and directly sequenced using dengue-virus-specific primers. This method can characterize reliably flavivirus field isolates at the molecular level without extensive virus propagation and molecular cloning, and will be a valuable tool for molecular epidemiological studies.

Lindberg, A. M. and A. Andersson (1999). "Purification of full-length enterovirus cDNA by solid phase hybridization capture facilitates amplification of complete genomes." Journal of Virological Methods **77**(2): 131.

<http://www.sciencedirect.com/science/article/B6T96-3VR1B1H-3/2/d12b4eab7656f410aa9b47c4a5375c63>

The aim of the study was to develop a method for the selective purification of full-length enterovirus single strand (ss) cDNA for subsequent amplification of complete enterovirus genomes by long distance PCR. As a model system we have used the prototype strain of echovirus 5 (EV5). Due to inefficient first strand cDNA synthesis using EV5 RNA as template, only a few molecules of EV5 sscDNA were completely reverse transcribed and no amplification products were observed when long distance polymerase chain reaction (LD-PCR) was used for amplification of complete EV5 genomes. To purify the complete EV5 cDNA present, an oligonucleotide, derived from the conserved 5' end of an enterovirus genome, was immobilized on paramagnetic beads and complete EV5 sscDNA was captured and purified from the less than full-length cDNAs. LD-PCR using the purified EV5 cDNA resulted in amplification of complete EV5 genomes. Transfection of the EV5 RNA transcribed from these uncloned amplicons resulted in production of replicating viruses. This demonstrates that solid phase hybridization capture of sscDNA is an efficient method that can be used for enrichment and purification of full-length enterovirus sscDNAs.

Lindstrom, A. and J. Albert (2003). "A simple and sensitive 'in-house' method for determining genotypic drug resistance in HIV-1." Journal of Virological Methods **107**(1): 45.

<http://www.sciencedirect.com/science/article/B6T96-46YXPVK-1/2/ed6de7176e753ff6aab9650b50d5d8b6>

Antiretroviral combination therapy is a major advance in the treatment of HIV infection, but development of antiretroviral resistance is still an important cause of treatment failure. Therefore, resistance testing was recommended recently for follow-up of HIV-1 infected individuals. The aim of this study was to develop a new genotypic resistance assay because simple and affordable assays with sufficient sensitivity for different genetic subtypes and low copy number samples are still lacking. Different methods and primers for RNA extraction from plasma, cDNA synthesis, nested PCR and sequencing on an ABI310 automated sequencer were evaluated and optimised. The PCR was designed to amplify a fragment covering the protease and the first half of the reverse transcriptase (RT), which harbour most known resistance mutations to licensed antiretroviral drugs. Resistance mutations were identified using resistance analysis tools available over the Internet. The optimised assay had a sensitivity of approximately 200 RNA copies per ml. The method was evaluated on plasma samples from treated patients infected with different subtypes of HIV-1 and appeared to have similar sensitivity for all subtypes. Samples that had failed previously in routine testing were analysed successfully with the new assay. The new assay is more sensitive and robust than the current routine method.

Liu, H.-J. and J. J. Giambrone (1997). "Amplification, cloning and sequencing of the [sigma]C-encoded gene of avian reovirus." Journal of Virological Methods **63**(1-2): 203.

<http://www.sciencedirect.com/science/article/B6T96-3RHM7T6-1H/2/5790e7b61c10fbfb2ba8900d41488400>

The [sigma]C-encoding cDNA of avian reovirus (ARV) 1733 strain was amplified, cloned and sequenced using double nested polymerase chain reaction (PCR). The ARV [sigma]C protein is a minor component of the outer capsid that induces type-specific neutralization antibodies. Four overlapping [sigma]C-encoding cDNA fragments were obtained. Together, the four fragments represented the whole coding sequence. The nucleotide and deduced amino acid sequences of [sigma]C-encoded gene of U.S. (S1133 and 1733) and Australian isolates (RAM-1 and SOM-4) were compared. The U.S. isolates were closely related, but different from Australian isolates. The degree of differences between the U.S. and Australian isolates was over 44.89% at both the nucleotide and deduced amino acid levels and suggested that the virus is evolving separately in different continents. The deduced amino acid sequences of ARV [sigma]C indicated a heptapeptide repeat in the N-terminal region of ARV [sigma]C existed in all ARVs. The results suggested that ARV [sigma]C is structurally related to mammalian reovirus (MRV) [sigma]1.

Liu, H.-J., J. J. Giambrone, et al. (1994). "Detection of genetic variations in serotype I isolates of infectious bursal disease virus using polymerase chain reaction and restriction endonuclease analysis." Journal of Virological Methods **48**(2-3): 281.

<http://www.sciencedirect.com/science/article/B6T96-476F6FR-F0/2/8d9eb2293f30db9da00d0aad2bf7aab>

Reverse transcription with polymerase chain reaction (PCR) followed by restriction endonuclease analysis detected genetic variations among serotype I isolates of infectious bursal disease virus (IBDV). Using a set of synthetic primers derived from the large genome segment of APHIS-IBDV, the hypervariable region (AccI-SpeI fragment) located in the VP2 gene was amplified. With all strains, a cDNA fragment of approximately 643 bp was amplified, indicating that there were no apparent deletions or insertions in this region among isolates. Fragments amplified from 9 isolates were digested with 14 restriction enzymes. Restriction fragment profiles generated by restriction enzymes NaeI, StuI, TaqI, and SacI, showed genetic variations among isolates. This study provided a simple and sensitive method for detection of genetic variations among isolates that are closely related serologically and could not be differentiated using current serologic methods.

Liu, H. J., J. J. Giambrone, et al. (1997). "Molecular characterization of avian reoviruses using nested PCR and nucleotide sequence analysis." Journal of Virological Methods **65**(2): 159.

<http://www.sciencedirect.com/science/article/B6T96-3RJG407-3/2/71d5c699c9ae427595bd1952d9d58c72>

A nested polymerase chain reaction (PCR) with subsequent nucleotide sequence analysis identified and differentiated avian reoviruses (ARVs). PCR products amplified from the S1 gene segment of ARV of USA isolates were 738 and 342 bp, respectively. PCR products were confirmed by Southern and dot blot hybridizations. The amplified cDNA fragments were cloned into the pUC18 vector and subjected to DNA sequencing. The nucleotide and deduced amino acid sequences of four USA (S1133, 1733, 2408, and CO8) and two Australian isolates (RAM-1 and SOM-4) were compared. Results of paired difference analysis and a predicted dendrogram

revealed that USA isolates were closely related, but different from, Australian isolates. The deduced amino acid sequences of the N-terminal region of ARV [sigma]C showed a heptapeptide repeat of hydrophobic residues in all ARV isolates.

Lokensgard, J. R., D. G. Thawley, et al. (1991). "Enzymatic amplification of latent pseudorabies virus nucleic acid sequences." Journal of Virological Methods **34**(1): 45.

<http://www.sciencedirect.com/science/article/B6T96-476RM08-6/2/71eb01468a54bc451de3ccf8eb88addf>

To investigate various aspects of the latency of pseudorabies virus in swine (PRV, suid herpesvirus 1) we developed in vitro nucleic acid amplification methods based upon the polymerase chain reaction. Primers flanking a 156-bp region of the pseudorabies virus gp II gene were annealed to purified PRV DNA as well as DNA isolated from the trigeminal ganglia of swine latently infected with PRV and subjected to PCR amplification. Following amplification, 100 fg of PRV DNA was visualizable on stained gels and 1 fg (equivalent to 6 viral genome copies) was detectable when amplification was combined with blot hybridization. PRV-specific DNA sequences which remained undetectable by direct blot hybridization assays were amplified to levels visualizable on ethidium-bromide-stained gels in 5 of 5 experimental latently infected animals. In addition, oligonucleotide primers specific for a 223-bp region of the PRV immediate-early gene (IE 180) were capable of amplifying overlapping latency associated transcripts (LATs), via a cDNA intermediate, in 6 of 6 latently infected swine. These nucleic acid amplification methods should be applicable to the investigation of PRV latency, and gene expression during latency and reactivation, in which few cells harbor latent virus.

Lovatt, A., J. Black, et al. (1999). "High throughput detection of retrovirus-associated reverse transcriptase using an improved fluorescent product enhanced reverse transcriptase assay and its comparison to conventional detection methods." Journal of Virological Methods **82**(2): 185.

<http://www.sciencedirect.com/science/article/B6T96-3XT0BN2-B/2/296d10f4df7d1baea340b4627345daf3>

The development and application of a novel, sensitive TaqMan fluorescent probe-based product enhanced RT test (F-PERT) for the detection of retrovirus are described. The assay allows discrimination between the amplification signals generated by genuine positive signals that result from retroviral RT activity and the RT-like activity from DNA polymerases. The RT-like activity from DNA polymerases was suppressed by the addition of activated calf-thymus DNA with no reduction in the RT activity. A linear relationship between threshold cycle (CT) and the number of virus particles was demonstrated, allowing quantification of retroviruses in unknown samples. The F-PERT assay was able to detect a wide range of retroviral RT activities, including that from porcine endogenous retrovirus (PoERV), murine leukaemia virus (MLV), simian foamy virus (SFV), simian immunodeficiency virus (SIVmac) and squirrel monkey retrovirus (SMRV). The detection limit of SMRV, MLV and PoERV was approximately 100 virion particles and the test was able to detect at least 102 molecules of purified RT enzyme. RT activity was not detected in cellular lysates and supernatants from MRC-5, BT, VERO, or Raji cells, whereas RT activity was detected in C1271, Mus dunni, K-Balb, BHK-21, CHO-K1, SP2/0-Ag14 and NSO cell supernatants. RT activity was also detected in the Spodoptera cell line Sf9.

M. Shamloul, A., N. A. Abdallah, et al. (2001). "Sensitive detection of the Egyptian species of sugarcane

streak virus by PCR-probe capture hybridization (PCR-ELISA) and its complete nucleotide sequence." Journal of Virological Methods **92**(1): 45.

<http://www.sciencedirect.com/science/article/B6T96-427JWSG-6/2/bb1896ce52bc9d2c82bef67844bcb837>

A rapid and sensitive assay for the specific detection of Sugarcane streak virus (SSV) using PCR-probe capture hybridization (PCR-ELISA) was developed. Nucleic acids suitable for PCR were extracted from SSV-infected tissue using organic solvents or Fast DNA kit. SSV cDNA was amplified using viral specific primers and the amplified SSV cDNA (amplicon) was DIG-labelled during the amplification process. The amplicon was then detected in a colorimetric hybridization system by a microtiter plate using a biotinylated cDNA (22 nt), cDNA (789 nt) or cRNA (789 nt) capture probe. This system combines the specificity of molecular hybridization, the ease of the colorimetric protocol, and is 10-100 fold more sensitive than agarose gel electrophoretic analysis in detecting the amplified product. Long cDNA or cRNA capture probe was 2-7 fold more sensitive than the oligo cDNA probe for the detection. Complete nucleotide sequence of SSV from Naga Hammady, Egypt, revealed that SSV-EG is a new species of SSV that shares 66% nucleotide identity with the virus species from Natal, South Africa.

Mackie, N., S. Dustan, et al. (2004). "Detection of HIV-1 antiretroviral resistance from patients with persistently low but detectable viraemia." Journal of Virological Methods **119**(2): 73.

<http://www.sciencedirect.com/science/article/B6T96-4C76GN5-2/2/f048012db030a1f2adef5984b4777052>

We modified the Abbott diagnostics HIV-1 Viroseq version 2 assay(TM) in order to detect the presence of HIV-1 drug resistance mutations in patients with viraemia below 1000 copies/ml of plasma. One hundred and forty-four patients with a detectable HIV-1 plasma viral load below 1000 copies/ml were selected and HIV-1 genetic analysis carried out using a modification of the Abbott Diagnostics Viroseq 2.0 assay(TM). The procedure differs from the standard protocol in that a nested PCR amplification step was introduced. The oligonucleotide primers for the first round of PCR were those supplied in the RT-PCR module of the kit. The nested PCR primers were primers A and H taken from the sequencing module. One hundred and twenty-eight out of 144 (89%) plasma samples with an HIV-1 viral load of less than 1000 copies/ml (ranging from 54 to 992 copies) were successfully sequenced. HIV-1 genotypes were obtained from 68 out of 81 (84%) samples with a viral load of greater than 50 but less than 300 copies/ml and 60/63 (95%) of samples with a viral load of greater than 300 but less than 1000 copies/ml. Serial dilution of a sample with a high viral load did not affect the detection of resistance mutations. Multiple sequencing of samples with low viral load did not result in detection of additional mutations, although, in one sample the K103N mutation was detected in 3/6 replicates while wild-type was detected in 2/6 and a mixture of wild-type/mutant in 1/6. Samples from patients infected with both clade B and non-B clades of HIV-1 could be genotyped at low copy number. Modification of the Abbott Viroseq assay allows reproducible sequencing of the HIV-1 genome from patients with low, but detectable, plasma virus burden.

Malet, I., M. Belnard, et al. (2003). "From RNA to quasispecies: a DNA polymerase with proofreading activity is highly recommended for accurate assessment of viral diversity." Journal of Virological Methods **109**(2): 161.

<http://www.sciencedirect.com/science/article/B6T96-4891C2T-1/2/71eda00073c8b9f038aed98b0ec9c9a5>

RNA viruses are characterized by their high rates of genetic variation. Their genetic diversity is generally studied by reverse transcription (RT) followed by polymerase chain reaction (PCR) amplification and nucleotide (nt) sequence determination. The misinterpretation of viral diversity due to copy errors introduced by the enzymes used in this two-step protocol has not yet been assessed systematically. In order to investigate the impact of such errors, we sought to bypass the intrinsic viral heterogeneity by starting from a homogeneous cDNA template. With this in mind, the hepatitis C virus (HCV) 5' non-coding region (5'NCR) was amplified either by PCR starting from a homopolymeric cDNA template or by RT-PCR starting from the in vitro RNA transcript derived from the same original cDNA template. Amplicons were cloned and the 17-20 individual clones were sequenced in each assay. Different quasispecies patterns were obtained with various commercially available DNA polymerases, resulting in different computed error rates. The non-proofreading Taq DNA polymerase provided the highest error rate which was seven times higher than that obtained with the most reliable of the proofreading polymerases tested. We, therefore, emphasize that the misleading interpretation of the observed heterogeneity for a given viral sample could be due to ignorance of the fidelity of the polymerase used for viral genome amplification, and thus that proofreading DNA polymerases should be preferred for the investigation of natural genetic diversity of RNA viruses.

Malmstrom, C. M. and R. Shu (2004). "Multiplexed RT-PCR for streamlined detection and separation of barley and cereal yellow dwarf viruses." Journal of Virological Methods **120**(1): 69.

<http://www.sciencedirect.com/science/article/B6T96-4CPM5P6-1/2/ae2085cad0e040878f54f4f6c0a38ab2>

Two novel multiplexed RT-PCR assays that can efficiently detect and distinguish among different barley and cereal yellow dwarf viruses (B/CYDVs) are described. The basic multiplex can produce two fragments simultaneously, a ~830-bp fragment indicating the presence of the BYDV-PAV, BYDV-MAV, or BYDV-SGV viruses and a ~372-bp fragment indicating the presence of the CYDV-RPV, BYDV-RMV, or BYDV-GPV viruses. The enhanced multiplex produces two additional fragments, which further differentiate between BYDV-PAV, BYDV-MAV, and BYDV-SGV. These assays fulfill the critical need for a streamlined diagnostic procedure for B/CYDVs that can be cost-effectively applied to large numbers of small samples. The assays are useful not only in the basic diagnosis of B/CYDVs, but also for studies examining the ecological roles of B/CYDVs in natural systems and for longer-term epidemiological studies of grasses and cereals.

Markowski-Grimsrud, C. J., M. M. Miller, et al. (2002). "Development of strain-specific real-time PCR and RT-PCR assays for quantitation of chicken anemia virus." Journal of Virological Methods **101**(1-2): 135.

<http://www.sciencedirect.com/science/article/B6T96-44M2HSJ-1/2/d513f8604d65fa9a9d84899d6a894076>

Chicken anemia virus (CAV) is a ubiquitous pathogen of poultry. A CAV specific TaqMan(TM)-based PCR and RT-PCR assay for real-time quantitation of viral load and relative quantitation of virus-specific transcript levels was developed. Detection of viral DNA copy number from infected MDCC-CU147 cells was determined by extrapolation from a CAV plasmid-based standard curve. Viral load increased proportionally with increasing cell number harvested, increasing from 4 x 10² copies in 250 cells with 38% virus positive cells in an indirect immunofluorescence assay to 8 x 10⁵ copies in 250,000 cells with 64% infected cells. The estimated average viral copy number per infected cell ranged from 5 to 14. Strain-specific primers were developed to distinguish between the Cux-1 and CIA-1 strains of CAV. These primers exhibited a 3 to 4 log differential in amplification comparing homologous versus heterologous virus-primer combinations. The

sensitivity of the real-time assay was found to be comparable to a nested PCR assay using DNA samples from a SPF poultry flock exposed to the SH-1 strain of CAV. The real-time PCR detected from 1.7 to 4.2 target molecules in three out of four samples that were positive by nested PCR using 50% of the DNA used in the nested PCR. Relative viral transcript levels for Cux-1 and CIA-1 infected cell cultures increased proportionally with increasing cell numbers harvested for RNA extraction. This assay will be important for both diagnosis and in understanding the complex pathogenesis of CAV infection.

Marshall, R. L., J. Cockerill, et al. (1998). "Detection of GB virus C by the RT-PCR LCx(R) system." Journal of Virological Methods **73**(1): 99.

<http://www.sciencedirect.com/science/article/B6T96-3T2PCND-F/2/a71c64db59aa0681dd9f0a9d2c65d436>

The recent publication of representative genomic sequences of GBV-C has permitted the selection of PCR primers for detection of GBV-C in clinical samples by PCR techniques. Traditional amplification methodologies which couple reverse transcription polymerase chain reaction (RT-PCR) and Southern blot detection are slow, cumbersome, and can be technique dependent. This has hampered studies to determine the clinical significance of GBV-C. We report the selection of highly conserved PCR primers and a probe useful for semi-automated RT-PCR using the Abbott LCx(R) system. This adaptation of the LCx(R) system expands its capabilities to include the detection of RNA by RT-PCR, in addition to DNA detection by ligase chain reaction (LCR).

Maudru, T. and K. Peden (1997). "Elimination of background signals in a modified polymerase chain reaction-based reverse transcriptase assay." Journal of Virological Methods **66**(2): 247.

<http://www.sciencedirect.com/science/article/B6T96-3RYCMYM-B/2/e6c2f0bca9b23a5dc1daf372cf6bedd1>

Three highly sensitive reverse transcriptase (RT) assays were recently published that are at least one million times more sensitive than conventional RT assays. These assays derive their high sensitivities through the ability to amplify the complementary DNA (cDNA) product of the RT reaction by the polymerase chain reaction (PCR). We describe a modified PCR-based RT (PBRT) assay that retains the high sensitivities of the original assays while reducing their inherent background signals. The background signal of the PBRT assay was found to be due to an intrinsic RNA-dependent DNA polymerase activity of the Taq DNA polymerase, the enzyme used for the PCR. It could be eliminated by inserting a ribonuclease digestion step prior to amplifying the cDNA product of the RT reaction by PCR and by using a thermostable DNA polymerase identified as having reduced RNA-dependent DNA polymerase activity. Comparable results were obtained using three RNA templates with two purified RT enzymes. This modified assay is capable of detecting reliably between 10 and 100 molecules of RT, which is equivalent to between 1 and 10 retrovirus particles.

Maunula, L., H. Piiparinen, et al. (1999). "Confirmation of Norwalk-like virus amplicons after RT-PCR by microplate hybridization and direct sequencing." Journal of Virological Methods **83**(1-2): 125.

<http://www.sciencedirect.com/science/article/B6T96-3XVPG88-G/2/8c72d648973bd6cfe8fc00cef880ed9b>

A large number of Norwalk-like viruses (NLVs) have been identified from stool samples by RT-PCR by amplifying part of the polymerase-coding gene. A set of probes were selected based on sequence analysis of the viruses circulating in Finland during the years 1996-97 for confirmation of the findings by hybridization. A microplate hybridization test, which provides a rapid semi-automatic detection for PCR products, was designed and compared with agarose gel electrophoresis. From the material of 210 stool samples, mainly from diarrheal outbreaks during years 1997-1998, three probes, one for NLV genogroup GGI and one for each of the two GGII subgroups (Toronto-like and Lordsdale-like), were sufficient to detect 87.8% (36/41) of GGI and 89.0% (49/55) of GGII samples positive by gel electrophoresis. Amplicon sequencing of the strains not detected by the above probes revealed genetic variability in the sequences. Biotin-streptavidin binding was used both for microplate hybridization assays and for direct sequencing to identify the amplicons. Based on the sequences three more probes for the hybridization panel were added so that all the different NLVs of this study could be recognized.

McCann, S. H. E., J. A. Mumford, et al. (1995). "Development of PCR assays to detect genetic variation amongst equine herpesvirus-1 isolates as an aid to epidemiological investigation." Journal of Virological Methods **52**(1-2): 183.

<http://www.sciencedirect.com/science/article/B6T96-3YXC151-14/2/3040affac2accaa52598832be6104e5f>

A search for variable restriction sites has been carried out for equine herpesvirus-1 (EHV-1) in an attempt to develop markers which can be used to group epidemiologically related viruses into groups, and to learn more about the dynamics of EHV-1 disease. Crude viral DNA extracts of EHV-1, prepared by Hirt extraction, were digested with AluI, HaeIII, or RsaI, and Southern blotted following electrophoresis. DNA fingerprints, produced by probing the Southern blots with the EHV-1 EcoR1-I fragment, separated 56 isolates into 16 groups. The variable sites within the EcoR1-I fragment were mapped approximately using fragments from within EcoR1-I, and the precise location of the variable sites determined from the DNA sequence of this fragment. Oligonucleotide primers flanking the variable sites were synthesized, and used in PCR assays to detect variable fragments. The AluI variable fragment was found to result from the presence or absence of a single AluI site. In contrast, the variable bands seen with HaeIII and RsaI, resulted from variation in the copy number of two tandemly repeated sequences, one of which had not previously been recognized. In addition, HaeIII digests of EHV-1 isolates probed with the glycoprotein B (gB) gene of EHV-1 also separated isolates into two groups. The variable HaeIII site was mapped towards the 5'-end of the gB gene and a PCR assay established. The distribution of the variable AluI site within the EcoR1-I fragment and the HaeIII site within the gB gene were estimated on a large number of clinical isolates using PCR on unpurified viral tissue culture medium. Both sites had a good distribution and together with additional variable sites should provide the basis for the rapid DNA fingerprinting of EHV-1 isolates.

McElhinney, L. M., R. J. Cooper, et al. (1995). "Multiplex polymerase chain reaction for human herpesvirus-6, human cytomegalovirus, and human [beta]-globin DNA." Journal of Virological Methods **53**(2-3): 223.

<http://www.sciencedirect.com/science/article/B6T96-3YYT72B-6/2/4f1f27b6af10e0e75dc01add9b99589d>

Human cytomegalovirus and human herpesvirus-6 are closely related viruses which cause similar diseases, have similar cellular repositories of latent infection, and may be detected largely in the same types of clinical specimens. DNA amplification appears likely to play an increasing role in the diagnosis of recent and remote infection with these agents. A sensitive multiplex polymerase

chain reaction was therefore developed for the two viruses and for human [beta]-globin DNA. Optimization of parameters such as the primers, primer concentrations, magnesium concentration, and buffer constituents was crucial in achieving a sensitive assay. Preliminary results indicated that the assay could simultaneously monitor DNA extraction from clinical specimens and allow detection of HCMV or HHV-6 in patients with diseases possibly caused by either pathogen.

Merel, P., I. Pellegrin, et al. (2001). "Comparison of capillary electrophoresis sequencing with the new CEQ 2000 DNA Analysis System to conventional gel based systems for HIV drug resistance analysis." Journal of Virological Methods **98**(1): 9.

<http://www.sciencedirect.com/science/article/B6T96-43VYKRP-2/2/a74ac886f6d28b6e5d854150025656d1>

To date the majority of sequencing technologies have been based on use of gel plates. In this study sequencing by capillary electrophoresis for HIV-1 genotyping on the CEQ 2000 sequencer (Beckman Coulter Inc.) has been investigated and compared to an 'in house' protocol on the Prism-377 sequencer (Applied Biosystems) and to the HIV-1 TruGene kit (Visible Genetics Inc.), two gel plate-based systems. Plasma from 20 HAART-treated patients with virological failure were analyzed for protease (PR) and reverse transcriptase (RT) genes. A total of 520 RT codons (26/patient) and 360 PR codons (18/patient) related to antiretroviral drug resistance were evaluated. The overall agreement between CEQ 2000 and Prism-377 results was 100% for the RT and PR primary and secondary mutations. The overall agreement between CEQ 2000 and TruGene was 100% for primary and $\geq 97\%$ for secondary mutations. Discrepant results would have never led to errors in genotype interpretation. Performances for a 24 patients/week/one technician genotyping throughput were analyzed. For Prism-377, TruGene and CEQ 2000, manual processing required 5, 4 and 2,5 days, sequence data analysis needed additional 3, 1 and 2 days and cost/patient was [ap]49, 214 and 39 \$, respectively. The CEQ 2000 sequencer offers a reliable alternative for fast and cost effective HIV drug resistance analysis.

Merzouki, A., T. Mo, et al. (1994). "Accurate and differential quantitation of HIV-1 tat, rev and nef mRNAs by competitive PCR." Journal of Virological Methods **50**(1-3): 115.

<http://www.sciencedirect.com/science/article/B6T96-47DKYF8-D/2/61a9db200bf9c6c8d8b4202c283ebd1c>

An accurate method is described for measuring the relative abundance of HIV-1 regulatory mRNAs directly in clinical specimens. Specimen RNA is reverse transcribed and coamplified with a common competitor containing tat, rev and nef internal standards using fluorescent primers and a competitive polymerase chain reaction. After amplification, individual products are separated and analyzed on a fluorescent DNA sequencer. Using this approach, it was possible to measure reproducibly two-fold differences in the relative abundance of mRNAs with coding potential for tat, rev and nef from as little as 0.2 ng of total RNA extracted from peripheral blood mononuclear cells of HIV-1 infected persons. The ratio method eliminates the need to account for variability in RNA recovery during sample processing and provides a powerful tool for measuring the differential expression of HIV-1 regulatory genes in vivo.

Meyer, R. F., G. D. Babcock, et al. (1997). "Baculovirus expressed 2C of foot-and-mouth disease virus has the potential for differentiating convalescent from vaccinated animals." Journal of Virological

Methods **65**(1): 33.

<http://www.sciencedirect.com/science/article/B6T96-3XY8R56-5/2/1ebf5bcac2100792c58b962740a63853>

Determining whether animals have been infected with foot-and-mouth disease virus or vaccinated is important because infected animals frequently become carriers of the virus, shed it intermittently and thus may be the source of new outbreaks of the disease. We had shown previously that the sera of convalescent animals contain antibodies to 2C, a highly conserved non-structural protein, whereas the sera of vaccinated animals do not. This is explained by the observation that 2C is retained on the membranes of cells used for growing the virus for vaccine production. In contrast, the non-structural protein 3D, which is released into the medium, is not removed by centrifugation or filtration during vaccine production and therefore stimulates an immune response in both vaccinated and convalescent cattle. In this study we produced 2C and 3D in insect cells infected with recombinant baculoviruses. As demonstrated by serology and electron microscopy, 2C is also retained on the membranes of the insect cells. Both expressed proteins react with sera of convalescent animals, indicating that they are conformationally similar, but the 2C does not react with sera from vaccinated animals. The baculovirus expressed 2C appears to be a suitable antigen for the development of a reliable diagnostic test.

Michael Lindberg, A., C. Polacek, et al. (1997). "Amplification and cloning of complete enterovirus genomes by long distance PCR." Journal of Virological Methods **65**(2): 191.

<http://www.sciencedirect.com/science/article/B6T96-3RJG407-6/2/fdeb3a40c6bc05cca66145b20fac4d7e>

A method for amplification and cloning of complete enterovirus cDNA genomes is described. Viral RNA was reverse transcribed using an optimized protocol and a reverse transcriptase with reduced RNase H activity. Amplicons corresponding to complete genomes of 14 prototype strains of group B coxsackieviruses and echoviruses were amplified using oligonucleotide primers derived from the Coxsackievirus B3 genomic sequence of the 5' and 3' ends and a mixture of thermostable DNA polymerases. Coxsackievirus B2 amplicon was then cloned and the terminal sequences of the insert were determined. Lipofection of individual clones resulted in productive Coxsackievirus B2 infection. The method described makes it possible to obtain large amounts of complete enterovirus cDNAs and simplifies the construction of infectious full-length cDNA clones. Successful amplification of all enterovirus prototype strains tested emphasizes the general use of the method described, which provides a rapid and efficient alternative to traditional cloning strategies.

Moen, E. M., J. Sleboda, et al. (2002). "Real-time PCR methods for independent quantitation of TTV and TLMV." Journal of Virological Methods **104**(1): 59.

<http://www.sciencedirect.com/science/article/B6T96-45CDM11-3/2/bf814f74647b9e35a57695929170d5be>

There is considerable interest in the possible clinical effects of the human circoviruses TT virus (TTV) and TTV-like mini virus (TLMV). Most people appear to have at least one of these viruses replicating actively in their bodies, thus mere correlation of the presence of virus and disease states are probably less informative than a quantitative analysis of viraemia. Real-time PCR based methods, with either SYBR Green or TaqMan probe, designed to quantitate selectively TTV and TLMV are described. The suggested TaqMan-based protocols were suitable for

quantitation of viruses in the range of 102-109 copies/ml of sample; and proved, by sequencing of PCR products, to be specific for each of the two viruses.

Molitor, T. W., K. Oraveerakul, et al. (1991). "Polymerase Chain Reaction (PCR) amplification for the detection of porcine parvovirus." Journal of Virological Methods **32**(2-3): 201.

<http://www.sciencedirect.com/science/article/B6T96-476KXFR-J7/2/5edd0839b161e30756147ad9d8ea6e68>

A polymerase chain reaction (PCR) amplification method was developed and evaluated to detect porcine parvovirus (PPV). A pair of 20-base primers and an oligonucleotide probe were derived from the DNA sequences common to two isolates of PPV, NADL-8 and NADL-2. The primers flanked 118-bp nucleotides within the region coding for the major structural protein VP2. After DNA amplification of PPV replicative form (RF), a 158-bp fragment was detected in agarose gels. This amplified fragment was shown to be specific for PPV DNA after Southern transfer and hybridization to a 20-base internal probe. The amplified fragment also contained a single EcoRI cleavage site. Various conditions, such as number of cycles and annealing temperature, were examined to optimize the conditions for detecting viral DNAs from infected cell cultures and swine fetal tissues. Four different isolates of PPV, NADL-8, NADL-2, KBSH and Kresse, and two other viruses, canine parvovirus (CPV) and pseudorabies virus (PRV), were included to determine specificity of amplification. Slot blot hybridization with a radiolabeled probe was used to evaluate the sensitivity of PCR amplification. The optimized protocol was specific for PPV detecting equally all four strains of PPV, but failing to amplify CPV or PRV sequences. The PCR method could detect at least 100 fg of viral replicative form (RF) DNA or the equivalent of 1 PFU of infectious virus. The applications of this method include routine detection of PPV in clinical samples and as a contaminant in mammalian cell lines.

Naito, H. and K. Abe (2001). "Genotyping system of GBV-C/HGV type 1 to type 4 by the polymerase chain reaction using type-specific primers and geographical distribution of viral genotypes." Journal of Virological Methods **91**(1): 3.

<http://www.sciencedirect.com/science/article/B6T96-426XYK0-J/2/e597903a5704609ffee9fb685bbe6a9b>

Based on variation in nucleotide sequence of 5'-untranslated region, GB virus type C (GBV-C) and hepatitis G virus (HGV) can be classified into three major genotypes. In addition to this classification, a novel genotype of GBV-C/HGV was identified and designated type 4. However, genotyping of GBV-C/HGV has been hampered by the lack of suitable assays. In this study, a simple and precise genotyping system based on PCR using the type-specific primers was developed for the determination of genotypes 1, 2, 3, and 4 of GBV-C/HGV. A total of 235 serum samples obtained from 11 different countries were tested by our PCR genotyping system of GBV-C/HGV. The results revealed that type 1 was prevalent mainly in Ghana, type 2 was prevalent in the USA, Spain, Egypt, Nepal and Myanmar, type 3 was prevalent in Japan and Bolivia, and type 4 was prevalent in Vietnam and Myanmar among the countries investigated in the present study. To confirm the specificity of the results of genotyping by PCR, phylogenetic analysis in the 5'-untranslated region of GBV-C/HGV was undertaken in 99 serum samples. By this analysis, the specificity of the genotyping system by PCR was confirmed. This assay system may be useful for rapid typing of GBV-C/HGV RNA when either epidemiological or transmission studies of this agent are carried out.

Najioullah, F., D. Thouvenot, et al. (2001). "Development of a real-time PCR procedure including an internal control for the measurement of HCMV viral load." Journal of Virological Methods **92**(1): 55.

<http://www.sciencedirect.com/science/article/B6T96-427JWSG-7/2/1e33f8b1e295ab442ae4f648e13142ed>

Human cytomegalovirus (HCMV) infections are frequent in immuno-compromised patients. The recent development of real-time PCR procedures that allow the rapid quantification of genome load will be helpful for accurate monitoring of these infections. Two extraction procedures were evaluated using 30 blood samples that were processed pure and diluted (1/10). Repeatability and reproducibility of the quantitative PCR procedure using an internal control for amplification were analysed, and its sensitivity compared to a qualitative PCR procedure using 50 HCMV culture positive blood samples. The real-time PCR and qualitative PCR procedures were positive in 46 and 48 of the samples tested, respectively. Discrepancies were observed for samples with a low viral load. The sensitivity of the real-time PCR procedure was evaluated at 500 HCMV DNA copies per ml of sera. The use of an internal control concomitantly processed during the HCMV quantification did not alter the sensitivity of the procedure, and was relevant for the detection of putative PCR inhibitors that may interfere with the amplification process. This procedure was used to measure genome load in two bone marrow transplant patients with HCMV disease, confirming that this new PCR procedure should be used widely for diagnosing and monitoring HCMV infections in transplant patients.

Navas-Castillo, J., J. A. Diaz, et al. (1998). "Improvement of the print-capture polymerase chain reaction procedure for efficient amplification of DNA virus genomes from plants and insect vectors." Journal of Virological Methods **75**(2): 195.

<http://www.sciencedirect.com/science/article/B6T96-3V5F5S6-7/2/ed24dcf3583c7e7ab5354f937afafa05>

A rapid and simple procedure is described to amplify efficiently geminivirus DNA genomes by improving the print-capture polymerase chain reaction (PCR) procedure reported recently for RNA viruses. This method, termed print-PCR (P-PCR), allows direct amplification of DNA from infected plant or whitefly tissues printed directly on Whatman 3MM paper, without the need of any grinding, incubation, or washing steps previous to the amplification reaction. P-PCR reduces sample manipulation and avoids previous extraction of nucleic acids, thereby diminishing the possibilities of cross-contamination between samples. P-PCR has been successfully applied to whiteflies and various plant species infected by two different tomato yellow leaf curl viruses, TYLCV-Sr and TYLCV-Is, and for the amplification of the full-length genome of TYLCV-Is from infected plants.

Nemchinov, L. and A. Hadidi (1998). "Specific oligonucleotide primers for the direct detection of plum pox potyvirus-cherry subgroup." Journal of Virological Methods **70**(2): 231.

<http://www.sciencedirect.com/science/article/B6T96-3SBVX4H-F/2/99c020d48a0c46e673cc680cfae9648c>

A specific polymerase chain reaction assay was developed for direct identification of the distinct subgroup of plum pox potyvirus (PPV) isolates from cherry trees (PPV-cherry, PPV-C) and its differentiation from other known subgroups of PPV. The specificity of the assay is based on using a pair of primers whose nucleotide sequences are located on the coat protein gene of PPV-sour

cherry (SoC) at regions of high nucleotide divergence between PPV-SoC and other isolates of PPV. The technique will be useful for studying the epidemiology of PPV-C as well as for practical testing in certification and quarantine programs worldwide.

Nie, X. and R. P. Singh (2002). "A new approach for the simultaneous differentiation of biological and geographical strains of Potato virus Y by uniplex and multiplex RT-PCR." Journal of Virological Methods **104**(1): 41.

<http://www.sciencedirect.com/science/article/B6T96-45CDM11-1/2/8361a181eef499c15344058b0eeb8ba3>

Two strains of Potato virus Y (PVY), the common (PVYO) and the tobacco vein necrosis (PVYN) have been known for decades. More recently, a tuber ringspot necrosis (PVYNTN), and several recombinants of PVYO and PVYN (designated here as PVYN:O) have been described. Further, the PVYN group of strains have been assigned to two geographical subgroups of European (EU) PVYN/NTN and the North American (NA) PVYN/NTN. The evolution of new PVYN strains, has complicated the diagnosis, which requires a combination of bioassay, serological and molecular assays. To simplify the identification and differentiation of various PVYN strain groups, a competitive (single antisense and multiple sense primers) reverse transcription-polymerase chain reaction (RT-PCR) was used, making use of minor differences in the variable region part of the PVY genome. Specifically, primers based on small variations in nucleotide stretches of P1 gene permitted a broad range separation of PVYO and PVYN groups and the specific detection of strain subgroups. The primer pairs designed for identifying PVYO, EU-PVYN/NTN, NA-PVYN and NA-PVYNTN are described. Primer pairs can be used in a uniplex (single pair of primer) or multiplex (duplex, tetraplex or pentaplex) competitive RT-PCR, allowing simultaneous testing for any combination of PVYO, EU-PVYN/NTN, NA-PVYN and NA-PVYNTN.

Nie, X. and R. P. Singh (2001). "A novel usage of random primers for multiplex RT-PCR detection of virus and viroid in aphids, leaves, and tubers." Journal of Virological Methods **91**(1): 37.

<http://www.sciencedirect.com/science/article/B6T96-426XYK0-P/2/e3a672edc650138234f031d660a2841f>

A multiplex reverse transcription polymerase chain reaction (m-RT-PCR) was developed for the simultaneous detection of five potato viruses and a viroid. The synthesis of cDNAs used for amplification was primed by hexanucleotides (random primers, RP). An RNA extraction procedure employing DNase I, is routinely used to isolate potato viruses and viroid (Potato virus S, PVS; Potato leafroll virus, PLRV; Potato virus X, PVX; Potato virus A and Y, PVA, PVY; and Potato spindle tuber viroid, PSTVd) from infected tissues. This extraction method produced deoxy-oligonucleotides, which in turn were used to prime the reverse transcription of RNA templates of all the viruses and the viroid. A time-course study from 15 s to 30 min showed optimal oligonucleotide generation by DNase I occurred at 10 min, an incubation time already incorporated in the extraction protocol. The presence of oligonucleotides capable of priming cDNA synthesis was also demonstrated in RNA preparations from aphids, leaves, and tubers. In order to duplicate the priming of templates by oligonucleotides, commercially available hexanucleotides were used as RP. When fragments were amplified from 5'- and 3'-ends of the random primed cDNA of PVY genome, bands of similar intensity were observed. In contrast, when two fragments (short and long) from the P1 gene region of the PVA genome were amplified, the yield of the short fragment was significantly higher in intensity than that of the long fragment in random primed cDNA. Irrespective of the origin of the primers (generated during extraction vs. commercially purchased), single or multiple viruses or the viroid were detected by amplification of random primed cDNAs present individually in the reaction or in a cDNA pool consisting of five

viruses and the viroid. The cDNA produced by RP or virus specific primers (SP) was used to detect PLRV and PVY from infected tubers in a duplex reverse transcription polymerase chain reaction (d-RT-PCR). The RP cDNA gave increased detection. Comparison of RP primed cDNAs from dormant or sprouted tubers and leaves showed that for some cultivars, such as 'Shepody', leaves were more reliable for PVY and PLRV detection than the tubers, in both the d- and m-RT-PCR.

Njouom, R., C. Pasquier, et al. (2003). "Assessment of HIV-1 subtyping for Cameroon strains using phylogenetic analysis of pol gene sequences." Journal of Virological Methods **110**(1): 1.

<http://www.sciencedirect.com/science/article/B6T96-488G8YB-3/2/3a9299f22aaf60d094f4d445fdd214ca>

Phylogenetic analysis of human immunodeficiency virus type 1 (HIV-1) pol gene is a useful method for subtyping European strains of HIV-1. The suitability of this method for genetically diverse African strains was evaluated by comparing HIV-1 subtyping of Cameroon strains using a long fragment of the pol gene sequence to the findings obtained using env gene sequences. When the pol gene could not be amplified, the reverse transcriptase (RT) or the protease (PR) genes were used. Phylogenetic analysis of the env C2/V3 gene sequences of 60 HIV-1 isolates showed 52 to be subtype A, 2 subtype G, plus one each of subtypes C, F2 and H, with 3 subtypes not determined. A long fragment of the pol gene was amplified successfully and sequenced in 23% of cases. The RT region was amplified for 42% of the samples that could not be typed by analysing the long fragment, and the PR gene was amplified for 40% of them. Thus, 63% of samples were typable. Env and pol gene subtypings were in agreement in 86% of cases. It is concluded that the phylogenetic analysis of pol gene sequences is not a practical method for HIV-1 subtyping in areas of high subtype diversity, despite the good agreement between the env and pol gene subtypings. However, it can be a useful method for HIV-1 subtyping, provided that the gene is amplifiable.

Nuanualsuwan, S. and D. O. Cliver (2002). "Pretreatment to avoid positive RT-PCR results with inactivated viruses." Journal of Virological Methods **104**(2): 217.

<http://www.sciencedirect.com/science/article/B6T96-45WGH3G-2/2/8fd0074a4e4ba9f4f3c80b21156d2545>

Enteric viruses that are important causes of human disease must often be detected by reverse transcription-polymerase chain reaction (RT-PCR), a method that commonly yields positive results with samples that contain only inactivated virus. This study was intended to develop a pretreatment for samples, so that inactivated viruses would not be detected by the RT-PCR procedure. Model viruses were human hepatitis A virus, vaccine poliovirus 1 and feline calicivirus as a surrogate for the Norwalk-like viruses. Each virus was inactivated (from an initial titer of [ap]103 PFU/ml) by ultraviolet light, hypochlorite or heating at 72 [deg]C. Inactivated viruses, that were treated with proteinase K and ribonuclease for 30 min at 37 [deg]C before RT-PCR, gave a negative result, which is to say that no amplicon was detected after the reaction was completed. This antecedent to the RT-PCR method may be applicable to other types of viruses, to viruses inactivated in other ways and to other molecular methods of virus detection.

Nunan, L. M. and D. V. Lightner (1997). "Development of a non-radioactive gene probe by PCR for detection of white spot syndrome virus (WSSV)." Journal of Virological Methods **63**(1-2): 193.

<http://www.sciencedirect.com/science/article/B6T96-3RHM7T6-1G/2/fcb9d76a3619ac6449cbbb965963b13b>

Combining primers created from the sequence information of two baculo-like viruses of penaeid shrimp, Baculovirus penaei (BP) and Monodon baculovirus (MBV), produced a 750 bp band on a 0.8% agarose gel using White Spot Syndrome Virus (WSSV), from *Penaeus monodon*, as the DNA template. The PCR fragment was ligated to a plasmid vector, (pGEM-T) and transformed, creating a 3.7 Kbp clone. The DNA insert was sequenced, and the original primer pair was located. Using restriction enzymes, the insert was isolated, excised and non-radioactively labeled. This cloned labeled fragment was tested by in situ hybridization for specificity and reactivity with BP, MBV and WSSV-infected shrimp tissues. The major advantage of this novel method of gene probe development is that no DNA sequence information of the targeted infectious agent needed to be known or available. In addition, tedious viral isolation and purification was circumvented. In this study, knowledge of the possible viral strain was important in limiting the PCR primer pairs investigated. The use of arbitrary primers designed for PCR assays from two other possibly related shrimp viruses, increased the likelihood that a generated PCR product would be specific for WSSV.

Nystrom, K., M. Biller, et al. (2004). "Real time PCR for monitoring regulation of host gene expression in herpes simplex virus type 1-infected human diploid cells." *Journal of Virological Methods* **118**(2): 83.

<http://www.sciencedirect.com/science/article/B6T96-4BVP8FY-1/2/ecdaff1c9d755a984c63b7f183a5585f>

Herpes simplex virus type 1 (HSV-1) induces prominent shifts in the rates of transcription of host cellular genes of relevance for the outcome of the viral infection. The quantitative analysis of transcription may be obscured by virus-induced alterations in the levels of RNA encoded by cellular housekeeping genes that are used commonly for normalisation of real time reverse transcription PCR (RT-qPCR). In the present study, we analysed [beta]-actin, GAPDH and 18S rRNA for their usefulness in normalisation of RT-qPCR analysis of the transcription of the HSV-1 gamma gB-1 gene and FUT5, a cellular gene induced by viral infection. The transcription of these genes was monitored in a TaqMan-based real time RT-PCR system over a 24 h interval of virus infection of human embryonic lung fibroblasts. The levels of gB-1 and FUT5 RNA were normalised via difference in the threshold cycle ([Delta]Ct) values relative to each and one of the housekeeping genes or calculated in relation to the number of infected cells without any further normalisation. The levels of RNA encoded by [beta]-actin or GAPDH were found to vary by several orders of magnitude during HSV-1 infection, introducing large errors in the estimation of the gB-1 and FUT5 RNA levels. In contrast, the variation of Ct values for 18S rRNA was less than one cycle during 24 h period of HSV-1 infection. The FUT5 and gB-1 RNA figures obtained by [Delta]Ct normalisation relative 18S rRNA were identical to those calculated in relation to the number of infected cells. These data recommend 18S rRNA for normalisation in HSV-1-infected human cells but discourage the use of [beta]-actin and GAPDH RNA for this purpose. By applying these procedures, it was shown that the transcription of FUT5 was increased by 50-fold 5-24 h after HSV-1 infection and 200-fold by the inhibition of viral DNA replication in HSV-infected cells.

O'Shea, M. K. and P. A. Cane (2004). "Development of a highly sensitive semi-quantitative real-time PCR and molecular beacon probe assay for the detection of respiratory syncytial virus." *Journal of Virological Methods* **118**(2): 101.

<http://www.sciencedirect.com/science/article/B6T96-4BY42V3-1/2/72cdebe02966a90458710dc3c2a18c4c>

Molecular beacons are a novel class of oligonucleotide probe capable of reporting the accumulation of target amplicon during real-time PCR by the emission of a fluorescent signal. A novel assay for the detection and estimation of respiratory syncytial virus (RSV) nucleic acid in clinical specimens based on real-time PCR utilising such a probe was developed. The probe consisted of two short arm sequences and a central loop sequence complementary to a region of the N gene (the target amplicon). The probe was characterised and a semi-quantitative nested real-time PCR using a LightCycler instrument was optimised. Standard curves were generated using cycle threshold (Ct) values calculated from several assays over a range of logarithmic RSV titres. Linear coefficient correlations were close to one ($r^2=0.998$) and the detection limit of the optimised assay was reproducibly shown to be 1×10^{-4} pfu/ml. The intra-assay coefficient of variation (CV) of Ct values of the optimal assay was 0.8% and the CV of quantification data was 6.6%. The interassay CV of Ct values was 2.0% and the quantification CV was 6.7%. The validity of the assay for the detection of RSV in clinical specimens was assessed by analysing ten specimens previously assayed in a different laboratory. Real-time PCR analysis was completely consistent with the results of prior analysis. The rapidity, sensitivity and specificity of the assay should greatly facilitate epidemiological studies, particularly in adults as existing methods perform better on clinical specimens from children.

Okamoto, H., S. Kobata, et al. (1996). "A second-generation method of genotyping hepatitis C virus by the polymerase chain reaction with sense and antisense primers deduced from the core gene." Journal of Virological Methods **57**(1): 31.

<http://www.sciencedirect.com/science/article/B6T96-3VXJ8FF-4/2/4f83ebd4df7935cae0f18c24b6941bdb>

A second-generation method of genotyping hepatitis C virus (HCV) was developed by the polymerase chain reaction (PCR) with sense as well as antisense primers deduced from the core gene. HCV RNA specimens extracted from sera were reverse-transcribed and amplified with universal primers in the first round of PCR to obtain fragments of 433 base pairs representing nucleotides 319-751. In the second round of PCR, portions of PCR products were amplified separately with sense and antisense primers specific for each of the five common genotypes prevailing across the world i.e., I/1a, II/1b, III/2a, IV/2b and V/3a. The specificity of the method was verified by a panel of 177 HCV isolates of various genotypes in the genetic groups 1-9. It allowed clear differentiation of genotype I/1a from II/1b which was not always accomplished by the previous method. When 501 sera from blood donors and hepatitis patients with HCV viremia from various countries were genotyped by the second-generation method, 478 (95.4%) were classified into the five genotypes. HCV RNA samples from 23 (4.6%) sera were not classifiable into any of the five common genotypes and, by sequence analysis, 22 were found to be of four genotypes in group 4 and one of genotype 1c in Simmonds' classification.

Okuda, M. and K. Hanada (2001). "RT-PCR for detecting five distinct Tospovirus species using degenerate primers and dsRNA template." Journal of Virological Methods **96**(2): 149.

<http://www.sciencedirect.com/science/article/B6T96-43DDGXG-5/2/9f7f4a76d586d0086922e829e8a244b5>

RT-PCR procedures for detection of multiple species of tospovirus from plant tissues were investigated. Downstream primers were designated from the 3' untranslated sequences of the S RNA. An upstream primer was designated from the degenerated sequences of the nucleocapsid protein. Approximately 450 bp DNA fragments were detected when Tomato spotted wilt virus (TSWV)- or Impatiens necrotic spot virus (INSV)- infected tissues were examined. Approximately 350 bp DNA fragments were detected when Watermelon silver mottle virus (WSMoV)- or Melon

yellow spot virus (MYSV)-infected tissues were examined. Template RNA was extracted using CF 11 cellulose powder, and nonspecific amplification became unnoticeable when double-stranded RNA was used. The amplified fragments of WSMoV were differentiated from those of MYSV by AluI or TaqI digestion. The amplified fragments of TSWV were differentiated from those of INSV by DraI or HindIII digestion. An alstroemeria plant that was infected with an unidentified tospovirus was also examined, and a 350 bp fragment that was 97% identical to Iris yellow spot virus was detected. This method, therefore, detected at least five distinct Tospovirus species.

Olvera, A., M. Sibila, et al. (2004). "Comparison of porcine circovirus type 2 load in serum quantified by a real time PCR in postweaning multisystemic wasting syndrome and porcine dermatitis and nephropathy syndrome naturally affected pigs." Journal of Virological Methods **117**(1): 75.

<http://www.sciencedirect.com/science/article/B6T96-4BMK1TM-1/2/6aad5437203cfcf9072aff5468feebfa>

Postweaning multisystemic wasting syndrome (PMWS) diagnosis is based on the presence of characteristic histopathological lymphoid lesions and porcine circovirus type 2 (PCV2) within these lesions. Previous studies indicate that PCV2 load is higher in PMWS affected than in PCV2 infected, healthy pigs. On the other hand, PCV2 has been suggested to play a role in porcine dermatitis and nephropathy syndrome (PDNS) pathogenesis. This study describes a new TaqMan(c) real time PCR assay and its use to quantify viral load in serum samples. Serum viral loads were related with different degrees of PMWS characteristic lesions and PDNS cases. DNA extracted from serum samples from 75 animals with mild, moderate and severe PMWS lesions and 12 animals with PDNS was used as template. PCV2 DNA was quantified in 69 of 75 PMWS cases and in 11 of 12 PDNS cases. Significant differences in PCV2 load were observed between animals with severe, moderate and mild PMWS lesions, although variability within each group was high, probably due to heterogeneity in disease progression. These results suggest that high viral load is a major feature of PMWS affected pigs. PDNS affected animals had lower PCV2 loads. No significant differences in viral load were found between animals suffering from PDNS and animals with mild PMWS characteristic lesions, which were unaffected clinically.

Oviedo, J. M., F. Rodriguez, et al. (1997). "High level expression of the major antigenic African swine fever virus proteins p54 and p30 in baculovirus and their potential use as diagnostic reagents." Journal of Virological Methods **64**(1): 27.

<http://www.sciencedirect.com/science/article/B6T96-3RHM7T6-1X/2/7af5f3d9c9d003c6323ebe27c7e37be9>

At present, the eradication of African swine fever (ASF) in affected countries is based only on an efficient diagnosis program because of the absence of an available vaccine. The highly antigenic ASF virus proteins p54 and p30, encoded by genes E183L and CP204L respectively, were expressed in baculovirus for diagnostic purposes. A sequence comparison analysis of these genes from different field virus strains which are geographically diverse and isolated in different years, revealed that both genes are completely conserved among the isolates. Partially purified baculovirus-expressed proteins were used in ELISA and Western blot for ASF antibody detection in sera from experimentally inoculated pigs and field sera from ASF innaparent carriers. These comparative analyses showed that p54 presents better reactivity than p30 in Western blot. However, recombinant p30 was more efficient for antibody detection by ELISA, improving the discrimination between positive and negative sera by this technique. These data suggest the convenience of using p30 as ELISA antigen, while p54 should be the selected antigen for ASF virus antibody detection by Western blot. The combined use of both antigens for serodiagnosis of ASF disease will improve the sensitivity of innaparent carriers detection, facilitating also the

interpretation of the tests, and eliminating the use of ASF virus in antigen production.

Paragas, J. and J. A. Blaho (1998). "Cosolvents facilitate DNA synthesis in the herpes simplex virus 1 unique short (Us) inverted repeat." Journal of Virological Methods **73**(1): 53.

<http://www.sciencedirect.com/science/article/B6T96-3T2PCND-6/2/250e8b0381b3b0894cc9aac7c4a6a214>

DNA synthesis under standard conditions is not successful within a portion of the Us1 gene of HSV-1 which is juxtaposed to an 86% G+C-containing tract in the Us inverted repeat sequence. We report that the independent addition of specific amounts of at least three different types of cosolvents is capable of facilitating DNA synthesis within this G+C-rich region. In addition, this strategy was used to successfully place a specific site-directed mutation in the Us1 gene. Consideration of these observations should enable future site-specific mutational analyses of portions of the HSV-1 genome which have traditionally been unamenable to genetic manipulations.

Parshionikar, S. U., J. Cashdollar, et al. (2004). "Development of homologous viral internal controls for use in RT-PCR assays of waterborne enteric viruses." Journal of Virological Methods **121**(1): 39.

<http://www.sciencedirect.com/science/article/B6T96-4CVR4D3-1/2/9bd36ffdfc5ee6c642d648e20707746>

Enteric viruses often contaminate water sources causing frequent outbreaks of gastroenteritis. Reverse transcription-polymerase chain reaction (RT-PCR) assays are commonly used for detection of human enteric viruses in environmental and drinking water samples. RT-PCR provides a means to rapidly detect low levels of these viruses, but it is sensitive to inhibitors that are present in water samples. Inhibitors of RT-PCR are concentrated along with viruses during sample processing. While procedures have been developed to remove inhibitors, none of them completely remove all inhibitors from all types of water matrices. This problem requires that adequate controls be used to distinguish true from potentially false-negative results. To address this problem, we have developed homologous viral internal controls for hepatitis A virus (HAV), poliovirus, Norwalk virus and rotavirus. These internal controls can be used in RT-PCR assays for the detection of the above viruses by competitive amplification, thereby allowing the detection of false negatives in processed water samples. The internal controls developed in this study were successfully tested with virus-seeded environmental water sample concentrates.

Pasquier, C., N. Millot, et al. (2001). "HIV-1 subtyping using phylogenetic analysis of pol gene sequences." Journal of Virological Methods **94**(1-2): 45.

<http://www.sciencedirect.com/science/article/B6T96-42Y12XF-6/2/d80f24b7001e51dc6faacfbf8b23f572>

HIV-1 pol gene sequencing is now used routinely in France to identify mutations associated with resistance to reverse transcriptase (RT) or protease (PR) inhibitors. These sequences may also provide other information, such as the HIV-1 subtype. HIV-1 subtyping was compared using the RT and PR gene sequences to heteroduplex mobility assay (HMA) of the envelope gene. The RT and PR genes of 51 samples that had been subtyped earlier by HMA were sequenced. Sequences were aligned and subtypes were determined by phylogenetic analysis with reference

HIV sequences. HMA gave the following subtypes: A (20), B (19), C (1), D (3), F (1), G (3) and CRF01-AE (4). Phylogenetic analysis of the RT gene gave: A (5), B (19), C (2), D (3), F (1), G (6), J (2), CRF01_AE (4), CRF02_AG (7) and undetermined (2). PR gene analysis did not infer subtypes with sufficient confidence. HMA and RT subtyping was not in agreement in nine cases. RT subtyping can identify CRF02_AG and CRF01_AE variants from A subtype RT. It was shown that phylogenetic analysis of the RT gene could provide a useful method for HIV-1 subtyping. The length of the amplicon and the relative performance of each primer pair used in this study favoured RT sequences as a subtyping tool. One potential advantage over env subtyping HMA is the ability to identify some circulating recombinant forms (CRFs).

Petrik, J., G. J. M. Pearson, et al. (1997). "High throughput PCR detection of HCV based on semiautomated multisample RNA capture." Journal of Virological Methods **64**(2): 147.

<http://www.sciencedirect.com/science/article/B6T96-3RJ9BMF-5/2/9cff2ed2ded22d32025f3dcb5ae1436b>

An amplification procedure based on a semiautomated 60-sample RNA capture, including combined reverse transcription/polymerase chain reaction (RT-PCR) and nested PCR/Tagman amplicon detection, is described. It can be completed within a working day and is suitable for the development of a fully automated system. HCV RNA-specific capture is independent of the sequence variations as it targets the poly(U) tract commonly present at the 3'-end of the HCV genome (U-capture). The type specificity of the assay determined in a panel of 56 confirmed HCV antibody-positive samples (genotypes 1-6) was slightly better when compared to a commercial assay. The sensitivity evaluated on serial dilutions of representative samples was equal for genotypes 1, 2, 5, 6, or increased for genotypes 3 and 4 with the U-capture assay.

Poon, H., E. Jimenez, et al. (1993). "Detection of bovine leukemia virus RNA in serum using the polymerase chain reaction." Journal of Virological Methods **41**(1): 101.

<http://www.sciencedirect.com/science/article/B6T96-476RMYJ-6K/2/5fef2abb18e0a655d107030563c44935>

A method was developed for detecting bovine leukemia virus (BLV) RNA in serum samples using a pair of primers from the BLV polymerase gene in the polymerase chain reaction (PCR). The PCR was able to detect 3800-7600 molecules of BLV RNA. At this level of sensitivity eleven pools of adult and one fetal bovine serum appeared free from BLV contamination.

Pratelli, A., V. Martella, et al. (2001). "Genomic characterization of pestiviruses isolated from lambs and kids in southern Italy." Journal of Virological Methods **94**(1-2): 81.

<http://www.sciencedirect.com/science/article/B6T96-42Y12XF-9/2/65624063a12db9b5ada050304765320d>

A nested polymerase chain reaction was used to identify 13 pestivirus strains isolated from small ruminants in several mixed (sheep and goats) flocks of Southern Italy, and for classification as bovine viral diarrhoea virus (BVDV) type 1, BVDV type 2, and Border disease virus (BDV) genotypes. Of the nine ovine isolates, two were characterized as BVDV type 1, and seven as BVDV type 2. The four pestiviruses isolated from kids belong to BVDV type 1. None of the pestivirus strains tested could be classified as 'true' BDV (genotype 3). Although BVDV type 2

has been described in Europe rarely, the characterization of BD/90-1M strain as BVDV type 2, isolated in Italy in 1990, demonstrates that this genotype has been circulating in Italy since the 1990s.

Pratelli, A., V. Martella, et al. (2003). "Genetic diversity of a canine coronavirus detected in pups with diarrhoea in Italy." Journal of Virological Methods **110**(1): 9.

<http://www.sciencedirect.com/science/article/B6T96-488G8YB-1/2/97c330073d15a9fb1cf3545d25dd2edf>

The sequence of the S gene of a field canine coronavirus (CCoV), strain Elmo/02, revealed low nucleotide (61%) and amino acid (54%) identity to reference CCoV strains. The highest correlation (77% nt and 81.7% aa) was found with feline coronavirus type I. A PCR assay for the S gene of strain Elmo/02 detected analogous CCoVs of different geographic origin, all which exhibited at least 92-96% nucleotide identity to each other and to strain Elmo/02. The evident genetic divergence between the reference CCoV strains and the newly identified Elmo/02-like CCoVs strongly suggests that a novel genotype of CCoV is widespread in the dog population.

Pratelli, A., V. Martella, et al. (2003). "Identification of coronaviruses in dogs that segregate separately from the canine coronavirus genotype." Journal of Virological Methods **107**(2): 213.

<http://www.sciencedirect.com/science/article/B6T96-47DTDTF-N/2/66948c55e9362947f2496c0e4bc5eafd>

The genetic diversity of 16 canine coronavirus (CCoV) samples is described. Samples were obtained from pups infected naturally living in different areas. Sequence data were obtained from the M gene and pol1a and pol1b regions. The phylogenetic relationships among these sequences and sequences published previously were determined. The canine samples segregated in two separate clusters. Samples of the first cluster were intermingled with reference strains of CCoV genotype and therefore could be assigned to this genotype. The second cluster segregated separately from CCoV and feline coronavirus genotypes and therefore these samples may represent genetic outliers. The reliability of the classification results was confirmed by repeating the phylogenetic analysis with nucleotide and amino acid sequences from multiple genomic regions.

Pratelli, A., M. Tempesta, et al. (1999). "Development of a nested PCR assay for the detection of canine coronavirus." Journal of Virological Methods **80**(1): 11.

<http://www.sciencedirect.com/science/article/B6T96-3WM5CT3-2/2/d5d4acb70659bd6b1089e8b5c3c06679>

A diagnostic test for canine coronavirus (CCV) infection based on a nested polymerase chain reaction (n-PCR) assay was developed and tested using the following coronavirus strains: CCV (USDA strain), CCV (45/93, field strain), feline infectious peritonitis virus (FIPV, field strain), transmissible gastroenteritis virus (TGEV, Purdue strain), bovine coronavirus (BCV, 9WBL-77 strain), infectious bronchitis virus (IBV, M-41 strain) and fecal samples of dogs with CCV enteritis. A 230-bp segment of the gene encoding for transmembrane protein M of CCV is the target sequence of the primer. The test described in the present study was able to amplify both CCV and TGEV strains and also gave positive results on fecal samples from CCV infected dogs. n-

PCR has a sensitivity as high as isolation on cell cultures, and can therefore be used for the diagnosis of CCoV infection in dogs.

Pratelli, A., A. Tinelli, et al. (2002). "PCR assay for the detection and the identification of atypical canine coronavirus in dogs." Journal of Virological Methods **106**(2): 209.

<http://www.sciencedirect.com/science/article/B6T96-46WSX8S-6/2/dbd1d38351561ef0cb23980d01cd8c23>

Comparative sequence analysis of the PCR products of the M gene and fragments of the pol1a and pol1b genes of canine coronavirus (CCoV) have demonstrated that two separate clusters of CCoV are present in dogs. This note describes a PCR assay to identify atypical CCoV strains with nucleotide substitutions in the M gene. A total of 177 faecal samples from dogs CCoV positive previously with the PCR assay were analysed. Sixty-two of the 177 samples were amplified with the PCR described in the present study and were thus considered atypical CCoVs. The specificity of the PCR typing assay was confirmed by sequence analysis of the PCR products.

Puig, M., S. Pina, et al. (2000). "Description of a DNA amplification procedure for the detection of bacteriophages of *Bacteroides fragilis* HSP40 in environmental samples." Journal of Virological Methods **89**(1-2): 159.

<http://www.sciencedirect.com/science/article/B6T96-429RNYH-J/2/0a8d63b24e9477e01fb6c69bcd067de4>

A molecular test based on DNA amplification by PCR was developed for the detection of bacteriophages of *Bacteroides fragilis* strain HSP40 in the environment. These specific phages are associated with faecal contamination of human origin. A homologous DNA region of 1.5 kb, identified previously by hybridisation, was used to design primers for the detection of *B. fragilis* HSP40 phages. A nested-PCR procedure for the DNA amplification of those phages was developed. The sensitivity of the nested-PCR was between 10⁻¹ and 10⁻² PFU for purified HSP40 phage solutions, sewage and seawater samples, and between 1 and 10 PFU for river water samples. Specific amplification of HSP40 phages was observed when viral suspensions of 103 PFU/ml or lower were used. Common levels of *B. fragilis* phages found in sewage are 101-102 PFU/ml. A total of 24 water samples (sewage, river water and seawater) were tested both by PCR and by plaque assay, to evaluate the efficiency of the molecular method in field samples. The data obtained by PCR in environmental samples showed good concordance with the PFU counts and a higher sensitivity.

Quan, C. M., M. Krajden, et al. (1993). "High-performance liquid chromatography to assess the effect of serum storage conditions on the detection of hepatitis C virus by the polymerase chain reaction." Journal of Virological Methods **43**(3): 299.

<http://www.sciencedirect.com/science/article/B6T96-476RMR1-59/2/839f0ee3725ce81e60bbf457cd804527>

The effect of various serum storage conditions on the detection of hepatitis C virus (HCV) by the polymerase chain reaction (PCR) was assessed. 50 [μl] aliquots of serum from four HCV PCR positive patients were subjected, in triplicate, to: (a) storage at -20[deg]C for 1, 5, 10 days; (b)

storage at room temperature (RT) for 1, 2, 7 days; (c) 1, 3, 5, and 10 successive freeze-thaw cycles; (d) incubation at 37[deg]C, and 56[deg]C for 1, 3, 24 h; and (e) storage in guanidium-thiocyanate extraction buffer at RT, and 4[deg]C for 1, 5, 10 days. PCR products were detected by agarose gel electrophoresis (AGE) and quantitatively by high-performance liquid chromatography (HPLC). Only storage in extraction buffer at RT for 5-10 days and incubation at 56[deg]C for 24 h appeared to result in a loss of [ges]50% of detectable HCV PCR product. Up to 10 successive freeze-thaw cycles, storage at -20[deg]C for up to 10 days or at RT for up to 7 days, storage in extraction buffer at RT for 1 day or at 4[deg]C for up to 10 days, and incubation at 37[deg]C for up to 24 h resulted in minimal PCR signal loss. HPLC was a reproducible method of detecting and quantitating HCV PCR products, and was more sensitive than AGE.

Ray, R., P. J. Cooper, et al. (1996). "Direct in situ reverse transcriptase polymerase chain reaction for detection of measles virus." Journal of Virological Methods **60**(1): 1.

<http://www.sciencedirect.com/science/article/B6T96-3VYV029-1/2/5c11b5c2bcb450ea425e8689d6c9641>

New methods are described for combined intracellular reverse transcription (RT) and polymerase chain reaction (PCR) using single primer pairs, with direct incorporation of digoxigenin-11-dUTP into amplicants (direct in situ RT/PCR). Routinely used fixatives and minimal pre-treatments were employed. Target sequences of measles virus nucleocapsid (N) and phosphoprotein genes were detected within measles virus infected Vero cells, both in suspension and in formalin-fixed sections, that had been treated by in situ reverse transcription and 30 cycles of direct in situ PCR. Uninfected cells, omission of Taq polymerase, and irrelevant primers were used as controls. Distribution of measles virus within infected cells was determined by in situ hybridisation and immunocytochemistry for measles virus N gene and protein, respectively. Confirmation of amplification within sections was by gel electrophoresis, Southern blotting and sequencing of extracted amplicons. In the majority of cases, measles-infected cells exhibited intense cytoplasmic signal after direct in situ PCR; this was not seen in uninfected cells or infected cells reacted either with irrelevant primers or without Taq polymerase. Unfixed cells in suspension required nested reaction. Measles-specific in situ hybridisation and immunocytochemistry gave an identical signal distribution in sections. Nuclear artifact occurred in some sections and was unpredictable, although it was greatest either in areas of cellular damage, following DNase predigestion, or with vigorous protease pre-treatment. In situ RT-PCR is feasible for measles virus in acutely infected cells both in sections and in suspension. Further work is required to improve the procedure and to eliminate artefactual nuclear signal.

Reetoo, K. N., S. A. Osman, et al. (1999). "Development and evaluation of quantitative-competitive PCR for quantitation of coxsackievirus B3 RNA in experimentally infected murine tissues." Journal of Virological Methods **82**(2): 145.

<http://www.sciencedirect.com/science/article/B6T96-3XT0BN2-6/2/603d7118f2bb100f7c234314488bba18>

A method is described for quantitation of enterovirus RNA in experimentally infected murine tissues. Viral RNA was extracted from tissue samples and amplified by reverse transcriptase PCR in the presence of an internal standard RNA. The ratio of PCR product derived from viral RNA and internal standard RNA was then determined using specific probes in a post-PCR electrochemiluminescent hybridization assay. This provided an estimate of the viral RNA copy number in the original sample, and detection of PCR product derived from internal standard RNA validated sample processing and amplification procedures. RNA copy number correlated with viral infectivity of cell culture-derived virus, and one tissue culture infective dose was found to

contain approximately 103 genome equivalents. The ratio of RNA copy number to infectivity in myocardial tissue taken from mice during the acute phase of coxsackievirus B3 myocarditis was more variable ranging from 104-107, and was dependent on the stage of infection, reflecting differential rates of clearance for viral RNA and viral infectivity. The assay is rapid, and could facilitate investigations which currently rely upon enterovirus quantitation by titration in cell culture. This would be useful for experimental studies of viral pathogenesis, prophylaxis and antiviral therapy.

Revilla-Fernandez, S., B. Wallner, et al. "The use of endogenous and exogenous reference RNAs for qualitative and quantitative detection of PRRSV in porcine semen." Journal of Virological Methods **In Press, Corrected Proof** <http://www.sciencedirect.com/science/article/B6T96-4FJGW2B-3/2/4b6e018f1b0d1f46b449b1acfc7a000f>

Semen is known to be a route of porcine reproductive and respiratory syndrome virus (PRRSV) transmission. A method was developed for qualitative and quantitative detection of the seminal cell-associated PRRSV RNA in relation to endogenous and exogenous reference RNAs. As endogenous control for one-step real-time reverse transcription (RT)-PCR UBE2D2 mRNA was selected. Particularly for the analysis of persistent infections associated with low copy numbers of PRRSV RNA, UBE2D2 mRNA is an ideal control due to its low expression in seminal cells and its detection in all samples analysed (n = 36). However, the amount of UBE2D2 mRNA in porcine semen varied (up to 106-fold), thus its use is limited to qualitative detection of PRRSV RNA. For quantitation, a synthetic, non-metazoan RNA was added to the RNA isolation reaction at an exact copy number. The photosynthesis gene ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcl) from *Arabidopsis thaliana* was used as an exogenous spike. Unexpectedly, PRRSV RNA was detected in a herd of specific pathogen-free (SPF) boars which were tested ELISA-negative for anti-PRRSV antibodies. Therefore, RT-PCR for seminal cell-associated PRRSV is a powerful tool for managing the SPF status during quarantine programs and for routine outbreak investigations.

Rijsewijk, F., S. Pritz-Verschuren, et al. (2005). "Development of a polymerase chain reaction for the detection of Anguillid herpesvirus DNA in eels based on the herpesvirus DNA polymerase gene." Journal of Virological Methods **124**(1-2): 87.

<http://www.sciencedirect.com/science/article/B6T96-4F1503P-2/2/9df0f56d5ef0c68078e3d12a1633c505>

Anguillid herpesvirus (AnHV, also known as Herpesvirus anguillae or HVA) is found in both Japanese and European eels. Based on restriction enzyme analysis a small number of differences were found between AnHV isolated from Japanese eels and from European eels. The total genome size of both is about 245 kb, which is confirmed by alternating-field electrophoresis. Using a set of degenerate primers based on conserved regions within DNA-directed DNA polymerase coding regions, a 463 base pair fragment was isolated from both Japanese and European AnHV. Nucleotide sequence analysis showed that the cloned regions of both viruses have identical sequences. Based on this part of the DNA-polymerase sequence, primers were selected and used to develop a sensitive PCR to detect AnHV DNA in eel tissue samples. To avoid false negative results and to estimate the number of AnHV genome copies found in tissues, 100 copies of an internal control plasmid were added to the tissue samples. This semi-quantitative AnHV PCR can be used for both the European and Japanese isolates of AnHV, detects as few as 10 genome copies and is 100 times more sensitive than standard virus isolation.

Rimstad, E. and K. Ueland (1992). "Detection of feline immunodeficiency virus by a nested polymerase chain reaction." Journal of Virological Methods **36**(3): 239.

<http://www.sciencedirect.com/science/article/B6T96-476RM6B-1S/2/1fd9de23b89049a52b4dc338f65dfe2b>

A specific and sensitive polymerase chain reaction (PCR) procedure for the detection of feline immunodeficiency virus (FIV) in peripheral blood mononuclear cells (PBMC) was developed. PBMC from both blood samples and cultures were digested by proteinase K in a lysis buffer, and after heat inactivation of the proteinase, the resultant material was used in a two step amplification protocol using nested sets of primers. Two independent amplifications, from the gag and pol genes respectively, were performed in each tube. The PCR was positive for six of 14 samples from FIV seropositive adult cats, while all 36 samples from seronegative cats were negative. In comparison with an antigen-capturing ELISA procedure, the PCR detected FIV infection in PBMC cultures on average two days earlier.

Roberts, C. A., R. G. Dietzgen, et al. (2000). "Real-time RT-PCR fluorescent detection of tomato spotted wilt virus." Journal of Virological Methods **88**(1): 1.

<http://www.sciencedirect.com/science/article/B6T96-40TY33T-1/2/d294efce2cdf87866a0c40d592f87db1>

A real-time reverse transcription-polymerase chain reaction assay based on TaqMan(TM) chemistry was developed for the detection and quantification of tomato spotted wilt virus (TSWV). This method enabled sensitive, reproducible and specific detection of TSWV in 'leaf soak' and total RNA extracts from infected plants. TaqMan reliably detected TSWV in as little as 500 fg total RNA. The assay was 10-fold more sensitive than visualisation of ethidium bromide-stained bands following agarose gel electrophoresis. TSWV isolates from various crops and locations were detected with a cycle threshold of 20-26 in 1 ng total RNA extracted from fresh or freeze-dried leaves. A dilution series of in vitro transcripts from a cloned 628 base pair fragment of TSWV S RNA served as standard for quantification of viral template in infected leaf samples. The TaqMan assay detected reproducibly 1000 molecules of the target transcript.

Rohayem, J., S. Berger, et al. (2004). "A simple and rapid single-step multiplex RT-PCR to detect Norovirus, Astrovirus and Adenovirus in clinical stool samples." Journal of Virological Methods **118**(1): 49.

<http://www.sciencedirect.com/science/article/B6T96-4BT7JK6-1/2/c783ae5d862a1aa5da9d4aa1aa5ea7e1>

A single-step multiplex reverse transcription-polymerase chain reaction (RT-PCR) assay that detects and identifies Norovirus, Astrovirus and Adenovirus in clinical stool samples is described. Four hundred sixty stool samples were tested from patients with non-rotavirus acute gastroenteritis, that were either stored at -80 [deg]C and tested retrospectively, or tested immediately after viral nucleic acid extraction in a prospective manner, including outbreaks of gastroenteritis that occurred in Germany during the winter of 2003. The multiplex RT-PCR was validated against simplex RT-PCR with published primers for Norovirus (JV12/JV13 and p289/p290) and Astrovirus (Mon340/348), and against simplex PCR for Adenovirus. In both retrospective and prospective settings, the multiplex RT-PCR was equally sensitive and specific in detecting non-rotavirus infections compared with simplex RT-PCR/PCR. The specificity of the multiplex RT-PCR was assessed by sequencing of the amplicons that showed high nucleotide

identities to Norovirus genogroup I/1, I/4, II/2, or II/4 clades, as well as to Astrovirus serotypes 1, 2, 4, or 8. The multiplex RT-PCR was also more sensitive than Astrovirus and Norovirus antigen enzyme immunoassays (IDEIA, Dako), as well as Astrovirus isolation in cell culture. This novel multiplex RT-PCR is an attractive technique for the rapid, specific, and cost-effective laboratory diagnosis of non-rotavirus acute gastroenteritis.

Ros, C., M. E. Riquelme, et al. (1999). "Improved detection of five closely related ruminant alphaherpesviruses by specific amplification of viral genomic sequences." Journal of Virological Methods **83**(1-2): 55.

<http://www.sciencedirect.com/science/article/B6T96-3XVPG88-7/2/d5c0abe062eee652b1ff5ac334d67da2>

The detection and discrimination of five closely related ruminant alphaherpesviruses, bovine herpesvirus 1 (BHV-1), bovine herpesvirus 5 (BHV-5), caprine herpesvirus 1 (CapHV-1), cervine herpesvirus 1 (CerHV-1), and rangiferine herpesvirus 1 (RanHV-1), were achieved by the development of specific PCR systems. The highly variable N-terminal of the glycoprotein C was chosen to select the diagnostic primers, except for the CerHV-1 primers, which targeted the glycoprotein D region. All the assays proved specific since no heterologous virus was amplified. BHV-1 and BHV-5 were detected by using the same PCR assay and the different sizes of the amplification products allowed their identification on agarose gels. The practical diagnostic applicability of the novel PCR assays, with special regard to the BHV-1 system, has been evaluated on clinical samples from experimentally infected animals.

Sagazio, P., M. Tempesta, et al. (1998). "Antigenic characterization of canine parvovirus strains isolated in Italy." Journal of Virological Methods **73**(2): 197.

<http://www.sciencedirect.com/science/article/B6T96-3TGVK28-7/2/95394439de447d154ef4dd58b6eac5ee>

28 isolates of canine parvovirus type-2 (CPV-2) were obtained from dogs with hemorrhagic gastroenteritis in Italy. The antigenic structure of CPV-2 isolates was characterized, using four discriminating monoclonal antibodies. In addition, four vaccinal strains were examined. Similar to reports from Australia and the United Kingdom, a much higher prevalence of CPV-2a (25/28 isolates) was observed than the other variant type, CPV-2b (3/28 isolates). DNA fragments (2.2 kbp) of representative strains of CPV-2, CPV-2a and CPV-2b were amplified by the polymerase chain reaction (PCR) and the products were digested by the restriction enzymes (RE) RsaI, HpaII, HindIII and PvuII. The RsaI enzyme allows the differentiation of CPV-2 from CPV-2a and CPV-2b.

Saito, T., S. Matsumoto, et al. (1998). "Multicyclic reverse transcription-polymerase chain reaction assay system for quantitation of GB virus-C/hepatitis G virus RNA in serum." Journal of Virological Methods **74**(2): 185.

<http://www.sciencedirect.com/science/article/B6T96-3V5082S-7/2/99c3cccab7fe3b48c34d9ef79a26f4d5>

A new quantitative reverse transcription-polymerase chain reaction (RT-PCR) method is described for analyzing the amount of GB virus-C (GBV-C)/hepatitis G virus (HGV) RNA in

serum. This multicyclic RT-PCR (MRT-PCR) method used oligonucleotide primers deduced from the 3' noncoding region (3'NCR) that is highly conserved among GBV-C/HGV isolates. Quantitation of GBV-C/HGV RNA using MRT-PCR ranged between 102 and 1010 copies/ml when PCR cycle number was regulated at exponential amplification of the products. Competitive RT-PCR (CRT-PCR) was carried out with mutant RNA and sample that had been measured by MRT-PCR. Quantitation of GBV-C/HGV RNA using both methods agreed. MRT-PCR detected viral RNA in a single step PCR, and demonstrated a high degree of sensitivity that was equal to that of the RT-PCR procedure, which used nested primers deduced from the non-structural (NS) 3 region. The MRT-PCR method for quantitation of GBV-C/HGV RNA in serum may prove useful for diagnosis.

Saito, T., Y. Munakata, et al. (2003). "Evaluation of anti-parvovirus B19 activity in sera by assay using quantitative polymerase chain reaction." Journal of Virological Methods **107**(1): 81.

<http://www.sciencedirect.com/science/article/B6T96-46YXPVK-6/2/6624b5ec4f802415c0141af6f7bcd2b>

Human parvovirus B19 (B19) infects cells of erythroid lineage. Production of neutralizing antibodies (Abs) is indispensable for recovery from B19-related disease state. In this study, we used a convenient method to measure neutralizing activities in human sera by using a real-time quantitative PCR based assay. Erythroid cell line KU812Ep6 was incubated with test sera before infection with B19 virus. The copy number of B19-DNA in cultures was decreased in the presence of the sera from patients who recovered from acute B19 infection, whereas no decrease in B19-DNA was in cultures incubated with sera from healthy volunteers who had no B19 infection. The decrease in B19-DNA copy number was calculated and the inhibition percentage was expressed as neutralizing activity to B19. A clinical study showed that the levels of neutralizing ability were high in patients who recovered soon after acute B19 infection, but were low in some patients with a prolonged clinical course for recovery from B19 infection. This method is simple and convenient compared with methods described previously, showing its usefulness to evaluate the neutralizing activity to B19.

Saiz, M., S. Castro, et al. (1994). "Serotype-specific detection of bean common mosaic potyvirus in bean leaf and seed tissue by enzymatic amplification." Journal of Virological Methods **50**(1-3): 145.

<http://www.sciencedirect.com/science/article/B6T96-47DKYF8-H/2/87f59a02642bd2455bef08fc0ec16279>

An assay involving reverse transcription and polymerase chain reaction (PCR) is described for specific detection of serotypes A and B BCMV isolates in bean leaf and seed tissues. Three oligonucleotide primers designed according to the sequence data available allow, in appropriate combination, serotype-specific detection of BCMV. The sensitivity of the method was sufficient to detect BCMV in as little as 100 fg and 50 pg of infected leaf and seed tissues, respectively.

Sanchez-Seco, M. P., D. Rosario, et al. "Generic RT-nested-PCR for detection of flaviviruses using degenerated primers and internal control followed by sequencing for specific identification." Journal of Virological Methods **In Press, Corrected Proof**

<http://www.sciencedirect.com/science/article/B6T96-4FKYDM9-1/2/578f3dab73d77e4adfd7444b2f611d7a>

Flaviviruses are a widespread and numerous group of arboviruses that can cause serious illness in humans. The continuous and slow spread of certain flaviviruses, such as Dengue viruses, and the recent entry and spread of West Nile virus to the American continent, point to the need to control these infections. This control requires the use of suitable techniques for diagnostic and surveillance programmes. A generic RT-nested-PCR that is, theoretically, able to detect each member of the group has been designed. The identification of the detected virus is carried out by sequencing. The introduction of an internal control would reduce the number of false negative results and could be used to quantify the viral load in clinical samples where the method works well.

Sanchez-Seco, M. P., D. Rosario, et al. (2001). "A generic nested-RT-PCR followed by sequencing for detection and identification of members of the alphavirus genus." Journal of Virological Methods **95**(1-2): 153.

<http://www.sciencedirect.com/science/article/B6T96-433NRB5-H/2/3f2609203a01d4d108621be750cc519d>

A specific and sensitive nested RT-PCR method was developed for the detection of members of the alphavirus genus. Based on available sequences, degenerated primers were selected in the nsP4 gene. Reaction components and thermal cycling parameters were investigated and standardised, and optimal ones were selected. As few as 25 pfu/tube could be detected. The identities of the amplified fragments were confirmed by sequencing, and phylogenetic analysis was carried out. The resulting phylogenetic tree could be applied to classify every alphavirus according to its serogroup. This technique is suitable for rapid, sensitive and reliable detection of these viruses and may be very valuable for diagnostic applications and surveillance.

Saulnier, P., M. Vidaud, et al. (2003). "Development and validation of a real-time PCR assay for the detection and quantitation of p53 recombinant adenovirus in clinical samples from patients treated with Ad5CMV-p53 (INGN 201)." Journal of Virological Methods **114**(1): 55.

<http://www.sciencedirect.com/science/article/B6T96-49SNFD4-1/2/83c3e27a4029aea7fba1d9122574acf1>

The purpose of this study was to assess the usefulness of real-time PCR as a quantitative, highly reproducible, and sensitive method, for detecting and quantifying p53 recombinant adenovirus in biological samples from cancer patients receiving injections of Ad5CMV-p53. The dynamic range of this real-time PCR-based assay was wide (at least five orders of magnitude). Our assay used an internal positive control in the same PCR tube that is capable of detecting residual PCR inhibitors. Serial spiked samples in plasma with known quantities of Ad5CMV-p53 were evaluated. The minimum detection limit was 2 pfu per PCR (~50 pfu per ml of plasma) and the quantification values were reproducible. A total of 2069 controls tested with 1780 plasma samples from 286 patients enrolled in gene therapy trials using Ad5CMV-p53 were investigated. Using calibrators to adjust the quantitation value, the results confirmed the good performance of the assay. In conclusion, the high sensitivity, simplicity and reproducibility of the real-time Ad5CMV-p53 assay, allowing screening of large numbers of samples, combined with its wide dynamic range, make this method particularly suitable for monitoring gene therapy trials.

Sears, J. F. and A. S. Khan (2003). "Single-tube fluorescent product-enhanced reverse transcriptase assay with Ampliwax(TM) (STF-PERT) for retrovirus quantitation." Journal of Virological Methods

108(1): 139.

<http://www.sciencedirect.com/science/article/B6T96-47T8W17-4/2/d01195ea63d2951ed22610babe264af9>

A TaqMan fluorescent probe-based product enhanced reverse transcriptase (RT) assay is described in which the RT and polymerase chain reaction (PCR) steps are set-up in a single tube, in two compartments separated by Ampliwax(TM) (designated as single-tube fluorescent product-enhanced reverse transcriptase assay (STF-PERT)). This simplification of the two-step method resulted in increased assay reproducibility and handling efficiency while maintaining the sensitivity of the PERT assay (<10 virions). The STF-PERT assay can be used to quantitate low amounts of retrovirus in clinical and research materials and to evaluate retrovirus contamination in cell substrates and biological products in human use.

Shafer, R. W., A. Warford, et al. (2000). "Reproducibility of human immunodeficiency virus type 1 (HIV-1) protease and reverse transcriptase sequencing of plasma samples from heavily treated HIV-1-infected individuals." Journal of Virological Methods **86**(2): 143.

<http://www.sciencedirect.com/science/article/B6T96-403W2YC-5/2/211fd2658611ede185f47b60f768fccd>

The reproducibility of population-based human immunodeficiency virus type 1 (HIV-1) protease and reverse transcriptase (RT) sequencing was assessed using replicate aliquots of cryopreserved plasma samples obtained from seven heavily treated HIV-1-infected individuals. The sequence of each sample replicate was compared with the consensus sequence for that sample and 99.4% of 35128 amino acids were found to be concordant with the sample consensus. Partial discordances were present at 0.5% of positions and complete discordances were present at <0.1% of positions. To assess the reproducibility at detecting mutations (defined here as differences from the subtype B consensus sequence), the proportion of sequences having a mutation when at least two sequences from that sample had the same mutation were examined. There was a median of 13 protease and 18 RT mutations per sample for a total of 3126 mutations; 95% of these mutations were detected. However, sequencing of multiple clones from two samples demonstrated that those mutations present in a minority of clones were often not detected by population-based sequencing. These results suggest that HIV-1 protease and RT sequencing of circulating plasma virus is highly reproducible but that the sensitivity at detecting mutations may be low if those mutations are present as minor variants.

Shamloul, A. M., F. Faggioli, et al. (2002). "A novel multiplex RT-PCR probe capture hybridization (RT-PCR-ELISA) for simultaneous detection of six viroids in four genera: Apscaviroid, Hostuviroid, Pelamoviroid, and Pospiviroid." Journal of Virological Methods **105**(1): 115.

<http://www.sciencedirect.com/science/article/B6T96-46041YT-1/2/4e56918384176482201d2cdf6a26b133>

A rapid and sensitive assay was developed for the detection and identification of viroids by standard or multiplex reverse transcription-polymerase chain reaction (RT-PCR)-probe capture hybridization (RT-PCR-ELISA). The assay was applied successfully for the detection and identification of the following six viroid species from infected tissues: Potato spindle tuber viroid (Pospiviroid), Peach latent mosaic viroid (Pelamoviroid), Apple scar skin viroid (Apscaviroid), Apple dimple fruit viroid (Apscaviroid), Pear blister canker viroid (Apscaviroid), and Hop stunt viroid (Hostuviroid). Total RNA was obtained from infected tissue by the Qiagen RNeasy kit and,

then viroid cDNA was synthesized using viroid specific complementary DNA primer. To identify and differentiate the amplicons of the six viroids, each amplicon was digoxigenin (DIG)-labelled during the amplification process, and then detected by a colorimetric system using a biotinylated cDNA capture probe specific for each viroid. The results revealed that each capture probe hybridized only to its complementary DIG-labelled amplicon. Thus the six viroids can be detected and differentiated in a multiplex RT-PCR-ELISA assay. In the multiplex assay, cDNAs of six viroids were synthesized simultaneously in one tube, DIG-labelled during amplification, then a portion of the DIG-labelled amplified products was hybridized with selected capture probe. All the six viroid capture probes hybridized to their respective complementary DIG-labelled RT-PCR-amplified product. These findings are important for viroid detection and identification for studying host-viroid interactions and for management and control viroid diseases.

Shamloul, A. M. and A. Hadidi (1999). "Sensitive detection of potato spindle tuber and temperate fruit tree viroids by reverse transcription-polymerase chain reaction-probe capture hybridization." Journal of Virological Methods **80**(2): 145.

<http://www.sciencedirect.com/science/article/B6T96-3X29D70-5/2/e3613f2ae2220c4fea31c8809811e72f>

A rapid and sensitive assay for the specific detection of plant viroids using reverse transcription-polymerase chain reaction (RT-PCR) -probe capture hybridization (RT-PCR-enzyme-linked immunosorbent assay (ELISA)) was developed. The assay was applied successfully for the detection of potato spindle tuber viroid, peach latent mosaic viroid, or apple scar skin viroid from viroid infected leaf tissue. Clarified sap extract from infected leaf tissue was treated first with GeneReleaser(TM) polymeric matrix to remove inhibitors of RT-PCR reactions. Viroid cDNA was then synthesized and amplified using viroid specific primers in RT-PCR assays and the amplified viroid cDNA (amplicon) was digoxigenin (DIG) -labelled during the amplification process. The amplicon was then detected in a colorimetric hybridization system in a microtiter plate using a biotinylated cDNA capture probe. This system combines the specificity of molecular hybridization, the ease of the colorimetric protocol, and is at least 100-fold more sensitive than gel electrophoretic analysis in detecting the amplified product. Viroid cRNA may replace viroid cDNA as the capture probe. The cRNA probe was several fold more sensitive than the cDNA probe for viroid detection. Six to seven hours are needed to complete the RT-PCR-ELISA for viroid detection from infected leaf tissue.

Shin, J., E. M. Bautista, et al. (1998). "Quantitation of porcine reproductive and respiratory syndrome virus RNA in semen by single-tube reverse transcription-nested polymerase chain reaction." Journal of Virological Methods **72**(1): 67.

<http://www.sciencedirect.com/science/article/B6T96-3SXD XR-8/2/03cca086219a7ca36f7b08cbf32d7738>

Porcine reproductive and respiratory syndrome virus (PRRSV) is in boar semen for extended periods of time as determined by reverse transcription-nested polymerase chain reaction (RT-nPCR) assay. The concentration of PRRSV RNA in semen and the biological significance of the detection level, however, remain to be resolved. In order to determine the concentration of PRRSV VR-2332 (a prototypic strain of North American isolates) in semen following infection, we established a 'standard curve'-quantitative competitive (SC-QC)-RT-nPCR assay as well as an equimolar QC-RT-nPCR assay. A deletion-type competitor RNA derived from the Lelystad virus, a European strain of PRRSV, ORF-7 gene standard which shares the nested sets of primer recognition sequences with the VR-2332 ORF-7 gene was used as an internal standard. The equimolar QC-RT-nPCR assay results revealed that the number of copies of PRRSV RNA in 1

TCID₅₀/ml of virus derived from CL-2621 cell culture supernatants varied depending upon the type of samples in which virus was added; 143±24.0 and 266.5±48.5 copies in serum and semen samples spiked with PRRSV VR-2332, respectively. For the establishment of SC-QC-RT-nPCR assay, a standard curve was generated from band intensity ratios versus a series of known initial numbers of wild-type RNA copies which were quantified by the equimolar QC-RT-nPCR assay. Various initial numbers of copies of wild-type PRRSV RNA and each band intensity ratio with 1000 copies of competitor RNA were well correlated within the range of 100 to 200000 copies (R²=0.947). A 'standard curve' quantitation assay using competitive single-tube RT-nPCR will offer a rapid and reliable way to quantify low concentrations of PRRSV RNA in semen.

Singh, M. and R. P. Singh (1996). "Factors affecting detection of PVY in dormant tubers by reverse transcription polymerase chain reaction and nucleic acid spot hybridization." Journal of Virological Methods **60**(1): 47.

<http://www.sciencedirect.com/science/article/B6T96-3VYV029-5/2/30e07e74fe28d8beae6c23e901c5df2>

A reverse transcription polymerase chain reaction (RT-PCR) protocol was developed using two 20-mer primers located in nuclear inclusion genes NIa and NIb of potato virus Y (PVY). A 1017 bp PCR-product was detected in dormant potato tubers, infected with PVYO, but not in tubers from healthy plants. The PCR product was specific to PVY, as determined by Southern blot detection by hybridization with a PVYO-specific probe. As little as 1 pg of purified PVYO-RNA can be detected after RT-PCR amplification. The presence of phenolics or polysaccharides in tuber nucleic acids inhibited PVYO amplification, which was eliminated by diluting nucleic acid preparations prior to cDNA synthesis, modifying the nucleic acid extraction procedure by isopropanol precipitation and using phosphate-buffered saline-Tween in the cDNA mix. Potato cultivars differed in PVYO concentration in tubers as much as 128-fold. Tuber parts used for nucleic acid extractions were important in potato cultivars with low virus titres and combining of saps from bud and stem ends improved the PVYO detection. Storage of tubers up to 90 days at 4 [deg]C did not result in reduced detection of PVYO by both nucleic acid spot hybridization and RT-PCR, but RT-PCR band intensity was lower at longer storage periods. The primer pair developed in this study exhibited broad specificities with field isolates from Peru, Scotland and North America.

Singh, R. P. (1999). "A solvent-free, rapid and simple virus RNA-release method for potato leafroll virus detection in aphids and plants by reverse transcription polymerase chain reaction." Journal of Virological Methods **83**(1-2): 27.

<http://www.sciencedirect.com/science/article/B6T96-3XVPG88-4/2/977b58dd229d4b6f7912a88e1a7867d2>

A one-step, rapid and economical method for potato leafroll virus (PLRV) RNA release that is applicable to the use on a microcentrifuge scale is described. Discs (3-6 mm diameter) from leaves, petioles, stems, and tubers of potato plants were incubated in microcentrifuge tubes with detergent solution. The supernatants were used directly for reverse transcription (RT) and polymerase chain reaction (PCR). Of the seven nonionic detergents of the TritonX series evaluated, Triton X405R was the most effective, although X-405 and X-100R were also effective in releasing PLRV RNA. Application of the detergent method for detecting PLRV in greenhouse-grown potato organs (leaves, petioles, stems, tubers) and in field-grown tubers was demonstrated and compared to the multi-step phenol method. When individual aphids, *Myzus persicae*, were ground in 20 [μl] of detergent solution and supernatants were used for RT-PCR, virus was detected in single aphids in undiluted solutions and up to a dilution of 1:4. The concentration of

PLRV RNA released by the detergent method was substantially lower than that released by the phenol method. However, the detergent method was sensitive enough to detect PLRV from potato leaves, petioles, and stems 2 weeks after graft inoculation. The detergent method was rapid and economical, and has potential for large-scale application. The extracts survived over 37 days at room temperature, thus making it possible to mail extracts from remote areas lacking specialised RT-PCR facilities to a central laboratory for PLRV testing.

Singh, R. P., J. Kurz, et al. (1995). "Detection of potato leafroll virus in single aphids by the reverse transcription polymerase chain reaction and its potential epidemiological application." Journal of Virological Methods **55**(1): 133.

<http://www.sciencedirect.com/science/article/B6T96-3YYTF0W-1D/2/01e92bca3148bbe3d53dafaec5171d9c>

A reverse transcription and polymerase chain reaction (RT-PCR) system was developed using two 20-mer primers located in the potato leafroll virus (PLRV) capsid gene. A 336-bp PCR product was detected from aphids (*Myzus persicae*) which had been fed on PLRV-infected plants. The PCR band was specific to PLRV as determined by Southern blots and detection by a PLRV-specific probe. As little as 5 min exposure of aphids to PLRV-infected leaves resulted in the presence of PLRV-specific bands in 13% of aphids. However, the percentage of PLRV-positive aphids increased with longer exposure to infected sources and reached 90% after 3-4 days of feeding. PLRV can be detected from a single viruliferous aphid or a single viruliferous aphid combined with up to 29 non-viruliferous aphids. PLRV can be detected from freshly collected aphids, those stored at -70 [deg]C, or those stored in 70% ethanol at room temperature for extended periods. This method is applicable to assess the viruliferous nature of aphids caught in yellow-pan traps during the growing season or stored for over a year.

Singh, R. P., X. Nie, et al. (2000). "Duplex RT-PCR: reagent concentrations at reverse transcription stage affect the PCR performance." Journal of Virological Methods **86**(2): 121.

<http://www.sciencedirect.com/science/article/B6T96-403W2YC-3/2/c4001009ce2ed15163382fd0658efd00>

Test conditions for the simultaneous detection of potato leafroll virus (PLRV) and potato virus Y (PVY) in dormant tubers and leaves by reverse transcription-polymerase chain reaction (RT-PCR) were optimized. Various factors optimized at the reverse transcription (RT) stage rather than at the amplification (PCR) stage affected the outcome. In the simplex RT-PCR a onefold dNTPs concentration (0.5 mM) was sufficient in yielding a PLRV or PVY band. In contrast, the duplex RT-PCR required a minimum twofold dNTPs concentration (1.0 mM) during RT to produce distinct bands in PCR. Similarly, various proportions of antisense primers of PLRV and PVY used during RT affected subsequent duplex RT-PCR. Optimal amplification of both viruses were obtained at a ratio of 0.90:0.49 [mu]M of PLRV:PVY antisense primers. An interaction of dNTPs and RNA template concentration was observed. A higher concentration of RNA was required at onefold dNTPs concentration than at twofold dNTPs. Dilutions down to 1:300 of RNA template yielded distinct bands of both viruses at twofold dNTPs concentration. At optimized conditions of the duplex RT-PCR both viruses were reliably detected in composite samples at a ratio of one part infected sap mixed with 399 parts of sap from healthy tubers. Application of optimized conditions to singly- and doubly-infected tubers detected both viruses from naturally infected field-grown tubers. A nearly perfect correlation ($r^2=0.99$) was observed between visible plant symptoms and the virus detection from leaves and tubers by the duplex RT-PCR.

Singh, R. P., X. Nie, et al. (2002). "Sodium sulphite inhibition of potato and cherry polyphenolics in nucleic acid extraction for virus detection by RT-PCR." Journal of Virological Methods **99**(1-2): 123.

<http://www.sciencedirect.com/science/article/B6T96-448BG21-F/2/e2ba6de7b19d265c3bcb9cec6271bd11>

Phenolic compounds from plant tissues inhibit reverse transcription-polymerase chain reaction (RT-PCR). Multiple-step protocols using several additives to inhibit polyphenolic compounds during nucleic acid extraction are common, but time consuming and laborious. The current research highlights that the inclusion of 0.65 to 0.70% of sodium sulphite in the extraction buffer minimizes the pigmentation of nucleic acid extracts and improves the RT-PCR detection of Potato virus Y (PVY) and Potato leafroll virus (PLRV) in potato (*Solanum tuberosum*) tubers and Prune dwarf virus (PDV) and *Prunus* necrotic ringspot virus (PNRSV) in leaves and bark in the sweet cherry (*Prunus avium*) tree. Substituting sodium sulphite in the nucleic acid extraction buffer eliminated the use of proteinase K during extraction. Reagents phosphate buffered saline (PBS)-Tween 20 and polyvinylpyrrolidone (PVP) were also no longer required during RT or PCR phase. The resultant nucleic acid extracts were suitable for both duplex and multiplex RT-PCR. This simple and less expensive nucleic acid extraction protocol has proved very effective for potato cv. Russet Norkotah, which contains a high amount of polyphenolics. Comparing commercially available RNA extraction kits (Catrimox(TM) and RNeasy(TM)), the sodium sulphite based extraction protocol yielded two to three times higher amounts of RNA, while maintaining comparable virus detection by RT-PCR. The sodium sulphite based extraction protocol was equally effective in potato tubers, and in leaves and bark from the cherry tree.

Singh, R. P., M. Singh, et al. (1998). "Use of citric acid for neutralizing polymerase chain reaction inhibition by chlorogenic acid in potato extracts." Journal of Virological Methods **74**(2): 231.

<http://www.sciencedirect.com/science/article/B6T96-3V5082S-F/2/70fd6d5044a0673a2a80a8866266f339>

Polyphenolics are a widely distributed class of plant constituents implicated in the inhibition of the polymerase chain reaction (PCR). Since chlorogenic acid forms the bulk of phenolics in the potato tuber, a range of chlorogenic acid concentrations were tested for inhibition of reverse transcription (RT) and PCR steps. The RT step was inhibited at chlorogenic acid concentrations of 6.0 to 7.5 [μ g], while the PCR step was inhibited at much lower concentrations of 1.2 to 1.8 [μ g]. Incorporation of 1.2% citric acid at the nucleic acid extraction step prevented darkening of the extract and neutralized the inhibitory effect of chlorogenic acid.

Singh, S., V. T. K. Chow, et al. (2000). "RT-PCR, nucleotide, amino acid and phylogenetic analyses of enterovirus type 71 strains from Asia." Journal of Virological Methods **88**(2): 193.

<http://www.sciencedirect.com/science/article/B6T96-411PGRW-9/2/a1b8376344b1295742393fd5e48e326c>

A specific and sensitive method based on RT-PCR was developed to detect enterovirus 71 (EV71) from patients with hand, foot and mouth disease, myocarditis, aseptic meningitis and acute flaccid paralysis. RT-PCR primers from conserved parts of the VP1 capsid gene were designed on the basis of good correlation with sequences of EV71 strains. These primers successfully amplified 44 strains of EV71 including 34 strains isolated from Singapore in 1997

and 1998, eight strains from Malaysia isolated in 1997 and 1998, one Japanese strain and the neurovirulent strain EV71/7423/MS/87. RT-PCR of 30 strains of other enteroviruses including coxsackievirus A and B, and echoviruses failed to give any positive amplicons. Hence, RT-PCR with these primers showed 100% correlation with serotyping. Direct sequencing of the RT-PCR products of 20 EV71 strains revealed a distinct cluster with two major subgroups, thus enabling genetic typing of the viruses. The genetic heterogeneity of these strains culminated in amino acid substitutions within the VP1, VP2 and VP3 regions. The sequencing of a 2.9 kb fragment comprising the capsid region and the major part of 5' UTR of two Singapore strains revealed that they belonged to a group distinct from the prototype EV71/BrCr strain and the EV71/7423/MS/87 strain. The dendrogram generated from 341 bp fragments within the VP1 region revealed that the strains of Singapore, Malaysia and Taiwan belong to two entirely different EV71 genogroups, distinct from the three genogroups identified in another recent study.

Smith, G. R., R. Van de Velde, et al. (1992). "PCR amplification of a specific double-stranded RNA region of Fiji disease virus from diseased sugarcane." Journal of Virological Methods **39**(3): 237.

<http://www.sciencedirect.com/science/article/B6T96-476F61S-67/2/e61cf83990890fba99d5dcd116139156>

A 450-bp region from one species of the segmented dsRNA genome of Fiji disease virus (FDV) was amplified from total nucleic acid extracts of diseased plants by reverse transcription with MMLV, followed by amplification with Taq DNA polymerase (RT-PCR). Other FDV-specific regions (c 150 bp and c 270 bp) were also amplified from the dsRNA template. FDV cDNA was only synthesised when the viral dsRNA template was boiled and quenched with FDV-specific or random hexamer primers. The reverse transcriptase/DNA polymerase enzyme rTth appeared to yield only the 150 bp fragment from the dsRNA template under the conditions used. The level of sensitivity of RT-PCR for purified FDV dsRNA was 100 ag, approximately 104-fold more sensitive than detection with biotinylated DNA probe.

Smith, J., L. M. McElhinney, et al. (2000). "Assessment of template quality by the incorporation of an internal control into a RT-PCR for the detection of rabies and rabies-related viruses." Journal of Virological Methods **84**(2): 107.

<http://www.sciencedirect.com/science/article/B6T96-3YDG9HS-1/2/e00ff628ace2e3a0272d32f5b1bf6c98>

A method is described to assess RNA template quality by the incorporation of a ribosomal RNA (rRNA) internal (in tube) control into a standard rabies and rabies-related virus specific RT-PCR. Specific virus and rRNA templates were co-amplified in a duplex reaction from RNA extracts derived from 60 isolates representing all six of the established lyssavirus genotypes. To ensure a wide species applicability of this technique we demonstrated that the rRNA assay was capable of functioning using the cells or tissues of 14 different mammals. Parallel studies between the duplex and the unlinked lyssavirus assay demonstrated only a minor reduction in the sensitivity of the former test. The ribosomal and viral targets (unlike [beta]-actin RNA) were shown to have similar degradation kinetics making rRNA amplification a good control for viral target integrity. As a consequence, the use of this system would reduce the likelihood of obtaining false negative RT-PCR results from lyssavirus infected material.

Stamey, F. R., M. DeLeon-Carnes, et al. (2003). "Comparison of a microtiter plate system to Southern

blot for detection of human herpesvirus 8 DNA amplified from blood and saliva." Journal of Virological Methods **108**(2): 189.

<http://www.sciencedirect.com/science/article/B6T96-47PGC4N-6/2/68d06de6865414884ae2c743798ae715>

The recent discovery of human herpesvirus 8 (HHV-8) as the etiologic agent of Kaposi's sarcoma (KS) has led to the interest in the development of PCR for this virus that is accurate, rapid, and convenient. We developed a sensitive PCR assay for HHV-8 with microtiter plate detection of amplimers. DNA was purified from white blood cells and saliva from HIV-infected men with and without Kaposi's sarcoma and one-step PCR was undertaken with primer sets specific for the N-terminal region of the glycoprotein B gene and open reading frame (orf) 26 of HHV-8. PCR was performed on 40 clinical specimens, followed by Southern blot and microtiter plate detection of amplimers. Results from the two methods of detection were nearly identical. Sensitivity for both methods based on serial dilution of a known standard was five to ten copies of HHV-8 per 400 ng of cellular DNA. In conclusion, microtiter plate detection of HHV-8 PCR amplimers is as sensitive and specific as Southern blot with much faster turnaround time at comparable cost, and utilizes common laboratory equipment.

Stocher, M., V. Leb, et al. (2003). "A convenient approach to the generation of multiple internal control DNA for a panel of real-time PCR assays." Journal of Virological Methods **108**(1): 1.

<http://www.sciencedirect.com/science/article/B6T96-47PGC4N-4/2/ff45cc19f684bd3f3af8a09818fdd0b3>

Real-time polymerase chain reaction (PCR) assays allow convenient detection and quantitation of virus-derived nucleic acids in clinical specimens. When specimens are assayed for the presence of virus-derived nucleic acids against external standards, sample adequacy is not monitored. This can be achieved by using internal controls that are co-amplified with the virus-specific DNA in competitive PCR. Each of the various real-time PCR assays in a routine clinical laboratory requires its specific internal control. In order to complement a panel of virus-specific real-time PCR assays with internal controls, a convenient approach is described to generate the several internal controls within single DNA fragment. By applying composite primer technology, PCR primer sequences used in real-time PCR assays were added in 5' and 3' of a stretch of heterologous DNA during consecutive preparative PCRs. The heterologous DNA was used for internal control specific detection by e.g. FRET-hybridisation probes. The presented example of such a multiple internal control DNA contained five internal controls for five competitive LightCycler-PCR assays. All five PCR products derived from the multiple internal control DNA were detected with a single pair of specific FRET-hybridisation probes. The example described proved useful in real-time PCR assays specific for the detection of EBV-, CMV-, VZV- HSV-, and HBV-DNA on the LightCycler instrument. This methodology should enable laboratories to conveniently complement their panel of existing real-time PCR assays with a single multiple internal control DNA.

Strappe, P. M., T. H. Wang, et al. (1998). "In situ polymerase chain reaction amplification of HIV-1 DNA in brain tissue." Journal of Virological Methods **70**(2): 119.

<http://www.sciencedirect.com/science/article/B6T96-3SBVX4H-1/2/110f9577f665297b8bc637b2348039f5>

A direct in situ polymerase chain reaction (IS-PCR) assay is described for the detection of HIV-1

proviral DNA in formalin fixed paraffin embedded brain tissue. Biotin-16-dUTP is incorporated during the PCR process and microwave pretreatment of tissue sections ensures that no non-specific incorporation into damaged or nicked genomic DNA occurs. Two methods are compared to detect the biotinylated amplified product, the use of an avidin-biotin-alkaline phosphatase complex (ABC) and the application of tyramide signal amplification (TSA) which allows both chromogenic and fluorescence detection. TSA detection enhances the sensitivity of IS-PCR, permitting fewer PCR cycles and preserving tissue morphology.

Syrjanen, S., B. Andersson, et al. (1991). "The use of polymerase chain reaction in generation of biotinylated human papillomavirus DNA probes for in situ hybridization." Journal of Virological Methods **31**(2-3): 147.

<http://www.sciencedirect.com/science/article/B6T96-47DM18P-H/2/5a673ca2c3dc4bbb3f5895972b7eca0>

The polymerase chain reaction (PCR) was used to produce biotin-labelled human papillomavirus (HPV) 16- and 18-specific DNA probes for in situ hybridization (ISH). PCR was performed by using AmplitaqTM DNA amplification reagent kit according to the manufacturer's instructions, except that dTTP was substituted by different concentrations of biotinylated dUTP (bio-11-UTP). As template DNA, DNA extracted either from CaSki or HeLa cells was used. The reaction mixture was taken through up to 40 cycles of amplification in a Perkin-Elmer Cetus Thermal Cycler. The highest yield was achieved when the concentrations of dTTP and biotinylated dUTP were 150 and 50 μ M, respectively. ISH results compatible with those obtained with biotinylated whole genomic HPV DNA probes were demonstrated when primers from E7 and E6 ORF of the HPV-18 genome were used to produce the biotinylated probe by PCR. With HPV-16, several areas of the genome had to be amplified to generate a PCR probe with equal sensitivity as the whole genomic probe. The background staining was always stronger with the PCR probes than with the whole genomic probes. The sensitivity of the PCR probes does not seem to bear a clear-cut correlation with the size or nucleotide content of the probe, but it might rather depend on the three dimensional structure of the probe and the availability of biotin for the detection system by ISH.

Szemes, M., M. M. Klerks, et al. (2002). "Development of a multiplex AmpliDet RNA assay for simultaneous detection and typing of potato virus Y isolates." Journal of Virological Methods **100**(1-2): 83.

<http://www.sciencedirect.com/science/article/B6T96-44M1FHH-8/2/469ee9271f622a5bae98781123d31802>

A multiplex AmpliDet RNA assay was developed for the specific detection of potato virus Y (PVY), and for the differentiation of the PVYN, PVYO/C strains and the tuber necrotic isolates (PVYNTN). The assay is based on the generic amplification of a region within the coat protein coding region of all known PVY isolates by nucleic acid sequence-based amplification (NASBATM) and strain-specific detection by molecular beacons. PVYNTN-specific diagnosis is achieved by detecting PVYN and PVYO-specific sequences flanking a recombination site that is associated with the tuber necrotic pathotype. The assay exhibited good specificity toward the various PVY strains in both single and mixed infections. The technique was validated by the use of 47 PVY isolates originating from six countries. The results of the AmpliDet RNA assay were identical or consistent with those of biological characterisation in the decisive majority of cases.

Tai, J. H., M. S. Ewert, et al. (2003). "Development of a rapid method using nucleic acid sequence-based amplification for the detection of astrovirus." Journal of Virological Methods **110**(2): 119.

<http://www.sciencedirect.com/science/article/B6T96-48R3KGG-1/2/da81470d0bd99e7695e005ccec634d79>

We have developed a rapid method to detect astrovirus in fecal specimens utilizing nucleic acid sequence-based amplification (NASBA) and several detection methodologies, including a sandwich hybridization assay based on DNA-tagged liposomes (liposome-strip detection assay). RNA was extracted from 65 stool specimens that were positive for astrovirus by enzyme immunoassay and was amplified by both NASBA and reverse transcriptase PCR (RT-PCR). Also extracted and amplified were 19 specimens containing rotavirus, 20 specimens containing norovirus, five specimens containing adenovirus, 15 water negative control specimens, and eight specimens containing astrovirus reference strains. NASBA products were detected by electrochemiluminescence detection (ECL) and by liposome-strip detection; RT-PCR products were detected by ethidium bromide staining following gel electrophoresis and by liquid hybridization assay (LHA). There was no significant difference in the detection rates of NASBA- and RT-PCR-based assays, with one exception in which the NASBA/ECL assay detected astrovirus in eight specimens that tested negative by the RT-PCR/LHA assay. These results suggest that these NASBA-based detection methods have detection rates that are as good as or better than those of RT-PCR-based methods. Both NASBA and liposome-strip detection may be useful for field studies and environmental testing because these methods are rapid and do not require specialized equipment.

Tasca, S. I., R. J. Hoffman, et al. (1993). "Detection of Moloney murine sarcoma virus in tissues and cultured cells by the polymerase chain reaction." Journal of Virological Methods **41**(3): 255.

<http://www.sciencedirect.com/science/article/B6T96-476F65D-84/2/de74827c1b229f85cf7653d7be953ecb>

The polymerase chain reaction was used for Moloney murine sarcoma virus (MoMuSV) detection in frozen and formalin-fixed, paraffin-embedded tissue sections and cultured cells isolated from MoMuSV-induced tumors. Rapid DNA extraction by proteinase K digestion, followed by CHROMA SPIN + TE-100 column purification proved to be satisfactory. Two pairs of overlapping primers, flanking 1026 base pair (bp) to 221 bp, allowed to choose among four different length of DNA-amplified segments. Although net amplification was obtained for frozen tissue and tumor cultured cells in all combinations of primers, the maximum specificity and sensitivity resulted with 602 bp fragment. This product was fully and adequately digestible using Apa I and Sau3A I restriction endonucleases. DNA extracted from paraffinembedded sections yielded an amplification product only when the primer pair which delineated a 221-bp segment was used. This reproducible method could be useful for diagnostic and for pathogenetic investigations of MoMuSV infections.

Telenti, A., P. Imboden, et al. (1992). "Competitive polymerase chain reaction using an internal standard: Application to the quantitation of viral DNA." Journal of Virological Methods **39**(3): 259.

<http://www.sciencedirect.com/science/article/B6T96-476F61S-69/2/ecc5c7eaa4887b555bee09b652206856>

A general strategy for the construction of an internal standard for the polymerase chain reaction (PCR) is described together with its application in the evaluation of clinical samples. This internal

standard is a plasmid containing a modified target sequence that is co-amplified with the native target using the same set of primers. The co-amplification reaction will generate two fragments of different size that are readily separated without the need for restriction enzyme digestion. Thereafter, they are detected and quantitated by hybridization to the same probe. Detection of HIV proviral DNA was chosen as a model for this competitive PCR. The assay proved to be a sensitive tool for the detection of PCR inhibitors and allowed quantitation of HIV with a 20-30% variation coefficient. Despite limitations that appear inherent to the amplification process, internal standards appear to be useful tools for quantitative analysis by PCR.

Thomson, D. and G. Smith (1999). "Herpesvirus genome mapping: a rapid generic approach." Journal of Virological Methods **82**(1): 1.

<http://www.sciencedirect.com/science/article/B6T96-3XBV608-1/2/84aeedf0b4d0df6502118200de3091e8>

A protocol for mapping the genome of the alphaherpesvirus macropodid herpesvirus 1 is described. This protocol greatly simplifies a similar protocol that was used to map the genome of the poxvirus molluscum contagiosum virus. A single restriction digestion is carried out on the viral DNA, and the fragments cloned into a plasmid vector. The ends of each cloned fragment are sequenced, translated, and used to search peptide sequence databases. Putative genomic maps are constructed by assembling contiguous fragments identified by the sharing of common open reading frames and through the demonstrated colinearity of herpesvirus genomes belonging to the same subfamily. Oligonucleotide primers designed from the nucleotide sequence at the ends of each cloned fragment enable confirmation of putative contiguous fragments by PCR. Fragments not identified by searches of peptide databases are subcloned using a rapid subcloning method. This approach involves restriction digestion of the cloned fragment with restriction enzymes present in both the multiple cloning site of the vector, and within the fragment. Digested fragments larger than the vector are recircularised and transformed into bacteria to generate subclones for sequence analysis. This subcloning method can also be used to order rapidly genes within large clones.

Thomson, D. and G. Smith (2001). "PCR-based plasmid vector construction for generation of recombinant viruses." Journal of Virological Methods **94**(1-2): 7.

<http://www.sciencedirect.com/science/article/B6T96-42Y12XF-2/2/e09addc730d9a9b95ea8228568f29b40>

A totally polymerase chain reaction (PCR)-based protocol for construction of plasmids for production of recombinant macropodid herpesvirus 1 (MaHV-1) is described. This protocol greatly simplifies traditional methods that use restriction enzyme-based cloning or a combination of restriction enzyme cloning and the PCR. PCR is used to amplify the vector backbone containing an origin of replication and selectable marker, and the inserts to be cloned (5' and 3' viral homologous recombination regions and the reporter gene green fluorescent protein (GFP)). The inserts are cloned in a sequential manner with the intermediate vectors then amplified to produce the next vector. At its most basic, this involves, after the initial PCR amplification of vector and inserts, two additional PCR amplifications and three ligation events. This protocol is however totally generic, and can be used not only for construction of plasmids for production of recombinant viruses, but also for any general cloning applications.

Trottier, M., B. P. Schlitt, et al. (2002). "Enhanced detection of Theiler's virus RNA copy equivalents in the mouse central nervous system by real-time RT-PCR." Journal of Virological Methods **103**(1): 89.

<http://www.sciencedirect.com/science/article/B6T96-453NT2F-4/2/0b435e59016aaecd22d4db3a142d416b>

Infection of mice by low-neurovirulence Theiler's murine encephalomyelitis virus (TMEV), such as BeAn and DA viruses, provides a relevant experimental animal model for multiple sclerosis (MS). As a step toward determining the kinetics of a persistent central nervous system (CNS) infection that leads to chronic demyelination, we adapted a rapid, accurate and highly specific real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assay for detection and quantitation of BeAn virus RNA copy equivalents in mouse tissues. The assay enabled detection of as few as 20-30 copies of BeAn virus RNA per [mu]g of total RNA from infected mouse tissues and results for spinal cord revealed the same high levels of BeAn RNA as detected by Northern hybridization during the first 4 months of the persistent infection, but also was able to detect virus RNA copies as late as 1 year post-infection. Real-time RT-PCR analysis of BeAn virus RNA copy equivalents in different parts of the CNS, analyses not possible by Northern hybridization, revealed the following cline of virus persistence: spinal cord>brainstem/cerebellum>cerebrospinal fluid (CSF)>cerebral hemispheres. Systemic organs, including heart, intestine and mesenteric lymph nodes of infected mice, showed no evidence of viral persistence at 4 months post-infection.

Tsuda, F., H. Okamoto, et al. (1999). "Determination of antibodies to TT virus (TTV) and application to blood donors and patients with post-transfusion non-A to G hepatitis in Japan." Journal of Virological Methods **77**(2): 199.

<http://www.sciencedirect.com/science/article/B6T96-3VR1B1H-9/2/e8194397a661b193f8b3f8313875d1a7>

Recently, a nonenveloped single-stranded DNA virus named TT virus (TTV) has been reported in association with non-A to G post-transfusion as well as sporadic acute and chronic liver disease. A method was developed for the detection of antibody to TTV (anti-TTV) by means of immune precipitation and detection of TTV DNA by the polymerase chain reaction. The test serum was incubated with TTV, recovered from feces of a carrier, and after incubation, the formed immune complexes were precipitated with goat antiserum to human IgG. TTV DNA was sought for by the polymerase chain reaction in both precipitate and supernatant. The detection of TTV DNA in the precipitate, but not in the supernatant, was considered to represent anti-TTV in the test serum. Of the 44 healthy blood donors in Japan, anti-TTV was detected in one of the six (17%) with TTV DNA and 11 of the 38 (29%) without TTV DNA. In the two patients with post-transfusion non-A to G hepatitis, free anti-TTV developed as they cleared TTV in serum. Anti-TTV complexed with TTV in serum, detectable by precipitating sera with goat anti-human IgG and testing for TTV DNA, elicited while the patients had elevated alanine transaminase levels. The determination of anti-TTV would be useful for detecting resolved infection in surveys for exposure to TTV in the general population, and for establishing the mechanism of liver injury associated with TTV infection.

Van Laethem, K., K. Beuselinck, et al. (1998). "Diagnosis of human immunodeficiency virus infection by a polymerase chain reaction assay evaluated in patients harbouring strains of diverse geographical origin." Journal of Virological Methods **70**(2): 153.

<http://www.sciencedirect.com/science/article/B6T96-3SBVX4H-4/2/a6e16bfe458047f13dfdf93acfaa1415>

Since the development of the highly sensitive polymerase chain reaction, PCR has been increasingly used for the diagnosis of viral infections, including the detection of human immunodeficiency virus (HIV), the causative agent of AIDS. In our laboratory a diagnostic PCR is carried out on proviral HIV-1 DNA using a standardised algorithm based on three HIV-1 primer sets. The three primer sets, amplifying a fragment in the LTR-gag gene, in the pol gene and in the env gene, are situated within conserved regions of the HIV-1 genome. These primers allow us to detect not only HIV strains from Belgian patients but also from African patients, who are, for historical reasons, a substantial part of the HIV-positive patients in Belgium. We are able to detect 1-5 copies of proviral HIV-1 DNA with each of the three nested primer sets. A sensitivity and specificity of 92 and 100%, respectively, were achieved when testing 24 Belgian and African HIV-1 seropositive samples. In our lab, the same PCRs are also used for the detection of viral RNA in cases of a doubtful undetectable viral load when using a commercial HIV-1 viral load assay. This is because present-day commercial assays are not entirely reliable with divergent strains. Both our 'in-house' diagnostic DNA and RNA-PCR can also be used semiquantitatively with limiting dilutions.

Vandamme, A.-M., S. Van Dooren, et al. (1995). "Detection of HIV-1 RNA in plasma and serum samples using the NASBA amplification system compared to RNA-PCR." Journal of Virological Methods **52**(1-2): 121.

<http://www.sciencedirect.com/science/article/B6T96-3YXC151-X/2/a3afbcd918ae8929dc7f40d7b7f89270>

The presence of HIV-1 RNA in the plasma and serum of European and African patients was monitored using RNA-polymerase chain reaction (RNA-PCR) and the new isothermal NASBA nucleic acid amplification system encompassing a gel-based detection assay (ELGA). Identical RNA extraction procedures, provided by the NASBA amplification system, were used for both methods. The detection limit for HIV-1 RNA, measured on a 10-fold dilution series of spiked HIVIIB in negative plasma, was about 0.05 CCID₅₀ per test for both methods. Both NASBA and RNA-PCR were more sensitive than a p24 assay for the detection of circulating HIV-1 virus in blood: 17 of the 34 (50%) p24 antigen-tested seropositives were p24-positive while 32 (94%) were positive by NASBA and 30 (88%) by RNA-PCR. Among the 45 seropositives, 34 of which were tested for p24 antigen, 43 (96%) were positive by NASBA and 41 (91%) by RNA-PCR. Almost all seropositives had a detectable viral load in 100 [µl] plasma. Lower viral loads were only encountered in some healthy seropositives with a higher CD4 count. There was no cross-reactivity with HIV-2 or HTLV-I with both the RNA-PCR and NASBA. The extraction method used permitted the detection of HIV-1 RNA equally well in serum and in plasma with heparin or EDTA.

Vanderhallen, H. and F. Koenen (1997). "Rapid diagnosis of encephalomyocarditis virus infections in pigs using a reverse transcription-polymerase chain reaction." Journal of Virological Methods **66**(1): 83.

<http://www.sciencedirect.com/science/article/B6T96-3RYCMYM-1D/2/85242a8bdd7275411681e02274e3e176>

Encephalomyocarditis virus (EMCV) is widespread and the economic losses caused by an EMCV outbreak in pig holdings and the similarity between a foot-and-mouth disease virus (FMDV) and an EMCV infection in young piglets stress the need for a rapid, specific and broad diagnostic assay. An alternative to the time-consuming seroneutralisation assay, currently used for the characterisation of EMCV, is described. An EMCV specific reverse transcription-polymerase chain reaction (RT-PCR), using primers located in a conserved region of the 3D gene of the viral genome, was developed and tested on 114 different EMCV isolates. The identity of the respective

amplicons was confirmed by sequencing. The potential of this assay for future diagnostic purposes was demonstrated by applying the RT-PCR on tissue samples collected from an experimentally infected piglet.

Venter, M., G. Napier, et al. (2000). "Cloning, sequencing and expression of the gene that encodes the major neutralisation-specific antigen of African horsesickness virus serotype 9." Journal of Virological Methods **86**(1): 41.

<http://www.sciencedirect.com/science/article/B6T96-3YS34HX-6/2/e135748986aec5916949874fdc8c4b72>

A marked improvement in the efficiency of cloning the large double stranded RNA (dsRNA) genome segments of African horsesickness virus (AHSV) was achieved when the dsRNA polyadenylation step was carried out with undenatured rather than strand-separated dsRNA. It is a prerequisite to use dsRNA of very high purity because in the presence of even trace amounts of single stranded RNA, the dsRNA appears to be poorly polyadenylated as judged by its effectiveness as a template for oligo-dT-primed cDNA synthesis. The full-length VP2 gene of AHSV-9, cloned by this approach, was sequenced and it was found to show the highest percentage identity (60%) to VP2 of AHSV-6, providing an explanation of why these two serotypes show some cross protection. The VP2 protein was also expressed in *Spodoptera frugiperda* (Sf9) cells by means of a baculovirus recombinant. The yield of the expressed VP2 was high, but the protein was found to be largely insoluble. Nine smaller, truncated VP2 peptides were subsequently expressed in insect cells, but no significant improvement in solubility of the peptides, as compared to that of the full-sized protein, was observed. A western immunoblot analysis of the overlapping peptides indicated the presence of a strong linear epitope located within a large hydrophilic domain between amino acids 369 and 403.

Verhofstede, C., K. Fransen, et al. (1996). "Isolation of HIV-1 RNA from plasma: evaluation of eight different extraction methods." Journal of Virological Methods **60**(2): 155.

<http://www.sciencedirect.com/science/article/B6T96-3W0NTKY-6/2/a3a12d1506e4ca8f8f19793c34cc7317>

The efficacy of eight different methods for the extraction of HIV-1 RNA from plasma was compared. The RNA preparation method that gave the best results by RT-PCR was the one described by Chomczynski and Sacchi (1987, *Anal. Biochem.* 162, 156-159). This method consists of a guanidine thiocyanate treatment followed by three phenol-chloroform-isoamylalcohol extractions and an ethanol precipitation. The disadvantage of this method is that it is time consuming and less suitable for the extraction of large series of samples. Moreover, due to the large number of procedural steps, there is a greater risk of sample mix-up or contamination. Of the single-step RNA purification methods, good results were obtained with the TRIzol method (Gibco Life Technologies, Paisley, UK) and with the extraction method offered by the NASBA kit (Organon Teknika, Turnhout, Belgium). The above single-step methods are recommended since both are sensitive enough to detect low copy numbers of HIV-RNA in the plasma of asymptomatic patients, and require only 2 h for completion. For most of the methods evaluated the inter-test variability was acceptable (mean variation coefficient between duplicate extraction varied between 17.3 and 47.3%). Inter-laboratory reproducibility was evaluated only for the TRIzol-method and found to be low (mean variation coefficient 63.4%).

Vesonen, M., H. Piiparinen, et al. (1996). "Detection of herpes simplex virus DNA in cerebrospinal fluid samples using the polymerase chain reaction and microplate hybridization." Journal of Virological Methods **59**(1-2): 1.

<http://www.sciencedirect.com/science/article/B6T96-3VYV049-D/2/81db02d5cb631156ed287cb194b19db9>

As conventional polymerase chain reaction (PCR) procedures are time-consuming and laborious, we developed and evaluated a rapid semi-automatic microplate method to detect the amplified PCR products. The use of PCR, with subsequent hybridization in microplates, is described for the detection of herpes simplex virus (HSV) DNA in cerebrospinal fluid samples. The principle of the method is based on two phases. Firstly, the amplification of the viral DNA in the sample is undertaken using a pair of primers of which one is biotinylated. Secondly, the amplified viral genomic sequences are bound to the wells of streptavidin-coated microplates and hybridized with digoxigenin-labeled oligonucleotide probes which are then detected using anti-digoxigenin antibody enzyme conjugates and either a photometric, fluorometric or luminometric substrate and microplate reader. The method is highly sensitive allowing the detection of as few as five purified DNA molecules. Compared to conventional gel electrophoresis followed by Southern blotting the established microplate hybridization is also much less time-consuming and involves less manual work. The applicability of the method is described for use as a routine diagnostic procedure for detection of early central nervous system infections caused by HSV-1 and HSV-2.

Vorndam, V., G. Kuno, et al. (1994). "A PCR-restriction enzyme technique for determining dengue virus subgroups within serotypes." Journal of Virological Methods **48**(2-3): 237.

<http://www.sciencedirect.com/science/article/B6T96-476F6FR-DT/2/6fe56633fe4d60a49583d278a9193c34>

The polymerase chain reaction (PCR) and restriction enzyme analysis were used to develop a rapid and simple procedure for identifying geographic subgroups of dengue virus within serotypes for epidemiologic investigations. The entire structural protein region of dengue viruses was amplified and the products were digested with the endonucleases AluI or DdeI. By comparing the restriction fragment length polymorphisms (RFLPs), we recognized dengue-2 and dengue-3 subgroups that corresponded to those previously determined by oligonucleotide fingerprinting or genomic sequencing. This procedure can be performed in 2 days without the use of radioisotopes, and results can be interpreted without computer analysis. For those analyses which require only subgroup affiliations, this is a useful tool for rapidly screening multiple virus isolates.

Weilguny, H. and R. P. Singh (1998). "Separation of Slovenian isolates of PVYNTN from the North American isolates of PVYN by a 3-primer PCR." Journal of Virological Methods **71**(1): 57.

<http://www.sciencedirect.com/science/article/B6T96-3SY3H5D-8/2/064363d0928e36dcee94b5a9422cbb11>

The potato tuber ringspot necrosis isolate of potato virus Y (PVYNTN) is a recently recognized and highly aggressive isolate of the PVYN group of strains. In order to screen specifically sources of resistance to PVYNTN a method to separate PVYNTN from PVYN is needed. To achieve this, 61 isolates from 13 imported and locally developed potato cultivars in Slovenia were studied. On the basis of the reactions in indicator plants *Nicotiana tabacum* cv. Samsun and *Solanum brachycarpum* and with a PVYN specific monoclonal antibody (4E7), all Slovenian isolates (SI-

NTN) were identified as PVYN. Using two primer pairs from the P1 gene of a Hungarian isolate of PVYNTN by a conventional single primer pair, reverse transcription polymerase chain reaction (RT-PCR) both PVYNTN and PVYN were amplified similarly. However, specific amplification of PVYNTN was achieved by a nested-PCR at an annealing temperature of 63[deg]C. A simplified form of the nested-PCR, termed 3-primer PCR was developed, which is applicable for large-scale testing of samples. Using the 3-primer PCR at annealing temperature of 63[deg]C, known mixtures of PVYNTN and PVYN were correctly separated. PVYNTN was detected in dormant tubers and leaves from all SI-NTN isolates. The 3-primer PCR was specific to PVYNTN and did not react with nine isolates of PVYN, 13 isolates of PVYo, one isolate of PVYC, six commonly occurring potato viruses and a viroid.

Weinberger, K. M., E. Wiedenmann, et al. (2000). "Sensitive and accurate quantitation of hepatitis B virus DNA using a kinetic fluorescence detection system (TaqMan PCR)." Journal of Virological Methods **85**(1-2): 75.

<http://www.sciencedirect.com/science/article/B6T96-3YGDCNX-8/2/ad601a0499d0fc4e745f78d7b4d9cd97>

The laboratory diagnosis of hepatitis B virus (HBV) infection is based mainly on serological assays. Yet the detection and quantitation of viral DNA is necessary when addressing directly the question of infectivity or when monitoring the viral load during therapy. Standard hybridization assays allow for exact quantitation, but their sensitivity is limited to 10⁵-10⁶ viral genomes per ml of serum. The most sensitive tests for HBV DNA are nested PCR systems, which recognize virtually one molecule of the target DNA per reaction. However, these assays only provide very coarse quantitative statements. To take advantage of both methods, a new assay for HBV DNA is described based on the commercial TaqMan(c) system. This assay is capable of quantifying HBV DNA from the theoretical lower limit up to 10¹⁰ genome equivalents per ml of serum and, thus, covers the complete range of naturally occurring states of infections. The method was calibrated on the basis of serial plasmid dilutions and compared with a well-established nested PCR system. More than 100 HBV positive sera and serial dilutions of the Eurohep standard for both ad and ay subtypes were analyzed. The assay reliably detected all HBV positive samples. It shows minimal run-to-run deviations, allows for quantitation that covers eight orders of magnitude, and finally, completely avoids the risk of cross-contamination by PCR products. Thus, this technique combines the sensitivity of PCR amplification and the quantitation potential of hybridization tests and it is time efficient and safer.

Whitby, J. E., P. R. Heaton, et al. (1997). "Rapid detection of rabies and rabies-related viruses by RT-PCR and enzyme-linked immunosorbent assay." Journal of Virological Methods **69**(1-2): 63.

<http://www.sciencedirect.com/science/article/B6T96-3S2BMBF-8/2/f1836ca8f6b43d0ddfedf1f8ae781af9>

A rapid detection method for the six established genotypes of rabies and rabies-related viral RNA using RT-PCR-ELISA is described. The detection of digoxigenin-labelled amplified products is performed by solution hybridization to two specific, biotin-labelled, capture probes, which are complementary to the inner region of the amplification products. The capture probe and amplified product hybrid are then immobilised on a streptavidin-coated microtitre plate, bound products are detected by an anti-DIG Fab fragment conjugated to peroxidase, and colorimetric reaction automatically measured. This method was up to 100-fold more sensitive than Southern blot hybridization, detecting 0.00002 TCID₅₀/ml of a genotype 1, classical rabies virus strain. The complete detection methodology from RT-PCR to PCR-ELISA detection could be completed within 10 h. Using this procedure, we were 100% successful in detecting 60 isolates from a

representative selection of the six established genotypes from all over the world. This test is a useful additional tool for the detection of the rabies and rabies-related viruses, which is easy to perform, rapid and highly sensitive.

Wilborn, F., T. Binder, et al. (1998). "Human herpesvirus type 6 variants identified by single-strand conformation polymorphism analysis." Journal of Virological Methods **73**(1): 21.

<http://www.sciencedirect.com/science/article/B6T96-3T2PCND-3/2/fd50b7c2d5c603a4b2e7bb4e2e164683>

Six human herpesvirus 6 (HHV-6) variants were analyzed for heterogeneity using the polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP). Two independent DNA regions were selected: a fragment of the gene U11 (position 18966-21578) coding for a basic phosphoprotein, the major antigenic structural protein pp100; and a fragment from an open reading frame (ORF) area of the gene U67, previously referred to as 13R (position 102458-103519), coding for a product of unknown function. The two PCR systems based on the above DNA sequences yielded products of 187 bp and 223 bp, respectively. DNA obtained from three laboratory reference strains (U1102, R104 and St.W.) and from HHV-6 infected peripheral white blood cells of bone marrow transplant patients and blood donors was used to test the applicability of two different SSCP analysis systems for the identification of HHV-6 variants using amplicons derived by PCR from the two genomic regions described above (U11 [pp100], U67 [13R-ORF]). The generation of characteristic SSCP patterns enables the rapid differentiation of HHV-6 A and B strains for the classification of variants derived from clinical samples, reducing the need for expensive and time-consuming direct sequencing analyses.

Williamson, A.-L. and S. J. Dennis (1991). "The use of the polymerase chain reaction for the detection of human papillomavirus type 13." Journal of Virological Methods **31**(1): 57.

<http://www.sciencedirect.com/science/article/B6T96-47DM172-6/2/06e1159bac2c9ff344416aed504f309b>

Human papillomavirus type 13 (HPV-13) is associated with oral focal epithelial hyperplasia (FEH). The purpose of this study was to establish conditions for the application of polymerase chain reaction (PCR) to the specific detection and amplification of HPV-13 DNA. To design primers for HPV-13 a part of the HPV-13 genome was sequenced first: the smallest BamHI fragment (597 bp) of HPV-13 was subcloned and sequenced. The sequence was found to be part of a large open reading frame and had significant homology with the L1 gene of other HPVs. HPV-13 specific primers were designed to amplify a 240 bp fragment from the L1 gene by PCR. Conditions for PCR were standardized for this set of primers.

Yamaguchi, Y., T. Hironaka, et al. (1992). "Increased sensitivity for detection of human cytomegalovirus in urine by removal of inhibitors for the polymerase chain reaction." Journal of Virological Methods **37**(2): 209.

<http://www.sciencedirect.com/science/article/B6T96-476F5YM-5P/2/ba31f393d5b9280ecefaea1201d709a8>

The presence of inhibitors in urine interferes with the enzymatic reaction of the polymerase chain reaction (PCR) for detection of human cytomegalovirus (HCMV). To remove inhibitors, HCMV

virions in urine were precipitated with polyethylene glycol, or DNA was extracted from urine by the use of glass powder and subjected to PCR followed by Southern blot hybridization with alkaline phosphatase-linked oligonucleotide probes. These simple, rapid methods increased significantly the sensitivity of PCR for detection of HCMV in urine.

Yang, J.-H., J.-P. Lai, et al. (2002). "Real-time RT-PCR for quantitation of hepatitis C virus RNA." Journal of Virological Methods **102**(1-2): 119.

<http://www.sciencedirect.com/science/article/B6T96-450HBM7-1/2/a9ecfe7916725d98c498b1dece24ac36>

A newly developed real-time RT-polymerase chain reaction assay for quantitation of hepatitis C virus (HCV) RNA in human plasma and serum was applied. A pair of primers and a probe (molecular beacon) were designed that are specific for the recognition of a highly conservative 5'-non-coding region (5'-NCR) in HCV genome. HCV real-time RT-PCR assay had a sensitivity of 1000 RNA copies per reaction, with a dynamic range of detection between 10³ and 10⁷ RNA copies. The coefficient variation of threshold cycle (Ct) values in intra- and inter-runs were less than 1.37 and 4.66%, respectively. The real-time RT-PCR assay on the HCV sero-positive samples yielded reproducible data, with less than 2.09% of the inter-assay variation. In order to determine its potential for clinical diagnosis, real-time RT-PCR was used to examine the HCV RNA levels in plasma from sero-positive and negative subjects, showing that the assay is highly sensitive and has specificity of 100%. It was demonstrated that the real-time RT-PCR was able to amplify HCV RNA in reference sera with seven genotypes (1A, 1B, 2B, 3A, 4, 5A and 6A) that include six major HCV genotypes circulated in the world. Since HCV is a major pathogen of post-transfusion and community-transmitted non-A, non-B hepatitis, this assay has a broad application for basic and clinical investigations.

Ying, C., J. Van Pelt, et al. (1999). "Use of digoxigenin-labelled probes for the quantitation of HBV-DNA in antiviral drug evaluation." Journal of Virological Methods **81**(1-2): 155.

<http://www.sciencedirect.com/science/article/B6T96-3XMPJNX-N/2/09b8fc47c5efc19fda48eb13900d78f5>

The use of digoxigenin-labelled probes was studied for quantitation of HBV-DNA during antiviral drug evaluation. Digoxigenin (dig)-labelled probes were generated either via incorporation of dig-dUTP in a polymerase chain reaction (PCR) or a random priming reaction. Using the PCR-labelled probe (delineating a 523 bp fragment in the core gene of the HBV) as little as 1 pg of immobilized HBV-DNA could be detected following an 8 h exposure of the hybridized membrane. A close correlation ($r=0.95$) was found between the amount of HBV-DNA (range 2.5-200 pg) and the signal generated by the probe hybridized to its target DNA. By using a probe that was labelled with digoxigenin via random priming, the minimal quantity of immobilized HBV plasmid DNA that could be detected following an 8 h exposure was 4 pg, whereas a ³²P-labelled probe, generated in parallel by random priming, allowed the detection of 16 pg of HBV plasmid DNA following a 4-day exposure. The PCR-generated digoxigenin-labelled probe proved to be useful for antiviral drug evaluation, i.e. to detect HBV-DNA in total cellular DNA from HBV-positive hepatoma cells (HepG2.2.15) that had either been treated with reference antiviral agents or left untreated. The 50% effective concentrations (EC₅₀) that were calculated for inhibition of HBV-DNA production by lamivudine (3TC), penciclovir (PCV), lobucavir (LBV), adefovir (PMEA) and tenofovir (PMPA) were comparable to those reported in the literature. The use of digoxigenin-labelled probes thus appears to be a simple, convenient, rapid, reliable and non-radioactive method for use for anti-HBV screening. In addition, and in contrast to ³²P-labelled probes, digoxigenin-labelled probes can be stored for >1 year without loss of specific activity, which makes these probes particularly

attractive for large-scale antiviral drug evaluation purposes.

Yu, Q., N. Hu, et al. (2001). "Rapid acquisition of entire DNA polymerase gene of a novel herpesvirus from green turtle fibropapilloma by a genomic walking technique." Journal of Virological Methods **91**(2): 183.

<http://www.sciencedirect.com/science/article/B6T96-426XYG8-B/2/4d5ace1b6ced9aff356ac5778285f744>

A 4837-bp sequence of a newfound green turtle herpesvirus (GTHV), implicated in the etiology of green turtle fibropapilloma, was obtained from tumor tissues of a green turtle with fibropapilloma using a genomic walking method based on restriction enzyme digestion, self-ligation and inverse polymerase chain reaction (IPCR). The 4837-bp sequence was 56.23% G/C rich and contained three nonoverlapping open reading frames (ORF). The largest ORF (3507-bp) encoded the DNA polymerase gene (pol gene), which exhibited a high degree of homology at both amino acid and nucleotide levels with the DNA pol genes of human and animal herpesviruses, with a predicted protein of 1169 amino acids and molecular weight of 132.6 kilodaltons. The ATG at 518 to 520 was the first initiation codon in the ORF and was presumed to be the first methionine codon of the pol gene. Phylogenetic analysis, based on the amino acid sequence of the GTHV DNA pol gene and the corresponding regions of other known human and animal herpesviruses, indicated that GTHV belonged to the Alphaherpesvirinae subfamily. The upstream ORF of the pol gene encoded the N-terminal region of the GTHV homologue of the DNA-binding protein gene, whereas the downstream ORF was the C-terminal region of a gene which was homologous to ORFs conserved in human and animal herpesviruses, i.e., herpes simplex virus 1 (HSV1) gene UL31, Epstein-Barr virus (EBV) gene BFLF2, equine herpesvirus 1 (EHV1) gene 29, and alcelaphine herpesvirus 1 (AHV1) hypothetical protein 69 gene. The arrangement of these three genes in GTHV genome was identical to that seen in other alphaherpesviruses. The sequence and location of the DNA pol gene in the GTHV genome should greatly facilitate future studies of the viral life cycle.

Zhang, H., B. Soteriou, et al. (1997). "Characterisation of genomic RNA of Coxsackievirus B3 in murine myocarditis: reliability of direct sequencing of reverse transcription-nested polymerase chain reaction products." Journal of Virological Methods **69**(1-2): 7.

<http://www.sciencedirect.com/science/article/B6T96-3S2BMBF-2/2/386b5d4dd1c82c240be7445ad7a5aba5>

SWR mice develop viral myocarditis histologically similar to the human disease following inoculation with a cardiovirulent Coxsackievirus B3 (CVB3), reactivated from a sequenced cDNA clone of Nancy strain. A sequence of 215 nucleotides, or 628 nucleotides in representative cases, of the 5'non-translated region (5'NTR) of CVB3 genome was amplified from myocardial samples of the infected mice by reverse transcription-nested polymerase chain reaction (RT-NPCR). In order to verify the viral nucleotide sequence and detect the mutation frequency of the viral RNA, the nucleotide sequence of NPCR products were determined by direct sequencing in both orientations. The amplified products from mouse heart on day 1-13 post-inoculation were sequenced and, in each case, the consensus sequence was identical to the published sequence of CVB3 (Nancy strain). To evaluate further the reproducibility of these techniques, three tissue samples from the same infected mouse heart were processed independently. Sequences of their RT-NPCR products were identical to each other as well as to the published sequence. When two attenuated CVB3 mutants were amplified and sequenced, single mutations were detected. To evaluate the overall fidelity of these two combined techniques, genomic RNA of a different CVB3 Nancy strain stock, Coxsackievirus A9 or poliovirus sabin 1 was amplified and the NPCR

products sequenced. Each product showed 100% homology with its published sequence. These results demonstrate that the coupled technique of the enterovirus RT-NPCR with direct sequencing of NPCR products generates accurate consensus sequence data and this technique proved to be useful in verification of enteroviral amplicons and in detection of nucleotide mutations. In addition, a low mutation frequency was found in the 5'NTR of CVB3 detected in myocardial samples of immunocompetent mice up to 13 days.

Zimmermann, W., R. Durrwald, et al. (1994). "Detection of Borna disease virus RNA in naturally infected animals by a nested polymerase chain reaction." Journal of Virological Methods **46**(2): 133.

<http://www.sciencedirect.com/science/article/B6T96-476F69S-BC/2/b2c6ea7294f02a31ba9e8a947b4de423>

Borna disease virus in naturally infected horses, a donkey and sheep was detected for the first time by amplification of viral RNA using PCR. In contrast to a control group of healthy horses, brain tissue was positive by this assay in all animals with neurological symptoms. The use of a second round of PCR with nested primers following Southern hybridization confirmed the specificity and increased the sensitivity of the test. Comparison with conventional methods recommends this technique for monitoring of BDV infections at a molecular level.

Zingg, W., W. Bossart, et al. (1999). "Detection and quantification of cell-free Epstein-Barr virus by polymerase chain reaction and subsequent DNA enzyme immunoassay." Journal of Virological Methods **79**(2): 141.

<http://www.sciencedirect.com/science/article/B6T96-3WJDTS6-3/2/20d346e54823d0c479dde2d2deef0326>

Amplification by polymerase chain reaction and subsequent DNA enzyme immunoassay (DEIA) were employed to determine the number of genome equivalents of cell-free Epstein-Barr virus (EBV) DNA in peripheral blood. The assay detected cell-free EBV DNA in the serum of 14 out of 18 patients with primary, productive EBV infection (sensitivity 77.7%) but not in healthy EBV carriers with latent infection (specificity 100%). Our assay has the potential for a clinical diagnostic tool to monitor patients at risk for EBV reactivation and productive infection with subsequent EBV-induced lymphoproliferative diseases.

Jpn. J. Clin. Oncol. (4)

Ishida, H., H. Furukawa, et al. (2002). "The Novel Germline Mutation of hMSH2 Gene in a Case of a Colon Cancer Patient Without Family History." Jpn. J. Clin. Oncol. **32**(7): 266-269.

<http://jjco.oupjournals.org/cgi/content/abstract/32/7/266>

Hereditary non-polyposis colorectal cancer (HNPCC) is generally found from the patient's family history. The functional disorder of mismatch repair genes has been reported to be responsible for HNPCC. The proband was a 28-year-old Japanese female who was admitted to our hospital with

a diagnosis of descending colon cancer. Although there was no previous or family history of malignant disorders within the first- and second-degree relatives, the early onset of colon cancer prompted genetic analysis with suspicion of HNPCC. PCR analysis of the primary tumor showed DNA replication errors at the six microsatellite regions. PCR/direct sequential analysis of the peripheral lymphocytes revealed a germline frameshift mutation due to deletion of TTCAA at nt. position from 650 to 654 in exon 4 of the hMSH2 gene. According to the Human Mutation Database and International Collaborative Group on HNPCC Database, this type of the frameshift mutation is the first report in the hMSH2 gene.

Kamoto, T., Y. Isogawa, et al. (2005). "Association of a Genetic Polymorphism of the E-cadherin Gene with Prostate Cancer in a Japanese Population." *Jpn. J. Clin. Oncol.* **35**(3): 158-161.

<http://jjco.oupjournals.org/cgi/content/abstract/35/3/158>

The E-cadherin gene has been identified as having a physiological role in cellular attachment, and is hypothesized to participate in carcinogenesis. A polymorphism (an A to C substitution) in the 5'-untranslated region has a direct effect on E-cadherin gene transcriptional regulation. We explored the association between E-cadherin gene polymorphism and the risk of prostate cancer in a Japanese population. The subjects consisted of 236 patients with prostate cancer, 209 benign prostatic hyperplasia (BPH) patients and 139 male controls. A marginally significant difference was found between prostate cancer patients and male controls ($P = 0.053$). No significant difference was observed between prostate cancer and BPH patients. When patients with prostate cancer were divided into two groups, stage A+B and stage C+D, a significant difference was observed between progressive cancer patients (stage C+D) and male controls (odds ratio = 1.93, $P = 0.016$). It is possible that the presence of one A allele resulted in an increased risk of cancer progression.

Nomoto, K., M. Maekawa, et al. (2002). "Methylation Status and Expression of Human Telomerase Reverse Transcriptase mRNA in Relation to Hypermethylation of the p16 gene in Colorectal Cancers as Analyzed by Bisulfite PCR-SSCP." *Jpn. J. Clin. Oncol.* **32**(1): 3-8.

<http://jjco.oupjournals.org/cgi/content/abstract/32/1/3>

Background: The expression level of human telomerase reverse transcriptase (hTERT) is correlated with telomerase activity and is expressed at high levels in malignant tumors. It is of interest whether expression of hTERT is regulated by methylation of the CpG island in the promoter of the hTERT gene. We examined hTERT expression and methylation status of the hTERT and other genes including p16. Methods: We analyzed methylation status by bisulfite treatment and polymerase chain reaction with single-strand conformation polymorphism analysis (PCR-SSCP) and expression of the hTERT by RT-PCR, in 13 cancer cell lines, eight white blood cell samples and 24 colorectal cancer tissues. Results: In the cancer cell lines, hTERT was expressed and the CpG island of the hTERT promoter was methylated. Most colorectal cancer tissues showed similar results. The promoter of hTERT was methylated in six cases, partially methylated in 17 cases and unmethylated in one case. All cases with methylation of hMLH1 or p16 also showed methylation of hTERT; however, some of the cases lacking p16 methylation also had hTERT methylation. Conclusion: Increased expression of hTERT is related to hypermethylation of hTERT in colorectal cancerous tissues as well as some cancer cell lines and discordant with hypermethylation of p16.

Tsurusako, S.-I., H. Tanaka, et al. (2002). "Low Incidence of p53 Mutations in Well-differentiated Tongue Squamous Cell Carcinoma in Japan." Jpn. J. Clin. Oncol. **32**(9): 327-331.

<http://jjco.oupjournals.org/cgi/content/abstract/32/9/327>

Background: Despite an increasing number of patients suffering from squamous cell carcinomas of the tongue, little is known about the molecular mechanisms involved in the origin and development of these neoplasms. Methods: We screened microdissected tongue squamous cell carcinoma (TSC) specimens from 28 consecutive, previously untreated, Japanese patients for mutations in the p53 tumor-suppressor gene single-strand conformation polymorphism analysis (exons 5, 6, 7, 8) and direct genomic sequencing. Results: Among them, 24 tumor specimens were well differentiated, three moderately and one poorly differentiated, according to the WHO classification. Mutations in the p53 tumor-suppressor gene were detected in only two out of the 28 (7%) tumor specimens. One was well differentiated and the other was poorly differentiated. Conclusions: Our results suggest that p53 gene mutations are less frequent in well differentiated TSC. These results indicate that mutations in the p53 gene may not be strongly involved in the development of well differentiated TSC.

Lebensmittel-Wissenschaft und-Technologie (1)

Berthoud, H., F. Chavagnat, et al. (2005). "Comparison of partial gene sequences encoding a phosphoketolase for the identification of bifidobacteria." Lebensmittel-Wissenschaft und-Technologie **38**(1): 101.

<http://www.sciencedirect.com/science/article/B6WMV-4CPD5SX-1/2/189b1b8e5513319bb0125a030d6c1c8b>

In this study, a new identification method for Bifidobacterium species based on sequencing of a partial xylulose-5-phosphate/fructose-6-phosphate phosphoketolase gene (xfp) was evaluated. Approximately 500 bp sequences from 68 different strains including 34 type strains were compared. The results showed that this method discriminated between all bifidobacteria, generally with greater accuracy than 16S rDNA sequence analysis. It even clearly distinguished B. longum biovar infantis from B. longum biovar longum and B. longum biovar suis, as well as B. animalis from B. lactis.

Legal Medicine(13)

Imaizumi, K., K. Noguchi, et al. (2005). "DNA typing of bone specimens--the potential use of the profiler test as a tool for bone identification." Legal Medicine **7**(1): 31.

<http://www.sciencedirect.com/science/article/B6W7W-4DFT078-3/2/c87afd52a4262b65ab827a09ce3454f7>

Twenty-six bone DNA identification cases are described. The postmortem periods of the studied remains ranged from three days to over 30 years, and the locations where the remains were found varied resulting in a variety of postmortem conditions. Nuclear DNA typing using an AmpFLSTR Profiler kit and mitochondrial DNA (mtDNA) typing of hypervariable regions 1 and 2 (HV1 and HV2) in a control region were performed both with decalcified and non-treated bone powder samples. Decalcification was shown to improve the success of DNA typing. The nucleotide sequences of the HV1 and HV2 regions were successfully determined in all cases examined. Nuclear DNA typing was very successful, more than half of the loci were typed during multiple amplifications (10 loci in one reaction) in 23 cases. Polymerase chain reaction (PCR) inhibition was observed in five cases including three samples that were found buried in soil. This inhibitory effect was identified as the result of unbalanced multiple PCR during the profiler test. These results revealed that DNA typing targeting nuclear DNA is a potentially powerful tool for bone identification.

Inagaki, S., Y. Yamamoto, et al. (2002). "Typing of Y chromosome single nucleotide polymorphisms in a Japanese population by a multiplexed single nucleotide primer extension reaction." Legal Medicine 4(3): 202.

<http://www.sciencedirect.com/science/article/B6W7W-46NY92R-7/2/74250d16c167ccd263fe2064cdb49697>

We have developed a new method for typing single nucleotide polymorphisms (SNPs) on the human Y chromosome based on a multiplexed single nucleotide primer extension. This method has the advantage that several SNPs are typed rapidly and simultaneously. We examined 15 different SNP loci on Y chromosome, M9, M105, M122, M125, M128, M130, SRY465, IMS-JST006241, IMS-JST006841, IMS-JST002611, IMS-JST003305, IMS-JST008425, IMS-JST021354, IMS-JST021355 and IMS-JST055457, in 159 Japanese males. From the typing results of these 15 loci, we found 13 haplotypes. Gene diversity for each locus ranged from 0.025 to 0.486 and the haplotype diversity was estimated to be 0.838. This method could be readily applied for personal identification and paternity testing.

Ishida, K., B.-L. Zhu, et al. (2002). "A quantitative RT-PCR assay of surfactant-associated protein A1 and A2 mRNA transcripts as a diagnostic tool for acute asphyxial death." Legal Medicine 4(1): 7.

<http://www.sciencedirect.com/science/article/B6W7W-44CXRJJ-2/2/2aad436a6d7b1bce2371b397c4fa1ba1>

Although an immunohistochemical investigation of pulmonary surfactant-associated protein A (SP-A) suggested a characteristic increase in fatal asphyxiation, no particular change was observed in the total amount of SP-A mRNA. SP-A is encoded by two highly similar genes, SP-A1 and SP-A2, which are differentially regulated in the expression. In the present study, to investigate the molecular pathology of SP-A, we established a method for quantitative RT-PCR assay of SP-A1 and SP-A2 mRNA transcripts. Using this method, fatalities from acute mechanical asphyxia (n=12) and drowning (n=9) were examined in comparison with control groups (n=17) of acute myocardial infarction (n=11) and peracute death due to brain lacerations (n=6). The SP-A1/A2 ratio (mean value) was markedly elevated in mechanical asphyxia (6.72) and drowning (5.64), whereas it was low in controls (acute myocardial infarction, 2.80; brain lacerations, 2.56). The analysis of the SP-A1/A2 ratio may assist interpretation of the molecular alterations of SP-A related to acute asphyxial death.

Kobayashi, T. and A. Akane (2000). "ABO genotyping by inverse PCR technique." Legal Medicine 2(1): 15.

<http://www.sciencedirect.com/science/article/B6W7W-44G8C9H-1X/2/9a4db775a139c33fa45c365758c3db6d>

Inverse PCR technique was applied to type three major alleles (A1, B and O1) of the ABO blood group by simultaneously detecting separated allele-determining nucleotides (the 261st base in exon 6 and the 796th and 803rd nucleotides in exon 7) of the ABO gene. A sequence of about 1.7 kb from exons 6 to 7 of each allele was amplified, both termini of the fragment ligated, and allele-typing performed by the inverse PCR-restriction fragment length polymorphism (IP-RFLP) and allele-specific inverse-PCR (ASIP) methods. For intramolecular ligation, primers for the first PCR were designed to have Acc I-restriction sites within the sequences, and both termini of the 1.7-kb fragment were digested with Acc I. Using the IP-RFLP method, the inverse PCR product was digested with Kpn I, Nla III and Dde I, A1, B, O1-standard (OA) and O1-variant (OG) alleles were detected as 365-bp, 272-bp, 193-bp and 128-bp fragments, respectively. By the ASIP method using four allele-specific primers, 222-bp, 124-bp and 232-bp fragments were amplified from A1, B and O1 templates, respectively. These techniques would be applicable to detecting separated polymorphic regions of some other genes.

Michino, J., Y. Hata, et al. "Demonstration of A antigen and A allele of ABO histo-blood group in nail in a case with the absence of A antigen and anti-A antibody in blood." Legal Medicine In Press, Corrected Proof <http://www.sciencedirect.com/science/article/B6W7W-4FHKK65-1/2/6b7a481100166eda9371a00055e5bc8c>

We report a case with the inconsistency that the red blood cells lacked both A- and B-antigens while the serum showed reactivity with control B-red cells but not with A-red cells. A- and B-antigens were examined by serological blood typing and immunohistochemical staining, and DNA analyses were performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), DNA sequencing, and hot-stop PCR. A-antigens were demonstrable in the nail of the subject by serological study and immunostaining. DNA analyses showed that the nail retained a small amount of A-allele comparing to that of O-allele. Those genomic analyses of ABO genes were useful for demonstration of A allele in the nail of an individual with the absence of A antigen on red blood cells and the corresponding antibody in serum.

Mitani, T. and A. Akane (2003). "Mung bean nuclease treatment improves Se genotyping by allele-specific inverse polymerase chain reaction amplification." Legal Medicine 5(4): 233.

<http://www.sciencedirect.com/science/article/B6W7W-49PRDRY-5/2/a28e9cfd0f4e22e42bddfe7f0273f440>

The allele-specific inverse polymerase chain reaction (PCR) technique, which has been explored to detect two linked polymorphic regions simultaneously, was applied to genotype the Se system. The major alleles of the Se system in Japanese are Se, sej defined by a single nucleotide substitution in the Se allele, and sefus generated by recombination between the Sec1 and FUT2 genes. The first PCR products using gene-specific primers were self-ligated, and each allele was detected by the second inverse-PCR using allele-specific primers. The 340, 331 and 353-bp products were finally amplified from Se, sej and sefus templates, respectively. The first PCR products without mung bean nuclease treatment were not self-ligated and non-specific fragments were amplified in the second PCR, suggesting that non-templated adenylation occurred at the termini during the first PCR. Nuclease digestion of the first PCR products that blunts their termini

was found to reduce interference of non-templated adenylation with the intramolecular ligation and to improve the genotyping markedly. This modified allele-specific inverse-PCR method is applicable to analyze haplotypes consisting of separated single nucleotide polymorphisms and recombinant genes.

Mitani, T., H. Tsujita, et al. (2000). "A case of DNA analysis of ABO blood group variant allele A2." Legal Medicine 2(4): 197.

<http://www.sciencedirect.com/science/article/B6W7W-44G8CDV-37/2/c0849594b3bd4abba78e9f99d49a28be>

The ABO phenotype of a bloodstain (B) on a knife that was used as a weapon in an attempted murder case was found to be different from that of the Peruvian victim's blood (AB). Serological analysis showed that the A-antigenicity was much weaker than B antigenicity, suggesting that the victim's phenotype was A2B or A3B. So, the ABO genotypes of the knife bloodstain and the victim's blood were determined by DNA analysis. The 261st G deletion, specific to the O1 allele, was not detected in the specimens by restriction fragment length polymorphism analysis. Also, the 871st A, specific to the A3 allele, was not found by the allele-specific amplification method. Amplified product length polymorphism and direct sequencing methods finally demonstrated that the typical B sequence was found in one allele and a single C deletion in the 1,059th-1,061st C stretch in the other allele, indicating that the ABO phenotype of the bloodstain and victim's blood were A2B.

Mitani, T., H. Tsujita, et al. (2002). "Se genotyping following allele-specific polymerase chain reaction amplification." Legal Medicine 4(3): 193.

<http://www.sciencedirect.com/science/article/B6W7W-46NY92R-5/2/c500ad24184da7d34998b0624088fcdf>

The polymorphism of the Sec2 gene, which determines Se blood type, has been reported. This study presents an Se genotyping system by the allele-specific polymerase chain reaction amplification method. The Se, sej and sefus alleles were amplified using allele-specific primers. The Sec1, Sec2 and sefus genes were analyzed by DNA sequencing. The 299-bp Se, 146-bp sej and/or 312-bp sefus allele-specific products were amplified and detected in the native polyacrylamide gel. The 314th-316th nucleotides of the Sec1 gene were CCC, which were different from the nucleotides GGG reported previously by Kelly et al. [J Biol Chem 270 (1995) 4640]. This Se genotyping system is a simple method available for the forensic science field in Japan. The crossover region of the sefus gene is a 164-bp stretch corresponding to the regions between the 253rd and 416th of the Sec1 gene and between the 211th and 374th of the Sec2 gene.

Ogata, Y., T. Yamaba, et al. "Improvement of a multiplex PCR system for DYS441, DYS442, DYS443, DYS444 and DYS445, and a population study in 340 Japanese males." Legal Medicine In Press, Corrected Proof <http://www.sciencedirect.com/science/article/B6W7W-4F29HMY-2/2/11ef3690e341fc2ef3f8841231af91de>

A multiplex PCR system for five Y-STRs (DYS441, DYS442, DYS443, DYS444 and DYS445) has been improved to increase the probability of obtaining a DNA typing result from aged samples. Newly designed PCR primers for amplification of the DYS441 and DYS442 loci and optimization

of PCR conditions enabled successful typing from blood and semen stains that had been stored for more than seven years at room temperature. Analysis of 340 Japanese males revealed 7, 5, 6, 5 and 4 alleles at the DYS441, DYS442, DYS443, DYS444 and DYS445 loci, respectively, yielding 122 haplotypes with a cumulative haplotype diversity of 0.97.

Sato, Y., M. Hayakawa, et al. (2003). "HLA typing of aortic tissues from unidentified bodies using hot start polymerase chain reaction-sequence specific primers." Legal Medicine 5(Supplement 1): S191.

<http://www.sciencedirect.com/science/article/B6W7W-47PG72T-VI2/b078826e1f8ff1c4773883cc890098dd>

This study investigated the identification of unidentified bodies through HLA DNA typing of aortic tissues. Eight aortas were collected at autopsy from six bodies found in water, one burnt body, and one mummified body, respectively. DNA was extracted from 10-20 mg of aortic tissue with a PUREGENE(R) DNA Isolation Kit and phenol/chloroform. HLA (A, B, DR or DQ locus) alleles were typed using the hot start polymerase chain reaction-sequence specific primers (PCR-SSP) method with a Dynal AllSet+(TM) SSP "low resolution" kit for each locus. The aorta was still retained in degraded samples, those from the drowned, the burnt, and the mummified bodies. In each case, approximately 0.04-3.84 [μ]g of DNA was recovered from 1 mg of fixed tissue. We typed 8/8 for the DR, 4/4 for the DQ, 3/3 for the A, and 3/3 for the B locus from the samples. Based on these results and the finding that DNA extraction is easier from the aorta than from other samples such as bone or tooth, we considered this method to be useful for forensic samples.

Tanaka, M., N. Yamashita, et al. (2001). "RHC/c genotyping based on polymorphism in the promoter region of the RHCE gene." Legal Medicine 3(4): 205.

<http://www.sciencedirect.com/science/article/B6W7W-44B1Y7K-2I2/7a11b975f09f6bf92139ccfcde7eebe9>

Designing of PCR tests for the RHC allele is difficult because of the high DNA sequence homology between RHC and RHD genes, which differ by only a one-nucleotide substitution at position 48 in exon 1 of the RHCE gene. We sequenced the promoter region of the RHCE gene, and compared our results with the reported sequence. Genomic DNA was prepared from blood samples collected from 656 Japanese donors. The DNA segment encompassing the promoter region and exon 1 of the RHCE gene from 30 donors was amplified by PCR and analyzed by DNA sequencing. Four nucleotide differences between RHC/c and RHD were found at positions -468, -304, -58, and -46. On the basis of the nucleotide differences at positions -468 (RHCE vs. RHD) and -292 (RHC vs. RHc), we then developed a novel polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method for RHC/c genotyping. Analysis of the genomic DNA from the 656 donors revealed that this method could discriminate RHC from RHc, irrespective of the RHD genotype, with only a few exceptions. The combination of our system and the intron 2-based PCR-RFLP method previously reported may prove to be more accurate than either of the methods alone, and therefore, useful and valuable for RHC/c genotyping.

Tsukada, K., K. Takayanagi, et al. (2002). "Multiplex short tandem repeat typing in degraded samples using newly designed primers for the TH01, TPOX, CSF1PO, and vWA loci." Legal Medicine 4(4): 239.

<http://www.sciencedirect.com/science/article/B6W7W-473VVB-4/2/38fbbc45d0eba3df5040f1a71ee36bbb>

We performed multiplex polymerase chain reaction (PCR) for the TH01, TPOX, CSF1PO, and vWA loci using a newly designed pair of primers that yield smaller fragments than reported previously [Fujii et al., J Hum Genet 45 (2000) 303; Lederer et al., Int J Legal Med 114 (2000) 87]. These loci can be detected in the range of 74-143 bp amplifying products. This system required genomic DNA in a range of 80 pg to 2 ng, and proved to be a sensitive typing method. We compared our system against the GenePrint Fluorescent STR Multiplex Systems CTTv (Promega, Madison, WI, USA), using DNA extracted from old bloodstains left to stand for 17-26 years at room temperature. With our designed system, all allele-typing efforts were successful in the range of 1-5 ng DNA, while no signal peaks were detected, even with when using 10 ng of DNA GenePrint Fluorescent STR Multiplex Systems CTTv.

Yasuda, T., R. Iida, et al. (2004). "A novel 56-bp variable tandem repeat polymorphism in the human deoxyribonuclease I gene and its population data." Legal Medicine 6(4): 242.

<http://www.sciencedirect.com/science/article/B6W7W-4D4CYM6-3/2/b6d2fc3fd19e3baff81a8299bb012a24>

This study confirms the presence of a novel variable number of tandem repeats polymorphism, designated as HumDN1, in intron 4 of the human deoxyribonuclease I (DNase I) gene. Genotyping was performed without difficulty by PCR-amplification and separation by agarose gel electrophoresis in 423 Japanese, originating from four geographically diverse areas in Japan, and 89 Germans. The HumDN1 allele variability was due to different numbers of 56-bp repeat sequences, and five different alleles were distinguished with apparent size between 364 and 588 bp. Although there was a general uniformity for the polymorphism in the Japanese population, significant differences in genotype distribution were found between the Japanese and German populations. Furthermore, linkage disequilibrium between the HumDN1 and DNase I protein polymorphisms was revealed.

Leukemia Research (39)

Baker, E. J., D. A. Gerard, et al. (2002). "HL-60 cell growth-conditioned medium is an effective inducer of myeloperoxidase expression in K-562 human leukemia cells." Leukemia Research 26(11): 1017.

<http://www.sciencedirect.com/science/article/B6T98-45F8XF0-2/2/9fbb55bc4814f88f0861b34705005cff>

K-562 cells were cultured in HL-60 cell growth-conditioned medium (GCM) for up to 96 h. Myeloperoxidase (MPO) mRNA was transiently detected by reverse transcription-polymerase chain reaction (RT-PCR) techniques at 12, 24, and 48 h. The de novo expression of MPO protein was subsequently detectable by intracellular flow cytometry at 24, 48, 72 and 96 h. Immunogold staining and cytochemical analysis demonstrated granularly-sequestered MPO in approximately 40% of HL-60 GCM-cultured cells after 48 h of culture. The sequential detection of MPO mRNA and MPO biosynthesis is considered an indicator of serial maturation evocative of myeloblastic cells, and suggest that K-562 cells maintain the ability to differentiate along this lineage.

Busque, L., J. R. Ilaria, et al. (1994). "Clonality analysis of childhood all in remission: No evidence of clonal hematopoiesis." Leukemia Research **18**(2): 71.

<http://www.sciencedirect.com/science/article/B6T98-4BY4PSJ-S/2/40bba214793b8b2f88a7a0b2ec272542>

We studied 98 female patients in remission (2-240 months) from childhood ALL to determine the clonality status of their hematopoiesis. Thirty-one (31.6%) were heterozygous at the PGK locus for the BstX1 endonuclease restriction site, permitting X-linked clonality assays to be performed. Two patients were in relapse at the time of study and were excluded. We used the PGK-PCR clonality assay (PPCA) to analyze DNA from PMN and mononuclear cells of the remaining 29 female patients. All (29/29) patients demonstrated polyclonal hematopoiesis. These data show that remission from childhood ALL involves reestablishment of polyclonally derived hematopoiesis in all patients studied.

Chim, C. S., Y. L. Kwong, et al. (2004). "Methylation profiling in multiple myeloma." Leukemia Research **28**(4): 379.

<http://www.sciencedirect.com/science/article/B6T98-49J8T38-5/2/d448bddef37302cc24b2f6fc4e107973>

Background: We analysed the methylation status of a panel of 10 genes including p15, p16, DAPK, p73, VHL, E-CAD, MGMT, RAR[beta], RIZ1, and ER. Methods: The gene promoter methylation status was studied by methylation-specific polymerase chain reaction (MSP) with primers for methylated (M-MSP) and unmethylated (U-MSP) DNA in the bone marrow of 13 patients with myeloma, and one patient with plasmacytoma. Result: None of the 10 genes tested were methylated in eight normal bone marrow samples. For the positive control, the sensitivity of M-MSP ranged from 1×10^{-2} for E-CAD and MGMT, to 1×10^{-4} for p73. Of the eight diagnostic myeloma marrow samples, hypermethylation of p15, p16, E-CAD, DAPK and ER occurred in six (75%), four (50%), seven (87.5%), eight (100%), and six (75%) patients. Similarly, of the five samples from patients who progressed from plateau phase, hypermethylation of p15, p16, E-CAD, DAPK, and ER occurred in five (80%), two (40%), five (100%), five (100%), and three (60%). None of the cases had hypermethylation of RIZ1, p73, VHL, RAR[beta], and MGMT. At diagnosis, all patients had concurrent hypermethylation of at least three genes, and five (62%) had concurrent methylation of four or more genes. One patient with plasmacytoma had methylation of E-CAD, ER, and DAPK. Conclusion: p15, p16, ER, DAPK, and E-CAD (but not RAR[beta], p73, VHL, RIZ1, and MGMT) were frequently methylated in MM at both diagnosis and disease progression. Future studies of larger scale are needed to identify the genes responsible for disease progression.

Curry, J. D. and M. T. Smith (2003). "Measurement of SIL-TAL1 fusion gene transcripts associated with human T-cell lymphocytic leukemia by real-time reverse transcriptase-PCR." Leukemia Research **27**(7): 575.

<http://www.sciencedirect.com/science/article/B6T98-47GHMRS-1/2/c9c5178d5b0a0e95caef2fe167764065>

TAL1 disruption at 1p32 [del(1p)] is a common rearrangement in the development of T-cell acute lymphocytic leukemia (T-ALL). The del(1p) are usually interstitial 90 kb deletions placing TAL1

under control of the SCL interrupting locus (SIL) gene forming the SIL-TAL1 fusion product. A reverse transcriptase real-time PCR assay to quantify SIL-TAL1 fusion genes is described. A SIL-TAL1 fusion gene RNA transcript was built that permitted absolute standard curves to be generated. Sensitivity of the RT-PCR assay was determined to be 10 cells (CEM cell line) in 10⁶ human lymphocytes. Peripheral blood lymphocytes from 10 healthy adults and 10 neonates were assayed. None of the samples showed any SIL-TAL1 expression. However, when lymphocytes from three adults were cultured in vitro the SIL-TAL1 transcript was detectable in the RNA isolates. No RAG2 expression was detected in these expanded samples, suggesting that the clones bearing the SIL-TAL1 fusion gene may have existed at low levels prior to the ex vivo expansion.

de Souza Fernandez, T., J. Menezes de Souza, et al. (1998). "Correlation of N-ras point mutations with specific chromosomal abnormalities in primary myelodysplastic syndrome." Leukemia Research **22**(2): 125.

<http://www.sciencedirect.com/science/article/B6T98-3T0S8SP-4/2/c46a2749dacc4c7ed348d4c7ded628bf>

A cytogenetic and N-ras point mutation study was done in patients with primary myelodysplastic syndrome (MDS) from Rio de Janeiro, Brazil, in order to evaluate the progression of preleukemic states to overt leukemia. Cytogenetic analysis was performed in 50 patients with MDS and clonal chromosomal abnormalities were detected in 19 (38%) of them. Patients with refractory anemia (RA) or with ringed sideroblasts (RARS) presented normal karyotypes or single abnormalities as del(5q) or -Y, while patients in more advanced states as RA with excess of blasts (RAEB), RAEB in transformation (RAEB-t) and chronic myelomonocytic leukemia (CMML) showed complex karyotypes and single abnormalities involving chromosomes 7 or 8, which were related to poor prognosis and elevated risk of transformation to acute myeloid leukemia (AML). The frequency of ras activation was studied in these 50 patients with MDS. Samples of bone marrow were screened for oncogenic point mutations by DNA amplification followed by oligonucleotide hybridization analysis (PCR-ASO) at codon 12 of N-ras proto-oncogene. We detected N-ras point mutations in 21 patients (42%). Progression from MDS to AML was observed in 9 patients (18%). The correlation analysis between N-ras point mutations and specific chromosomal abnormalities indicated that although mutated N-ras was found in cells with del(5q) and monosomy 7, cells with those abnormalities and normal N-ras were also identified. Otherwise trisomy of chromosome 8 showed a correlation with N-ras point mutations and in all cases, patients showed progression of MDS to AML during the follow-up study. MDS comprises a heterogeneous group of hematopoietic disorders and probably several steps are implicated in the evolution to AML. In this work we suggest that one possible pathway of leukemogenesis in MDS includes N-ras point mutations in association with trisomy of chromosome 8.

Delforge, M., V. Raets, et al. (2003). "Amifostine does not preferentially stimulate the growth of residual polyclonal progenitor cells in myelodysplastic syndromes." Leukemia Research **27**(7): 593.

<http://www.sciencedirect.com/science/article/B6T98-46RD13X-2/2/f61e97d9e530df63640f976c892d5d04>

Amifostine is a phosphorylated aminothiols that has besides anti-oxidative and cytoprotective properties, also survival- and growth-promoting effects on hematopoietic progenitor cells. Clinical studies have demonstrated that infusions with amifostine are able to increase erythro-, myelo-, and thrombopoiesis in some patients with myelodysplastic syndromes (MDS). Since clonal and non-clonal progenitors can coexist in early phase MDS, we have studied if amifostine exerts a selective growth-promoting effect on clonal or non-clonal cells. For this purpose, purified CD34+

marrow progenitors from nine female MDS patients were grown in short- and long-term cultures. Clonality was studied on individual colonies using polymorphisms in the human androgen receptor assay (HUMARA) locus. Three patients had growth of residual non-clonal progenitors at baseline. Continuous exposure to 100 nM amifostine exerted a growth-promoting effect on progenitors in 50% of the patients. HUMARA patterns of individual colony-forming unit granulocyte macrophage (CFU-GM; 5/9) and 5 week long-term culture-initiating cells (LTC-IC; 2/9) were compared without and with amifostine exposure. We did not observe preferential stimulation of clonal or non-clonal progenitors. Based on these results, the stimulation of committed and immature progenitor growth in MDS by amifostine, is non-selective and does not favor nor suppress the growth of residual non-clonal cells.

Diamant, M., M. B. Hansen, et al. (1996). "Differential interleukin-6 (IL-6) responses of three established myeloma cell lines in the presence of soluble human IL-6 receptors." Leukemia Research **20**(4): 291.

<http://www.sciencedirect.com/science/article/B6T98-3VXNHRT-2/2/2dd731b53de8a77a0a3e24ae6c9c4daf>

We investigated the possible influence of recombinant (r) sIL-6R on the growth of three IL-6 non-responsive or weakly IL-6 responsive long-term myeloma cell lines. The three cell lines chosen for the study (U266, L363 and Fravel) all expressed gp130 but differed in their expression of IL-6R and IL-6. mRNA analysis by northern blot and reverse transcriptase polymerase reaction showed that the cell line U266 was the only one that expressed IL-6 mRNA. Only U266 and L363 expressed IL-6R mRNA. 125I-rIL-6 binding studies and FACS analysis, using biotinylated IL-6 and antibodies directed against the IL-6R and gp130, showed corresponding results on the protein level. Addition of rIL-6R resulted in induction of IL-6 responsiveness in L363 cells, whereas the 3H-thymidine incorporation of the cell lines U266 and Fravel was unaffected by rIL-6R addition. In conclusion, the IL-6 unresponsive growth of several long-term myeloma cell lines in vitro can in some, but not all cases, be due to a deficiency in exogenous sIL-6R.

Dybkaer, K., B. Pedersen, et al. (2000). "Identification of acute myeloid leukemia patients with diminished expression of CD13 myeloid transcripts by competitive reverse transcription polymerase chain reaction (RT-PCR)." Leukemia Research **24**(6): 497.

<http://www.sciencedirect.com/science/article/B6T98-40378GB-7/2/5c0820c31098d0aaa34f5d17ff9fcc52>

Normal myeloid cells of monocytic and granulocytic origin express the metallopeptidase cluster of differentiation 13 (CD13) on the surface just as leukemic blasts in most acute myeloid leukemias (AML). A minor percentage of AML patients, however, lack the surface expression of CD13 antigen. To study this difference in CD13 surface expression, specific CD13 mRNA from 44 individuals were quantified by competitive reverse transcription polymerase chain reaction (RT-PCR). Absolute values for CD13 transcripts were normalised against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcript levels to control for variations in sample preparation and mRNA degradation. By correlating normalised CD13 transcript levels and CD13 surface expression, a subgroup of AML patients was identified, having simultaneous diminished levels of myeloid CD13 transcripts and surface expression of the corresponding antigen. For this subgroup we suggest CD13/aminopeptidase N (APN) gene expression to be restricted primarily by limited amounts of transcripts. For the majority of AML patients determinants in addition to transcript levels must be involved in regulating CD13/APN gene expression.

Ek, S., E. Ortega, et al. (2005). "Transcriptional profiling and assessment of cell lines as in vitro models for mantle cell lymphoma." Leukemia Research **29**(2): 205.

<http://www.sciencedirect.com/science/article/B6T98-4D99K2T-2/2/d9c824d4e2e03f82ed7ca85bcba47afc>

Mantle cell lymphoma (MCL) is an aggressive malignancy and new treatment modalities must be established to increase patient survival time. In the search for new therapeutic targets, reliable and well-characterised in vitro models are essential. In this study, we have characterised three MCL cell lines (SP53, Granta 519 and NCEB1) in comparison with primary tumours from MCL, follicular lymphomas (FL), a FL cell line (RL), a Burkitt lymphoma cell line (RAJI) and five different B cell populations from healthy individuals. Expression profiling was used to determine the relative expression of >12000 transcripts in these samples, and flow cytometry analysis was performed to establish a phenotypic signature for each of the cell lines. In addition, the cell lines were sequenced, and the frequency of somatic mutations and immunoglobulin (Ig) variable heavy chain (VH) usage were determined. We show by hierarchical clustering that the cell lines retain a genetic signature similar to primary MCL, which readily separated the MCL samples from the other lymphoma cell lines and the FL tumours. Furthermore, the MCL cell lines showed differences in the frequency of VH somatic mutations (0-2.1%). The increased number of mutations in NCEB1, compared to the other MCL cell lines, was in agreement with a decreased expression of CD31, CD44, CXCR5, CCR7 and CCR6. Taken together, our data show that the cell lines are clearly derived from MCL tumours and expressed similar genetic and phenotypic signatures compared to primary tumours, which confirmed their usefulness as in vitro models.

Hanawa, H., T. Koike, et al. (1997). "Novel analysis of minimal residual disease in leukemia with TCR [beta] rearrangement--Detection of monoclonality by single strand conformation polymorphism and PCR using a clonotype primer of leukemic T cell receptor [beta]-chain RNA." Leukemia Research **21**(3): 201.

<http://www.sciencedirect.com/science/article/B6T98-3RGSWRB-J/2/c2ac4410559a3c8c2f9d738f43de5a26>

Several means of analyzing minimal residual disease (MRD) in leukemia involving the rearranged T cell receptor (TCR) gene have been described. We investigated MRD in leukemia with TCR [beta] rearrangement by examining TCR [beta]-chain RNA. A complementary DNA (cDNA) corresponding to the variable region of the TCR [beta]-chains originating from the peripheral blood or bone marrow from four patients was amplified. Single strand conformation polymorphism (SSCP) analysis of amplified cDNA showed that all four patients had monoclonal leukemia with TCR [beta] rearrangement; two patients had V[beta]2+ leukemia, another patient had V[beta]14+ leukemia and the other had V[beta]9+ leukemia. Flow cytometry supported this finding. Sequencing of the V[beta]2-complementarity determining region 3 (CDR3), V[beta]9-CDR3 and V[beta]14-CDR3 revealed monoclonality. To investigate MRD using TCR [beta]-chain RNA, cDNA from each patient was diluted with the cDNA of a healthy person and amplified using a specific CDR3 clonotype primer. A band in the ethidium bromide-stained agarose gel was detected from samples diluted 10 000-fold. SSCP analysis determined which V region gene was utilized in monoclonal leukemic cells. The leukemic cell specific TCR, determined in such a manner, may be a target for immunotherapy. Because the MRD of T cell malignancy can be easily examined once the CDR3 clonotype primer is made, this novel analysis is considered to be a useful method.

Harashima, A., Y. Matsuo, et al. "Transcription factor expression in B-cell precursor-leukemia cell lines: preferential expression of T-bet." Leukemia Research **In Press, Corrected Proof**
<http://www.sciencedirect.com/science/article/B6T98-4FJTP45-1/2/bf4ea7c12d7ce1ec4cb23a79293a70ca>

A number of transcription factors (TFs) have been reported that play crucial roles in hematopoiesis. However, only little is known about how these factors are involved in the mechanisms of hematopoietic development and lineage commitment. To investigate the roles of TFs in human B-cell precursors (BCPs), the present study analyzed the expression of the following 16 hematopoietic TFs: AML1, C/EBP[alpha], C/EBP[beta], C/EBP[gamma], C/EBP[epsilon], E2A, Ets-1, GATA-1, GATA-2, GATA-3, Ikaros, IRF-1, Pax5, PU.1, T-bet and TCF-1 in 30 human BCP-leukemia cell lines. All BCP-leukemia cell lines were found to be positive for the expression of AML1, C/EBP[gamma], E2A, Ets-1, IRF-1, Pax5 and PU.1 at the mRNA level. The mRNA expression of C/EBP[alpha], C/EBP[beta], C/EBP[epsilon], GATA-2, Ikaros, T-bet and TCF-1 was detected in 2 to 29 of the cell lines. Eight BCP-cell lines showed positivity for the dominant negative Ikaros isoform Ik6, while others were positive for expression of Ik1, 2, 3 and 4. GATA-1 and GATA-3 were universally negative. The expression of C/EBP[alpha], PU.1 and T-bet was positive at the protein level in five, 29 and four out of 30 BCP-cell lines, respectively. Cell lines were stimulated with interleukin (IL)-7 and/or interferon (IFN)-[gamma] to investigate the regulation of TF expression. T-bet was clearly induced in the two cell lines NALM-19 and NALM-29 after stimulation. C/EBP[beta] and IRF-1 were up-regulated in both cell lines and TCF-1 was down-regulated in NALM-19. No significant changes were observed for the other 12 TFs. The present report could provide useful information in the study of the role of TFs on normal and malignant human BCPs.

Horikoshi, T., H.-J. Lenz, et al. (1994). "Quantitative determination of the ratio of mutated to normal ras genes in the blood of leukemia patients by allele-specific PCR." Leukemia Research **18**(9): 693.

<http://www.sciencedirect.com/science/article/B6T98-4BP9WBF-MV/2/a7288214b97a21de39a5c77e2bed4b2d>

By combining allele-specific PCR amplification and a PCR-based quantitation approach, a method has been developed to estimate the mutated K-ras gene content in the blood of AML patients as a percentage of total K-ras. One PCR primer set was designed not to discriminate between mutant K-ras and wild-type K-ras and thus amplified the total K-ras gene. The other PCR primer set was designed to be allele-specific for K-ras genes containing a G to C mutation at codon 12. This primer set could discriminate the mutant and wild-type genes when the proportion of the mutated sequence was 0.2% of the total K-ras gene. To test the method on biological specimens, genomic DNA samples were analyzed from the peripheral blood of a patient who had secondary AML with the same codon 12 K-ras mutation. Two samples taken from this patient 2 months apart during follow-up had myeloblast cell contents of 67 and 80%. However, the percentage of mutated K-ras was 50% in both samples, suggesting that this patient may be inherently heterozygotic in this particular mutation. This ratio of mutated to normal K-ras in the patient's cells was confirmed by RNA-SSCP analysis and RNA sequencing. This quantitation method can provide a sensitive and specific estimation of the content of mutated K-ras alleles in patient samples.

Illmer, T., M. Schaich, et al. (1999). "A new PCR MIMIC strategy to quantify low *mdr1* mRNA levels in drug resistant cell lines and AML blast samples." Leukemia Research **23**(7): 653.

<http://www.sciencedirect.com/science/article/B6T98-3WM54SD-7/2/b6c8713c0156735b57abd18936a82790>

Determination of the MDR-phenotype in patients suffering from AML is an important hallmark of treatment outcome but is often complicated by technical problems in P-gp assessment. A PCR-MIMIC strategy was employed to construct PCR-fragments for a competitive and quantitative *mdr1* reverse transcription-PCR-assay. Using K562 cells, which had been selected for drug resistance to the epipodophyllotoxin VP16, a stepwise increase of *mdr1* levels depending on the concentration of VP 16 was shown with the MIMIC technique. Comparison of *mdr1* levels in drug selected K562 cells with the corresponding levels for P-gp and functional data indicated a mRNA threshold that has to be exceeded for the full expression of the MDR-phenotype. Subsequently *mdr1* levels of 34 samples of de novo acute myeloid leukemia were determined with the PCR-MIMIC strategy. Ten patient samples could be identified with elevated *mdr1* levels which were substantially lower than the levels observed in the MDR-cell line K 562 0.7 [μ]M VP16. Outcome analysis revealed that eight of the ten patients had an unfavourable prognosis and did not achieve CR after induction chemotherapy. Coexpression of *mdr1* and CD 34 was not associated with CR in all examined cases. Moreover all these patients had unfavourable cytogenetic aberrations. These data indicate a sensitive technique with applicability in patient material.

Jahn, B., L. Bergmann, et al. (1995). "Bone marrow-derived T-cell clones obtained from untreated acute myelocytic leukemia exhibit blast directed autologous cytotoxicity." *Leukemia Research* **19**(2): 73.

<http://www.sciencedirect.com/science/article/B6T98-3YB565M-3R/2/22f2fbee714bbc9d4d290d932ca16e54>

The eradication of minimal residual blast populations by activation of autologous cytotoxic cells with interleukin 2 (IL-2) is a new promising tool in the treatment of acute myelocytic leukemia (AML). However, the immunological effector cells are not yet clearly defined. The present study was designed to investigate the presence of cytotoxic precursor cells in active AML and to identify phenotypical and functional characteristics of autologous anti-leukemic cytotoxic effector cells. For this purpose, mononuclear cells (MNC) containing at least 70% leukemic blasts were isolated from bone marrow of untreated AML and cultured in the presence of 3000 IU/ml recombinant IL-2 (rIL-2) for 6-8 weeks. Under these conditions, T-cells were selected in the bone marrow cultures and overgrew the leukemic blasts. The resulting T-cell populations were cloned by limiting dilution and the clones obtained were characterized for their phenotypical and functional patterns. Totally, cloning resulted in 68 clones and a few cell lines. The clonality was verified by RT PCR analysis of TCR V[beta] gene expression. All clones obtained stained positive for CD2, CD3, DR and CD56. The vast majority (68%) of T-cell clones/lines was CD4+, a few clones expressed CD8 (19%) or CD4 and CD8, and four clones were of TCR[gamma][delta] origin. Seven of 15 clones tested, including three CD4+, two CD8+ and two TCR[gamma][delta]+-clones were found to be cytotoxic against autologous leukemic blast cells. All except one clone expressed oncolytic activities against allogeneic blasts too. One of the TCR[gamma][delta]+-clones demonstrated NK activity by lysis of K562 targets. The majority of the T-cell-clones released IL-2, IL-8, TNF-[alpha], GM-CSF but only a few IFN[gamma] and expressed high levels of mRNA for IL-2, TGF-[beta] and IL-10. None of the clones was found to produce IL-3, IL-4, IL-7 and TNF-[beta]. The data provide evidence of the existence of T-cell precursors in untreated AML bone marrow differentiating to cytotoxic cells with activity against autologous and allogeneic AML blast cells.

Jordan, J.-H., R. Fritsche-Polanz, et al. (2001). "A case of 'smouldering' mastocytosis with high mast cell burden, monoclonal myeloid cells, and C-KIT mutation Asp-816-Val." *Leukemia Research* **25**(7): 627.

<http://www.sciencedirect.com/science/article/B6T98-433NP9N-G/2/7cf951a8077be0a8f660f02ed4267058>

Mastocytosis is a term used for a group of disorders characterized by abnormal growth and accumulation of tissue mast cells (MC) in one or more organ systems. In patients with systemic mastocytosis (SM) the clinical course may be indolent or aggressive or even complicated by leukemic progression or an associated clonal hematologic non mast cell lineage disease (AHNMD). However, at first presentation (diagnosis) it may be difficult to define the category of disease and the prognosis. We report on a 48-year-old female patient with SM with urticaria pigmentosa-like skin lesions and mediator-related symptoms. She was found to have splenomegaly, a high infiltration grade (MC) in bone marrow biopsies (>30%), mild anemia, and a high serum tryptase level (>500 ng/ml). In addition, she exhibited discrete histologic signs of myeloproliferation in the 'non-affected' marrow and monoclonal blood cells established by C-KIT 2468A -> T mutation (Asp-816-Val) -analysis and HUMARA assay. Despite these findings, however, the clinical course was stable over years and no AHNMD or organ impairment developed. Because of the 'intermediate' clinical signs and absence of progression to aggressive disease, we proposed the term 'smouldering mastocytosis'.

Kawasaki, H., K. Shindou, et al. (1996). "Deoxycytidine kinase mRNA Levels in leukemia cells with competitive polymerase chain reaction assay." Leukemia Research **20**(8): 677.

<http://www.sciencedirect.com/science/article/B6T98-3W258FG-8/2/41d24b57616471a39726f20dae6ae01f>

Deoxycytidine kinase (dCyd kinase) is important for the phosphorylation of several different nucleoside antimetabolites. To understand the significance of dCyd kinase levels in chemotherapy, dCyd kinase mRNA levels were evaluated in several cells with a quantitative competitive polymerase chain reaction (PCR) assay. dCyd kinase catalytic activity and intracellular ara-CTP production were also compared with the levels of dCyd kinase mRNA. The assay was able to show: (i) that dCyd kinase catalytic activity and dCyd kinase mRNA levels were correlated in cells; (ii) that dCyd kinase mRNA levels were more sensitive in lower levels of 10 amol/[μg] of total RNA; and (iii) in cytosine arabinoside (ara-C)-resistant cells, both dCyd kinase mRNA levels and intracellular ara-CTP levels were lower compared with levels in sensitive cells. The PCR assay for dCyd kinase mRNA could be useful in the selection and monitoring of patients treated with nucleosides that are activated by this enzyme.

Kishino, K., K. Muroi, et al. (2002). "Evaluation of engraftment by ABO genotypic analysis of erythroid burst-forming units after bone marrow transplantation." Leukemia Research **26**(1): 13.

<http://www.sciencedirect.com/science/article/B6T98-44J720S-3/2/acfe39f022efd1b367dea2746722a0e5>

Six patients received an allogeneic bone marrow transplant from HLA-identical ABO-mismatched donors. ABO genotype of erythroid burst-forming units (BFU-E) from peripheral blood was analyzed using polymerase chain reaction with sequence specific primers (PCR-SSP). After bone marrow transplantation (BMT), engraftment of donor cells by ABO genotypic analysis of BFU-E was compared with ABO phenotypic analysis of red blood cells (RBCs). During the early stage after BMT, ABO genotype of BFU-E in the recipients converted to that of the donors. In contrast, mixed ABO phenotype of RBCs persisted for about 3 months. In one patient, autologous hemopoietic cell recovery was detected by the ABO genotypic analysis before clinical manifestation. ABO genotypic analysis of BFU-E is relevant for engraftment after ABO-mismatched BMT.

Koiso, H., N. Tsukamoto, et al. (2002). "Quantitative analysis of Cyclin D1 and CD23 expression in mantle cell lymphoma and B-chronic lymphocytic leukemia." Leukemia Research **26**(9): 809.

<http://www.sciencedirect.com/science/article/B6T98-458P438-2/2/2520d3c3099a04d5806a9a689400e4d9>

We studied Cyclin D1 (CyD1) and CD23 mRNA expression with real-time quantitative reverse transcription polymerase chain reaction (RQ-PCR) method. CyD1 expression in peripheral blood of seven mantle cell lymphoma (MCL) patients was found to be 1305.4 times higher than in 24 B-chronic lymphocytic leukemia (CLL) patients. CD23 expression in CLL was found to be 54.8 times higher than in MCL. These differences were statistically significant, and no overlap was found in CyD1 expression intensities between MCL and CLL. RQ-PCR allows rapid, simple and accurate quantification of CyD1 and CD23 expression, even from small samples, and is thus useful for the diagnosis of MCL and CLL.

Kristensen, T., H. K. Jensen, et al. (1994). "Overexpression of human thymidine kinase mRNA without corresponding enzymatic activity in patients with chronic lymphatic leukemia." Leukemia Research **18**(11): 861.

<http://www.sciencedirect.com/science/article/B6T98-4C118PD-T/2/d2befeeaa1057c3d44522c4688537835>

The level of cytosolic thymidine kinase (TK1) mRNA in lymphocytes from six healthy people and in lymphocytes from five patients with untreated chronic lymphatic leukemia (CLL) was determined with competitive polymerase chain reaction (competitive PCR). Using this procedure we have shown that in patients with CLL, there is an overexpression of TK1 mRNA without corresponding enzymatic activity. The TK1 mRNA level is approximately 100-fold higher in lymphocytes from CLL patients than in lymphocytes from healthy persons. A high level of TK1 mRNA without corresponding enzyme activity may indicate a defect in the processing of the enzyme. This may disturb the cells' normal feedback system and thereby influence the development of malignant conditions.

Min, Y. H., G. X. Li, et al. (2002). "Long-term bone marrow culture-derived stromal fibroblasts as a potential target for gene therapy in acute myelogenous leukemia." Leukemia Research **26**(4): 369.

<http://www.sciencedirect.com/science/article/B6T98-452RBCH-7/2/7d00300d5750cdaba78f48d78f718258>

As a part of our continuing efforts to develop gene therapy for acute myelogenous leukemia (AML), this study was undertaken to evaluate the possibility of using autologous bone marrow stromal fibroblasts (BMSFs) as a target cell population. Autologous BMSFs in AML were isolated from the stromal layers of long-term bone marrow culture (LTBMC) using immunomagnetic beads. BMSFs exhibited rapid proliferation even in the absence of growth factors. Cultures stimulated with bFGF produced significantly increased numbers of BMSFs than cultures without added growth factors. Using LNC/LacZ retroviral vector, the transduction efficiency of BMSFs was 13+/-4% at a 5 multiplicity of infection (MOI). LNC/interleukin-2 (IL-2)-transduced BMSFs produced between 1200 and 4800 pg of IL-2/106 cells per 24 h. Using adenoviral vector AdV/LacZ, the transduction efficiency was 84+/-10% at 100, and 92+/-8% at a MOI of 1000. Although the addition of basic fibroblast growth factor, epidermal growth factor, or platelet-derived growth factor did not affect the transduction efficiency, they increased the numbers of transduced

cells significantly (P6 cells per 24 h. Our finding that the genetically engineered autologous BMSFs of AML could be successfully established in vitro implies that BMSFs obtained from LTBMK might be considered as a target cell population for certain types of clinical gene therapy in AML.

Miyauchi, J., Y. Inatomi, et al. (1997). "The in vitro effects of all-trans-retinoic acid and hematopoietic growth factors on the clonal growth and self-renewal of blast stem cells in acute promyelocytic leukemia." Leukemia Research **21**(4): 285.

<http://www.sciencedirect.com/science/article/B6T98-3RGT92H-2/2/68fb30d0061f3dc42b5a8141be4b4e16>

All-trans-retinoic acid (ATRA) has been used as a potent therapeutic agent to induce differentiation of acute promyelocytic leukemia (APL) cells, and granulocyte colony-stimulating factor (G-CSF) has been reported to enhance this effect of ATRA in vitro. We investigated the effects of ATRA and three myeloid growth factors, including G-CSF, on the growth of the leukemic stem cells of 10 APL patients. G-CSF was the most powerful stimulator of leukemic colony formation in five out of 10 patients, but was neither the major stimulant of self-renewal of the blast stem cells nor an inducer of maturation. In contrast, ATRA was highly effective in inducing morphological maturation of leukemic promyelocytes, but variable results were obtained in regard to its effects on the growth of blast stem cells: ATRA suppressed both clonal growth and self-renewal in some patients, but was inactive or even had stimulating effects in the other patients. Similar variable effects were observed with the combination of ATRA and G-CSF. These findings indicate that the differentiation-inducing effect of ATRA is not always associated with growth inhibition of leukemic stem cells in vitro and justify the use of chemotherapy in conjunction with ATRA in the treatment of APL.

Nakagawa, Y., S. Yamaguchi, et al. (2004). "Differential expression of survivin in bone marrow cells from patients with acute lymphocytic leukemia and chronic lymphocytic leukemia." Leukemia Research **28**(5): 487.

<http://www.sciencedirect.com/science/article/B6T98-4B5JM6V-6/2/1136f69233c135c369191fdbcb166f915>

Survivin, a member of the inhibitor of apoptosis protein (IAP) gene family, has been detected widely in fetal tissue and in a variety of human malignancies. In the current study, we investigated the expression of IAP family proteins in bone marrow samples from acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL) and control cases by quantitative real-time RT-PCR method and an immunohistochemical approach. Overexpression of survivin and cIAP2 mRNA was significant in CLL bone marrow cells ($P < 0.05$, respectively) compared with control samples. By immunohistochemistry, survivin was detected in a few scattered myeloid cells in all cases of control bone marrow. Concerning the ALL bone marrow, more than half the cases demonstrated positive expression of survivin (8 out of 13), while the majority of CLL cases (20 out of 21) exhibited intense expression of survivin. The differential subcellular localization of survivin was distinct between ALL and CLL cases. ALL cells essentially revealed nuclear localization of survivin as well as cytoplasmic signals in some cases, while CLL cells from the majority of cases predominantly showed cytoplasmic expression. Next, RT-PCR was performed for the expression of survivin and its splicing variant, survivin-2B and survivin- Δ Ex3 in ALL and CLL cells, as the distribution of these variants would be regulated by nuclear/cytoplasmic transport system. In both ALL and CLL bone marrow samples, the expression of wild-type survivin was more predominant than that of survivin-2B or survivin- Δ Ex3, although the expression of survivin- Δ Ex3 was prominent in samples from survivin-expressing ALL cases. Thus, the splicing of

survivin mRNA may be differently regulated in ALL and CLL cells, causing distinct manners of nuclear/cytoplasmic transport of survivin protein. In conclusion, our observations indicate a differential regulatory mechanism for the expression of IAP family proteins in ALL and CLL cells, although the functions of IAP families and the mechanisms of nuclear/cytoplasmic transport of survivin should be clarified in future studies.

Niemeyer, P., O. Tureci, et al. (2003). "Expression of serologically identified tumor antigens in acute leukemias." Leukemia Research **27**(7): 655.

<http://www.sciencedirect.com/science/article/B6T98-47K2J0N-2/2/4311d691880f917ee6e25676eef094f0>

Cancer/testis antigens (CTA) are an expanding family of immunogenic proteins selectively expressed in human neoplasms. As little is known about the expression of serologically identified CTA in leukemias so far, we investigated the expression of 5 CT genes (SSX-1, HOM-MEL-40/SSX-2, HOM-TES-14/SCP-1, SCP-3 and NY-ESO-1) in leukemic blood samples obtained from patients with either acute lymphatic leukemias (ALL) or myelocytic leukemia (AML). RT-PCR-analyses showed no expression of any of the CT-genes in the leukemia samples of 19 patients with AML, whereas frequent expression was found in ALL. In the 17 ALL cases studied, SCP3a, SSX-1, HOM-MEL-40/SXX-2 and HOM-TES-14/SCP-1 were expressed in 47, 29, 29 and 12%, respectively, whereas no case was positive for NY-ESO-1. 65% of patients with ALL showed expression of at least one, 41% of two or more of the five CT-genes investigated. We conclude that a majority of the ALLs might be amenable for specific immunotherapeutic interventions. However, the identification of additional antigens with a frequent expression in leukemias is warranted to allow the development of widely applicable polyvalent leukemia vaccines.

Pallis, M., C. Seedhouse, et al. (2003). "Flow cytometric measurement of phosphorylated STAT5 in AML: lack of specific association with FLT3 internal tandem duplications." Leukemia Research **27**(9): 803.

<http://www.sciencedirect.com/science/article/B6T98-47YH3V0-7/2/0c199dfb3517c8bcc8105056d0971133>

STAT5 phosphorylation has been noted in 69-95% of AML cases by Western blotting. We used flow cytometry to measure phosphorylated STAT5 on a semi-quantitative scale. The method was validated on K562 cells, which constitutively express phosphorylated STAT5, but lose this when BCR-abl tyrosine kinase activity is blocked by STI571. Phosphorylated STAT5 was found to measure 2.22 ± 0.09 relative fluorescence units (RFU) falling to 0.925 ± 0.005 RFU in the presence of STI571. Phosphorylated STAT5 expression was 0.99 to 2.09 RFU in 28 primary AML samples. There was no logical cut-off point between positive and negative fluorescence. FLT3 internal tandem duplications, found in 11/28 samples, were not significantly associated with the level of phosphorylated STAT5 expression. We conclude that STAT5 phosphorylation can be measured sensitively by flow cytometry in AML and that its expression should not be dichotomised as present or absent.

Perkel, J. M., M. C. Simon, et al. (2002). "Identification of a c-myc attenuator-binding factor." Leukemia Research **26**(2): 179.

<http://www.sciencedirect.com/science/article/B6T98-44PK59K-7/2/fee056022186d26f0c890ae0e894629a>

Expression of the c-myb proto-oncogene is developmentally regulated at the level of transcription elongation. In pre-B cells, complete c-myb transcripts are produced, whereas transcripts are attenuated near or within a 300-base pair (bp) interval of the first c-myb intron in mature cells. Hypothesizing that transcription attenuation results from a protein complex that physically impedes the progress of RNA polymerase II through the intron, we used electrophoretic mobility shift assays (EMSA) to search for DNA-binding activities that correlated with downregulation of c-myb transcription. We identified a stage-specific DNA binding activity, termed ABF, present in mature B cells but not in pre-B cells. ABF binds to a 15-bp DNA element located within a 300-bp BstEII-XbaI fragment. DMSO-treatment of murine erythroleukemia cells results in rapid downregulation of c-myb transcription and upregulation of ABF DNA binding activity. Thus, ABF binding activity correlates with downregulation of c-myb transcription in two systems. Preliminary biochemical characterization of ABF from mature B cells demonstrates that its primary DNA-binding component is a 64-kDa-protein. We hypothesize that this factor may represent a member of the transcriptional attenuation complex.

Scott, S., T. Kimura, et al. (2003). "Microsatellite mutations of transforming growth factor-[beta] receptor type II and caspase-5 occur in human precursor T-cell lymphoblastic lymphomas/leukemias in vivo but are not associated with hMSH2 or hMLH1 promoter methylation." Leukemia Research **27**(1): 23.

<http://www.sciencedirect.com/science/article/B6T98-460WG9P-1/2/5a29859452a0816e29dd5c0a99988491>

In solid cancers, defective DNA mismatch repair (MMR) is most commonly caused by hMSH2 or hMLH1 mutations, or epigenetic silencing of hMLH1 by promoter hypermethylation, and results in the acquisition of characteristic frameshift microsatellite mutations of mononucleotide repeats located within the coding regions of defined target genes. We previously identified hMSH2 mutations in T-cell lymphoblastic lymphoma (T-LBL) patient tumor samples and others have reported coding region microsatellite mutations in T-cell acute lymphoblastic leukemia (T-ALL) cell lines. Thus, while MMR gene mutations are known to occur in some human T-lymphoblastic tumors in vivo, it is still unknown if the coding region microsatellite mutations detected in human cell lines also occur in vivo or if hMLH1 or hMSH2 promoter hypermethylation contributes to defective MMR in these tumors. We analyzed the TGF[beta]RII (A)10 and caspase-5 (A)10 coding region repeats in 16 human T-LBL/ALL patient tumor samples and identified six with microsatellite mutations in one or both repeats. There was no evidence of hMSH2 or hMLH1 promoter methylation as assessed by standard methylation specific PCR or by a novel temporal temperature gradient electrophoresis (TTGE) method that analyzed 25 and 30 CpG sites in the hMLH1 and hMSH2 promoters, respectively. Our results indicate that coding region microsatellite mutations characteristic of defective MMR occur in some human T-LBL/ALL in vivo but not as a consequence of hMLH1 or hMSH2 promoter hypermethylation. Furthermore, the identification of TGF[beta]RII and caspase-5 coding region mutations in vivo implicates these genes in the pathogenesis of human T-LBL/ALL.

Sotlar, K., C. Fridrich, et al. (2002). "Detection of c-kit point mutation Asp-816 -> Val in microdissected pooled single mast cells and leukemic cells in a patient with systemic mastocytosis and concomitant chronic myelomonocytic leukemia." Leukemia Research **26**(11): 979.

<http://www.sciencedirect.com/science/article/B6T98-45JPFS6-2/2/15310005c90f62c646ffb6a8e9040709>

The c-kit mutation Asp-816->Val is detectable not only in neoplastic mast cells (MCs) in patients with systemic mastocytosis (SM) but also in most associated hematologic non-MC lineage disease (AHNMD). In order to prove a monoclonal disease evolution we investigated DNA of pooled microdissected single cells for the presence of the mutation in a patient with SM and concomitant chronic myelomonocytic leukemia (CMML). LightCycler melting curve analysis and direct sequencing of nested polymerase chain reaction (PCR) products revealed the c-kit mutation in tryptase-positive MC and in leukemic CD15-positive cells in bone marrow infiltrates, but not in colonic epithelial cells, thus, suggesting a monoclonal evolution of SM and concurrent CMML on the basis of a somatic mutation in a common hematologic progenitor.

Stacchini, A., M. Aragno, et al. (1999). "MEC1 and MEC2: two new cell lines derived from B-chronic lymphocytic leukaemia in prolymphocytoid transformation." Leukemia Research **23**(2): 127.

<http://www.sciencedirect.com/science/article/B6T98-3VWP0J1-10/2/f36f4b109d79d2e8544506a86ea45e4e>

We report the establishment and characterization of two cell lines, MEC1 and MEC2, that grew spontaneously on two subsequent occasions from the peripheral blood (PB) of a patient with B-chronic lymphocytic leukemia (B-CLL) in prolymphocytoid transformation. The patient was EBV-seropositive, his leukemic cells were EBNA negative, but the spontaneously grown cell lines are EBNA-2 positive. In liquid culture MEC1 cells grow adherent to the vessel wall and as tiny clumps; MEC2 cells do not adhere and form large clumps. The doubling time of MEC1 is 40h and of MEC2 is 31h. Both cell lines express the same light ([kappa]) and heavy chains ([mu], [delta]) as the fresh parental B-CLL cells at the same high intensity, share the expression of mature B cell markers (CD19, CD20, CD21, CD22), differ in the expression of CD23 and FMC7, are CD11a+, CD18+, CD44+, CD49d+, CD54+ and express at high levels both CD80 and CD86. CD5 is negative on MEC1 cells (as on the vast majority of parental cells) and it has been lost by MEC2 cells after several months of culture. The cells have a complex karyotype. The tumour origin of MEC1 and MEC2 has been demonstrated by Southern blot analysis of the IgH loci and by Ig gene DNA sequencing. They use the VH4 Ig family and have not undergone somatic mutations (94.8% homology with germline Ig gene 4-59). Cytofluorographic analysis and RT-PCR reveal that MEC1 and MEC2 overexpress Bcl-2 together with Bax, express large amounts of Bcl-xL and trace amounts of Bcl-xS.

Toba, K., H. Hanawa, et al. (2003). "Fc[epsilon]R1 and CD22 mRNA are expressed in early B-lineage and myeloid leukemia cell lines." Leukemia Research **27**(2): 173.

<http://www.sciencedirect.com/science/article/B6T98-461XG0N-4/2/dab009b9623e60e262c6385c8f94042f>

CD22, one of the important markers for diagnosing B-lineage acute leukemia, was expressed in mature basophil granulocytes. We then investigated the expression of CD22 and other B cell- and basophil-related molecules in 25 human acute leukemia cell lines to find the phenotype of the virtual common progenitor of B and myeloid lineage. Surface and cytoplasmic expressions of antigens were analyzed using a flow cytometer and an essential antibody panel used for diagnosing acute leukemia as well as cytokine receptors and basophil-related enzymes. Messenger RNA expression of Fc[epsilon]R1 and CD22 was also analyzed. Peroxidase-positive and -negative myeloid leukemias showed eosinophil- and basophil-type expression of enzymes, respectively. Early myeloid and B-lineage cells expressed basically similar combinations of cytokine receptors and various combinations of mRNA listed above, while T-lineage cells did not. The virtual common progenitor of B and myeloid lineage cells may be defined as immature cells simultaneously expressing B and basophil phenotypes.

Tokura, Y., M. Shikami, et al. (2002). "Augmented expression of P-gp/multi-drug resistance gene by all-trans retinoic acid in monocytic leukemic cells." Leukemia Research **26**(1): 29.

<http://www.sciencedirect.com/science/article/B6T98-44J720S-5/2/b4e49ab605ef5bc6cf990a9619272c2e>

P-glycoprotein (P-gp)/multi-drug resistance 1 (MDR1) gene is recognized to be, at least in part, responsible for the refractoriness to chemotherapy of leukemia. The transcriptional mechanism of MDR1 gene is largely unknown. However, recent reports have clarified that early growth response 1 gene (Egr1) positively regulates MDR1 transcription, while Wilms' tumor suppressor gene (WT1) does negative regulation of MDR1 gene expression in 12-O-tetradecanoylphorbol-13-acetate treated K562 cells. In addition, Egr1 and WT1 are structurally related transcription factors and bind to quite similar DNA sequences. Our study of mRNA expression profile of Egr1, WT1 and MDR1 in fresh AML samples demonstrated that there are disease-specific patterns. Egr1 mRNA was frequently and strongly expressed in monocytic leukemia cells, especially in AML M4 cells. WT1 mRNA was undetectable in t(8;21) AML cells. mRNA expression of MDR1 was frequent in AML M1 and t(8;21) AML cells, in which the expression level was highest in AML M1 and was low in monocytic leukemia (M4 and M5). Then, expression level of MDR1 was inversely correlated with Egr1. By liquid culture of leukemia cell lines and fresh AML cells with the addition of all-trans retinoic acid (ATRA), modulation of P-gp/MDR1 and Egr1 was observed and the pattern of modulation was divided into four groups: (1) blastic AML type, in which distinct expression of P-gp/MDR1 and CD34 was not influenced by ATRA; (2) t(8;21)AML type, in which P-gp/MDR1 expression was augmented by ATRA, while CD34 was kept high; (3) AML M3 type, in which P-gp/MDR1 expression was reduced with granulocytic differentiation by ATRA; (4) monocytic AML type, in which P-gp/MDR1 expression was augmented by ATRA, while CD34 expression decreased, and strong Egr1 expression was downregulated just prior to the augmentation of P-gp/MDR1 expression. WT1 expression was not influenced by the addition of ATRA in each group. Previous reports have suggested that P-gp/MDR1 plays an important role in resistance to chemotherapy, and is recognized as one of the stem cell marker. However, P-gp/MDR1 expression augmented by ATRA, which was observed in monocytic AML, was recognized as a functional molecule of mature monocyte/macrophage, because CD34 expression decreased and CD13 expression increased by ATRA. Finally, expression of P-gp/MDR1 in monocytic leukemia, which was functionally confirmed by Rh123 efflux study, was thought to be closely related to the characteristic modulation of Egr1 expression by ATRA.

Ullmannova, V., P. Stockbauer, et al. (2003). "Relationship between cyclin D1 and p21Waf1/Cip1 during differentiation of human myeloid leukemia cell lines." Leukemia Research **27**(12): 1115.

<http://www.sciencedirect.com/science/article/B6T98-48XCCH3-2/2/a9f4ee6a1ed1b17866109baea76c4e75>

Expression of cell cycle-regulating genes was studied in human myeloid leukemia cell lines ML-1, ML-2 and ML-3 during induction of differentiation in vitro. Myelomonocytic differentiation was induced by phorbol ester (12-o-Tetradecanoyl-phorbol-13-acetate, TPA), tumor necrosis factor [alpha] (TNF[alpha]) or interferon [gamma] (INF[gamma]), or their combination. Differentiation (with the exception of TNF[alpha] alone) was accompanied by inhibition of DNA synthesis and cell cycle arrest. Inhibition of proliferation was associated with a decrease in the expression of cdc25A and cdc25B, cdk6 and Ki-67 genes, and with increased p21Waf1/Cip1 gene expression, as measured by comparative RT-PCR. Expression of the following genes was not changed after induction of differentiation: cyclin A1, cyclin D3, cyclin E1 and p27Kip1. Surprisingly, cyclin D1 expression was upregulated after induction by TPA, TNF[alpha] with INF[gamma] or BA. Cyclin D2 was upregulated only after induction by BA. The results of the expression of the tested genes

obtained by comparative RT-PCR were confirmed by quantitative real-time (RQ) RT-PCR and Western blotting. Quantitative RT-PCR showed as much as a 288-fold increase of cyclin D1 specific mRNA after a 24 h induction by TPA. The upregulation of cyclin D1 in differentiating cells seems to be compensated by the upregulation of p21Waf1/Cip1. These results, besides others, point to a strong correlation between the expression of cyclin D1 and p21Waf1/Cip1 on the one hand and differentiation on the other hand in human myeloid leukemic cells and reflect a rather complicated network regulating proliferation and differentiation of leukemic cells.

Verstovsek, S., F. J. Giles, et al. (2004). "Telomerase activity is not a prognostic factor in chronic lymphocytic leukemia." Leukemia Research **28**(7): 707.

<http://www.sciencedirect.com/science/article/B6T98-4CC7RWX-1/2/1a9d7b04f3f19635cab58b22d4959038>

We measured telomerase activity (TA) in bone marrow samples from 214 patients with CLL and correlated it with patients' characteristics and survival. In >50% of cases (126/214; 59%) no detectable TA was found. There was no difference in TA between previously treated (n=153) and untreated (n=61) patients (P=0.4), or patients with various Rai (0-IV) stages (P=0.85). TA correlated significantly with white blood cell and lymphocyte count (P=0.02 and 0.01, respectively) but not with bone marrow cellularity, [beta]2-microglobulin ([beta]2M), or other patient characteristics. Patients who had no TA had slightly lower [beta]2M and lower lymphocyte counts (P=0.5 and 0.04, respectively) as compared with patients with detectable TA. However, there was no correlation between TA and survival. This data suggests that TA may not play a significant role in the clinical behavior of CLL.

Wan, M., J. Chow, et al. (2004). "Allelotyping of gastrointestinal nasal-type NK/T-cell lymphoma." Leukemia Research **28**(4): 339.

<http://www.sciencedirect.com/science/article/B6T98-49J8T38-3/2/f236d83bc38692b07017a31131c66cf1>

Nasal-type natural killer/T-cell lymphoma (NKL) is a rare but distinct malignancy that often involves the mid-facial region and the gastrointestinal tract. This study is the first genome-wide allelotyping analysis on this rare lymphoma. We applied 382 microsatellite markers covering loci which spanned 22 autosomes to screen for allelic imbalances (AI) in six intestinal NKL. The most common chromosomal regions of allelic imbalances were found in 11p, 9q and 13q. Novel spots of allelic losses were found at 2p21, 2q37.22, 18p11.21 and 18q12.1. In spite of presence of a few recurrent loci of imbalances, the allelotyping results show that NKL is heterogeneous.

Wang, J. C. and C. Chen (1998). "N-RAS oncogene mutations in patients with agnogenic myeloid metaplasia in leukemic transformation." Leukemia Research **22**(7): 639.

<http://www.sciencedirect.com/science/article/B6T98-3V4JR3R-R/2/d4bee82a0c748588b745e316a3062f8f>

From 5% to 20% of patients with agnogenic myeloid metaplasia (AMM) will evolve into a terminal leukemic phase; N-RAS gene mutations are the most common gene abnormalities detected in patients with leukemia. The present study was designed to see if N-RAS gene mutations are associated with the leukemic transformation in AMM. Over a 9 year period, in a single institution,

43 patients with AMM were studied. Of these, ten patients were found to be in leukemic phase. The results showed that none of the patients in chronic phase (40 patients) had N-RAS gene mutations, while two patients in leukemic phase showed this gene mutation. One patient was found to have a codon 12 mutation with arginine substituting for glycine (GGT->CGT); the other was a codon 12 mutation with glutamine substituting for glycine (GGT->GAT). The present study suggests that N-RAS mutations are rare events in the chronic phase of AMM, and are only occasionally found when patients have evolved into leukemic transformation. Further studies to search for other gene abnormalities in AMM may be warranted.

Wu, S., A. Korte, et al. (2005). "Interaction of bone marrow stromal cells with lymphoblasts and effects of prednisolone on cytokine expression." Leukemia Research **29**(1): 63.

<http://www.sciencedirect.com/science/article/B6T98-4D48XF7-1/2/17a581d689f0dea6e71df5c0b4a167c9>

Cytokines play a key role in the differentiation, growth and survival of hematopoietic cells in the bone marrow (BM) stroma microenvironment. The mechanisms by which stromal derangements may contribute to the evolution of hematopoietic neoplasias are largely unknown. Here, we characterized BM stromal cells isolated from children with acute lymphoblastic leukemia and determined the effect of the interaction between stromal cells and lymphoblasts on cytokine expression as well as the effect of prednisolone using mono- and co-culture models. The analyses demonstrate that (1) stromal cells and lymphoblasts display different patterns of cytokine gene expression individually. (2) Stromal cells influence gene expression of cytokines in lymphoblasts and vice versa. (3) Glucocorticoid substitution inhibit cytokine gene expression in stromal cells. These findings indicate that stromal cells are important components involved in malignant hematopoiesis and also in response to therapy.

Yamamoto, K., S. Abe, et al. (2004). "Expression of IAP family proteins in myelodysplastic syndromes transforming to overt leukemia." Leukemia Research **28**(11): 1203.

<http://www.sciencedirect.com/science/article/B6T98-4CJV8YV-7/2/e2860d193f75b9263669da1ab8a92086>

Bone marrow cells of patients with myelodysplastic syndromes (MDS) frequently undergo apoptosis, though the apoptotic cell ratio decreases when overt leukemia (OL) develops. Thus, we compared the expression of the inhibitor of apoptosis protein (IAP) gene family proteins in bone marrow samples from control, MDS, OL transformed from MDS (MDS -> OL), and de novo acute myelogenous leukemia (AML) subjects by the quantitative real-time RT-PCR method and an immunohistochemical approach. Overexpression of mRNA for survivin, cIAP1, NAIP and XIAP was significant in MDS bone marrow cells compared with control samples. However, the expression of mRNA for survivin, cIAP1 and cIAP2 exhibited a remarkable decrease after the development of OL (MDS -> OL). By immunohistochemistry, survivin was found to localize to the nucleus of myeloid cells in the majority of MDS cases. Next, the chronological changes in the expression of IAPs were determined in cases of MDS with evolution of OL. Although the expression of cIAP1 and cIAP2 revealed a sudden or gradual decrease as OL developed, survivin in many cases and XIAP in the majority of cases exhibited a peak of expression before a decline, indicating that these IAPs could be associated with the early events in the development of OL.

Yashima, A., C. Maesawa, et al. (2003). "Quantitative assessment of contaminating tumor cells in autologous peripheral blood stem cells of B-cell non-Hodgkin lymphomas using immunoglobulin heavy chain gene allele-specific oligonucleotide real-time quantitative-polymerase chain reaction." Leukemia Research **27**(10): 925.

<http://www.sciencedirect.com/science/article/B6T98-48JC9WM-2/2/d4faba8ea800b8eb0154204cdbcdf87c>

A real-time quantitative-polymerase chain reaction (RQ-PCR) targeting the immunoglobulin heavy chain (IgH) gene has been used for the quantification of minimal residual disease (MRD) in B-cell hematological malignancies. In non-Hodgkin lymphoma (NHL), experimental costs are increased, as a large number of primer-probe sets are required because of diversity, due to somatic and ongoing mutations of the IgH gene. We developed an allele-specific oligonucleotide (ASO) combined with a germline consensus probe-based RQ-PCR assay and examined MRD in peripheral blood stem cells (PBSC). The IgH consensus probes were adapted in seven (50%) of 14 amplifiable cases. Patients with heavily contaminating tumor cells in PBSC relapsed after PBSC transplantation. Our strategy will contribute to the development of a cost-efficient, precisely quantitative and systemic detection assay for MRD in NHL.

Yokohama, A., M. Karasawa, et al. (2001). "Molecular detection of tumor cells at diagnosis invading the bone marrow and peripheral blood of patients with aggressive or indolent lymphomas." Leukemia Research **25**(9): 749.

<http://www.sciencedirect.com/science/article/B6T98-4599B2H-3/2/a15755c7fc0c884b564b67fcd7c90479>

We studied tumor cell invasions of bone marrow and peripheral blood in patients with various types of advanced non-Hodgkin's lymphoma by amplifying complementarity determining region III using the polymerase chain reaction (PCR) method and developing patient-specific probes. After molecular engineering, we could detect tumor cells in bone marrow from seven of 11 cases and in peripheral blood from six of 11 cases, despite negative results in four cases studied morphologically. Indolent cases were more likely to yield positive results than aggressive cases. The reason may be different biological behaviors among the histological types.

Zhou, R., M. Frostvik Stolt, et al. (2002). "Real-time RT-PCR for the determination of topoisomerase II mRNA level in leukaemic cells." Leukemia Research **26**(5): 487.

<http://www.sciencedirect.com/science/article/B6T98-45CX73K-D/2/628b2ca821241d47ba9852010e2a9eb6>

We developed a real-time RT-PCR assay for the quantification of topoisomerase II (topo II) mRNA level. It was applied on peripheral leukaemic cells from 23 patients with acute myelogenous leukaemia (AML) and 23 with chronic lymphocytic leukaemia (CLL). RNA template dilutions from 0.25 to 25 ng per reaction were used as standard curves for topo II[alpha], [beta] and the internal control 18S rRNA. About 57% (26/46) and 26% (12/46) of the specimens had detectable topo II[beta] and [alpha] mRNA, respectively. The correlation between these two factors was $r=0.7$ and $P=0.0001$. No relationship between topo II[alpha] or [beta] mRNA level and response to chemotherapy was found in AML patients ($n=19$ assessable for response). Our method is rapid and convenient for quantification of topo II[alpha] and [beta] mRNA levels, and could be suitable for investigation in a larger population.

Bogdarina, I., H. C. Murphy, et al. (2004). "Investigation of the role of epigenetic modification of the rat glucokinase gene in fetal programming." Life Sciences **74**(11): 1407.

<http://www.sciencedirect.com/science/article/B6T99-4B42FPW-4/2/a9308159457ab5a29ef64848d6018514>

Fetal malnutrition is associated with development of impaired glucose tolerance, diabetes and hypertension in later life in humans and several mammalian species. The mechanisms that underlie this phenomenon of fetal programming are unknown. We hypothesize that adverse effects in utero and early life may influence the basal expression levels of certain genes such that they are re-set with long-term consequences for the organism. An excellent candidate mechanism for this re-setting process is DNA methylation, since post-natal methylation patterns are largely established in utero. We have sought to test this hypothesis by investigating the glucokinase gene (Gck) in rat offspring programmed using a maternal low protein diet model (MLP). Northern blot reveals that fasting levels of Gck expression are reduced after programming, although this distinction disappears after feeding. Bisulphite sequencing of the hepatic Gck promoter indicates a complete absence of methylation at the 12 CpG sites studied in controls and MLP animals. Non-expressing cardiac tissue also showed no DNA methylation in this region, whereas brain and all fetal tissues were fully methylated. These findings are not consistent with the hypothesis that programming results from differential methylation of Gck. However, it remains possible that programming may influence methylation patterns in Gck at a distance from the promoter, or in genes encoding factors that regulate basal Gck expression.

Caronti, B., C. Calderaro, et al. (1998). "Dopamine receptor mRNAs in the rat lymphocytes." Life Sciences **62**(21): 1919.

<http://www.sciencedirect.com/science/article/B6T99-3T0TY5N-2/2/20f3c6d6de1728310fc1466e4fcd090e>

It has been suggested that dopamine might play a role in the regulation of the immune system. In this study, reverse transcriptase-polymerase chain reaction (RT-PCR) was used to investigate the expression of mRNA for the different subtypes of dopamine receptors in the rat lymphocytes. D1, D3 and D5 receptor mRNAs were identified. These results provide further evidence for the interaction of dopamine systems and the immune system, and suggest to further investigate whether the immunosuppressive actions of dopamine and dopaminergic drugs might depend on a direct interaction with dopamine receptors on the lymphocyte membrane. Moreover, they suggest the suitability of this animal species to further investigate the correlation between changes in the expression of central and peripheral dopamine receptors produced by manipulations of the dopamine systems.

Chang, S. L., J. Bersig, et al. (2000). "Chronic cocaine alters hemodynamics and leukocyte-endothelial interactions in rat mesenteric venules." Life Sciences **66**(24): 2357.

<http://www.sciencedirect.com/science/article/B6T99-40B7XB5-5/2/0e750bce2094b175774c2b5a74ca74aa>

We investigated the effects of chronic cocaine exposure on the microcirculation in the rat mesenteric venules under both non-inflammatory and FMLP-induced inflammatory conditions. Chronic cocaine significantly increased WBC rolling flux in both conditions, and potentiated FMLP-induced leukocyte-endothelial cell adhesion (LEA). In cocaine-treated animals, total WBC number increased by 91%, and the ratio of white blood cell to red blood cell velocity was significantly lower, while vessel diameter was unchanged. Chronic cocaine decreased serum levels of tumor necrosis factor alpha (TNF-[alpha]) and interleukin-6 (IL-6), but had no effect on interleukin-1 beta (IL-1[beta]). Expression of intercellular adhesion molecule-1 (ICAM-1) was increased in mesenteric venules following chronic cocaine exposure, and may be one of the mechanisms underlying enhancement of FMLP-induced LEA. The increase in WBC count, WBC flux and LEA, and the change in cell velocity seen in the cocaine-treated animals could cause a decrease in effective vessel diameter and a change in intravascular resistance, and may underlie the progressive vascular damage seen in chronic cocaine-abusing individuals.

Cushing, D. J., M. Baez, et al. (1994). "Serotonin-induced contraction in canine coronary artery and saphenous vein: Role of a 5-HT_{1D}-like receptor." *Life Sciences* **54**(22): 1671.

<http://www.sciencedirect.com/science/article/B6T99-4751TMH-2NR/2/e8c4e22c9af4e5021bce604d3757f5d3>

The identity of the serotonin (5-HT) receptor(s) that mediate(s) contraction in canine coronary artery and saphenous vein remains controversial. Ring segments of endothelium-denuded coronary artery and helical strips of saphenous vein were suspended in organ chambers for measurement of isometric force. 5-HT, [alpha]Me-5-HT and sumatriptan contracted both coronary artery and saphenous vein and the non-selective 5-HT receptor antagonist 1-naphthylpiperazine (100nM) blocked 5-HT- and sumatriptan-induced contraction in both tissues. The agonist rank order potency for contraction (5-HT>sumatriptan>[alpha]Me-5-HT>5-MeOT>5-MeT) was similar in both tissues and was consistent with that for a 5-HT_{1D} receptor. Oligonucleotide primers specific for the 5-HT_{1D} receptor sequence were designed for use in a polymerase chain reaction (PCR). cDNA derived from total RNA or mRNA from canine tissues was used in the PCR. PCR resulted in the amplification of a 632 base pair sequence in both canine coronary artery and saphenous vein; consistent with that expected for the 5-HT_{1D} receptor. Southern blot analysis, with an oligonucleotide probe internal to the sequence amplified by the PCR primers, confirmed that the sequence amplified by PCR was the 5-HT_{1D} receptor. Thus, the 5-HT_{1D} receptor is expressed in canine coronary artery and saphenous vein and taken together with the pharmacological data, supports the possibility that a 5-HT_{1D}-like receptor mediates contraction in these two tissues.

Farooqui, S. M. (1994). "Induction of adenylate cyclase sensitive dopamine D₂-receptors in retinoic acid induced differentiated human neuroblastoma SHSY-5Y cells." *Life Sciences* **55**(24): 1887.

<http://www.sciencedirect.com/science/article/B6T99-4754KHC-2/2/cdd8ff78ab5e53087ad8ea0f96dcfc3d>

Dopamine D₂ receptor (D₂-receptor) expression and its coupling to Gi sensitive adenylate cyclase was investigated in human neuroblastoma SHSY-5Y cells. Incubation of SHSY-5Y cells in the presence of 100 nM retinoic acid (RA) for 24 hours resulted in phenotypic differentiation accompanied by a 47% increase in D₂-receptor mRNA and a significant increase in the specific binding of a D₂-receptor antagonist, [³H]YM09151-2. Stimulation of D₂-receptors in differentiated

cells by LY171-555, a D2-agonist, attenuated cellular cAMP levels by 30%. The effect of LY171-555 on cAMP levels was blocked by the D2-antagonist, (-)-sulpride. Application of these drugs to control undifferentiated cells or differentiated cells incubated with vehicle only had no effect on cellular cAMP levels. These studies suggest that differentiated SHSY-5Y cells express functional D2-receptors and will provide a useful model for future studies on the regulation of expression and function of D2-receptors in cellular differentiation of neuronal cells.

Fujii, T., T. Okuda, et al. (2003). "Detection of the high-affinity choline transporter in the MOLT-3 human leukemic T-cell line." *Life Sciences* **72**(18-19): 2131.

<http://www.sciencedirect.com/science/article/B6T99-47X0WMJ-1/2/bd4a23563cf1c232fb845e187c9bc057>

We previously showed that lymphocytes possess the necessary components to constitute an independent, non-neuronal cholinergic system; these include acetylcholine (ACh) itself, choline acetyltransferase (the ACh-synthesizing enzyme), and both muscarinic and nicotinic ACh receptors (AChRs). In addition, we showed that stimulation of AChRs with their respective agonists elicits a variety of biochemical and functional effects, suggesting that lymphocytic cholinergic system is involved in the regulation of immune function. In nerve terminals, choline taken up via the high-affinity choline transporter (CHT1) is exclusively utilized for ACh synthesis. In the present study, therefore, we investigated the expression of CHT1 in T-lymphocytes. Reverse transcription-polymerase chain reaction analysis revealed that MOLT-3 cells, a human leukemic T-cell line used as a T-lymphocyte model, expressed CHT1 mRNA, but that the CEM and Jurkat T-cell lines did not. Consistent with that finding, specific binding of [3H]hemicholinium-3 (HC-3), an inhibitor of CHT1, and HC-3-sensitive [3H]choline uptake were also detected in MOLT-3 cells. These results suggest that CHT1 plays a role in mediating choline uptake in T-lymphocytes and provides further evidence for the presence of an independent lymphocytic cholinergic system.

Funaki, N., J. Tanaka, et al. (1995). "Highly-sensitive identification of [alpha]-fetoprotein mRNA in circulating peripheral blood of hepatocellular carcinoma patients." *Life Sciences* **57**(17): 1621.

<http://www.sciencedirect.com/science/article/B6T99-3YYTH72-41/2/8c8e01ec8bc3030febce7238e62e5249>

In order to capture hepatocellular carcinoma (HCC) cells in circulating peripheral blood, we made analysis to see if a -fetoprotein (AFP) mRNA exists in the peripheral blood obtained from patients with HCC and also, as a control, from hepatitis-viral-marker-positive patients without HCC and a healthy volunteer. As the number of HCC cells in peripheral blood and the quantity of AFP mRNA are expected to be very small, the analysis was performed by the reverse transcription followed by an original three-step polymerase chain reaction. By this highly sensitive method, 5 of 7 HCC patients were positive for AFP mRNA. These 5 positive patients consisted of three with clinically apparent recurrence, one preoperative patient with tumor thrombus in the portal vein and one recurrence-free patient who developed clinically detectable recurrence three months after this analysis. Neither 4 patients with positive viral markers nor a healthy volunteer was positive. The results suggest that detection of AFP mRNA from HCC patients' peripheral blood by our highly-sensitive RT-PCR may be a practical and powerful tool to diagnose the preoperative spreading of HCC and to monitor its recurrence.

Funaki, N. O., J. Tanaka, et al. (1998). "Quantitative analysis of alpha-fetoprotein mRNA in circulating peripheral blood of patients with hepatocellular and alpha-fetoprotein-producing gastric carcinomas." Life Sciences **62**(21): 1973.

<http://www.sciencedirect.com/science/article/B6T99-3T0TY5N-9/2/b812097676b1bdb7537297815312ae12>

In conjunction with strategies introduced in recent years to identify cancer micrometastasis through amplification of cancer-associated mRNA, we developed a highly sensitive system to detect [alpha] -fetoprotein mRNA in circulating peripheral blood of hepatocellular carcinoma patients. The aim of the present study was to make our original system quantitative. Peripheral venous blood from patients with hepatocellular carcinoma and [alpha] -fetoprotein-producing gastric carcinoma was subjected to reverse transcription followed by our original three-step polymerase chain reaction co-amplifying both the original sequence and our synthetic competitor. We succeeded in modifying our system for quantitative analysis, and investigated the perioperative change, the postoperative change and the change after chemotherapy in order to illustrate the possible application of this method. The quantitative analysis of [alpha]-fetoprotein mRNA present in the peripheral blood represents a useful tool for analyzing the relationship of surgery to recurrence, the effect of chemotherapy, and to predict impending recurrence in patients with hepatocellular and [alpha]-fetoprotein-producing gastric carcinomas.

Funaki, N. O., J. Tanaka, et al. (1997). "Detection of colorectal carcinoma cells in circulating peripheral blood by reverse transcription-polymerase chain reaction targeting cytokeratin-20 mRNA." Life Sciences **60**(9): 643.

<http://www.sciencedirect.com/science/article/B6T99-3RHNJK5-N/2/02703f4bc1c5fff59b100070ea40087f>

For the detection of circulating colorectal carcinoma cells, we investigated the presence of cytokeratin 20 (CK 20) mRNA in the peripheral blood of colorectal carcinoma patients. Application of our published technique resulted in analysis by reverse transcription followed by three-step nested polymerase chain reaction. This analysis could detect a single Colo 205 colon cancer cell mixed with 1 ml of blood. Our system also successfully detected the presence of CK 20 mRNA in actual patients' peripheral blood samples. Our highly sensitive and specific system for the detection of CK-20 mRNA from patients' peripheral blood thus seems to be useful for screening for circulating colorectal carcinoma cells.

Hattori, M.-a., T. Fujioka, et al. (2002). "Preferential expression of ADP-ribosylation factor gene in the chick embryonic gonads." Life Sciences **70**(17): 2031.

<http://www.sciencedirect.com/science/article/B6T99-459BCHR-1/2/5c86818754f4e6794f8644e39de94a3d>

cDNA cloning from chick embryonic gonads subtracted from tissues of the brain, heart, liver, gizzard, mesonephros and skeletal muscle was performed to identify genes with expression unique to embryonic gonads. Several cDNA clones encoding characterized as well as many uncharacterized genes were obtained. ADP-ribosylation factor (ARF) of these identified genes was preferentially expressed in the chick embryonic ovary and testis as revealed by reverse transcription-polymerase chain reaction analysis. Expression of the ARF was evaluated through embryonic development, but no difference in the transcript (relative to glyceraldehyde-3-phosphate dehydrogenase transcript) was observed between the left and right ovaries, and

between the ovary and testis. In addition, the ARF transcript was detected in the gonads on embryonic days 5 to 21. These findings indicate that the ARF is constantly, but preferentially expressed in the embryonic gonads during development.

Hou, M., M. Malmsjo, et al. (1999). "Increase in cardiac P2X1- and P2Y2-receptor mRNA levels in congestive heart failure." Life Sciences **65**(11): 1195.

<http://www.sciencedirect.com/science/article/B6T99-3X9YSFN-C/2/3370cafd4258c51d9079609e7f1413b8>

We wanted to study the expression of P2-receptors at the mRNA-level in the heart and if it is affected by congestive heart failure (CHF). To quantify the P2 receptor mRNA-expression we used a competitive RT-PCR protocol which is based on an internal RNA standard. The P2 receptor mRNA-expression was quantified in hearts from CHF rats and compared to sham-operated rats. Furthermore, the presence of receptor mRNA was studied in the myocardium from patients with heart failure. In the sham operated rats the G-protein coupled P2Y-receptors were expressed at a higher level than the ligand gated ion-channel receptor (P2X1). Among the P2Y-receptors the P2Y6-receptor was most abundantly expressed (P2Y6 > P2Y1 > P2Y2 = P2Y4 > P2X1). A prominent change was seen for the P2X1- and P2Y2-receptor mRNA levels which were increased 2.7-fold and 4.7-fold respectively in the myocardium from the left ventricle of CHF-rats. In contrast, the P2Y1-, P2Y4- and P2Y6-receptor mRNA levels were not significantly altered in CHF rats. In human myocard the P2X1-, P2Y1-, P2Y2-, P2Y6- and P2Y11-receptors were detected by RT-PCR in both right and left atria and ventricles, while the P2Y4-receptor band was weak or absent. In conclusion, most of the studied P2-receptors were expressed in both rat and human hearts. Furthermore, the P2X1- and P2Y2-receptor mRNA were upregulated in CHF, suggesting a pathophysiological role for these receptors in the development of heart failure.

Inoue, K., S. Koizumi, et al. (2003). "Signaling of ATP receptors in glia-neuron interaction and pain." Life Sciences **74**(2-3): 189.

<http://www.sciencedirect.com/science/article/B6T99-49W2118-2/2/24d745003441ab7ebfb44ddabb096dbb>

ATP causes the activation of p38 or ERK1/2, mitogen activated protein kinases (MAPKs) resulting in the release of tumor necrosis factor-[alpha] (TNF) and Interleukin-6 (IL-6) from microglia. We examined the effect of TNF and IL-6 on the protection from PC12 cell death by serum deprivation. When PC12 cells were incubated with serum-free medium for 32 hr, their viability decreased to 30 %. IL-6 alone slightly protected the death of PC12 cells, whereas TNF alone did not show any protective effect. In the meanwhile, when PC12 cells were pretreated with TNF for 6 hr and then incubated with IL-6 under the condition of serum-free, the viability of PC12 cells dramatically increased. TNF induced an increase of IL-6 receptor (IL-6R) expression in PC12 cells at 4-6 hr. These data suggested that 6 hr pretreatment with TNF increased IL-6R expression in PC12 cells, leading to an enhancement of IL-6-induced neuroprotective action. To elucidate the role of p38 in pathological pain, we investigated whether p38 is activated in the spinal cord of the neuropathic pain model. In the rats displaying a marked allodynia, the level of phospho-p38 was increased in the microglia of injury side in the dorsal horn. Intraspinal administration of p38 inhibitor suppressed the allodynia. These results demonstrate that neuropathic pain hypersensitivity depends upon the activation of p38 signaling pathway in microglia in the dorsal horn following peripheral nerve injury.

Kageyama, H., T. Osaka, et al. (2003). "Fasting increases gene expressions of uncoupling proteins and peroxisome proliferator-activated receptor-[gamma] in brown adipose tissue of ventromedial hypothalamus-lesioned rats." Life Sciences **72**(26): 3035.

<http://www.sciencedirect.com/science/article/B6T99-487F0PJ-2/2/4938700a27ee12f04f24566883c266c9>

Uncoupling proteins (UCPs) are supposed to be involved in diet-induced thermogenesis. Their activities are usually elevated by feeding and reduced by fasting in normal animals. To investigate whether fasting affects the expression of UCPs mRNA in brown adipose tissue (BAT) of bilateral ventromedial hypothalamus (VMH)-lesioned rats, we determined the gene expression of UCP1, UCP2 or UCP3 in BAT of VMH-lesioned rats and examined oxygen consumption in these rats under fed or 48-h fasted conditions. Northern blotting revealed no difference in the expression of UCPs mRNA in BAT between VMH-lesioned and sham-operated rats under the fed condition, however, expressions were increased markedly in BAT of VMH-lesioned rats under the fasted condition. Under the fed condition, no difference in oxygen consumption was observed between VMH-lesioned and sham-operated rats. Under the fasted condition, oxygen consumption decreased in both rats, however, it decreased in VMH-lesioned less than in sham operated rats. To explore the mechanism that fasting elevated BAT UCPs mRNA in VMH-lesioned rats, we measured peroxisome proliferator-activated receptor (PPAR)-[gamma] mRNA and protein in BAT, because PPAR-[gamma] agonist can elevate UCPs mRNA levels in BAT. Under the fed condition, no differences in the expression of PPAR-[gamma] mRNA and protein content were observed between in BAT of VMH-lesioned and sham-operated rats. Under the fasted condition, however, both increased in BAT of VMH-lesioned rats. These results suggest that VMH-lesions enhance the gene expression of UCPs in BAT under long-term fasting as a defensive reaction to inhibit the reduction of body temperature through an increase in PPAR-[gamma] activity.

Katavic, V., D. Grcevic, et al. (2003). "Non-functional Fas ligand increases the formation of cartilage early in the endochondral bone induction by rhBMP-2." Life Sciences **74**(1): 13.

<http://www.sciencedirect.com/science/article/B6T99-49STN7H-3/2/6e893ea6358ff4848b9dad50ce6aacb0>

It has previously been shown that mice with a defect in Fas ligand-mediated apoptosis have an enhancement of ectopic bone formation. We investigated the expression of bone-related markers - alkaline phosphatase, collagen, bone sialoprotein, osteocalcin, osteopontin, and bone morphogenetic proteins (BMP) -2, -4, and -7; and cytokines interleukin-1[alpha] (IL-1), IL-1[beta], and tumor necrosis factor-[alpha] (TNF-[alpha]) in ectopic new bone induced by recombinant human (rh) BMP-2 in mice without functional Fas-ligand (gld mice). At day 6 after rhBMP-2 implantation, gld mice formed more cartilage and mesenchyme compared with their wild type littermates. At later stages, gld mice did not differ from the control mice in the volume of newly formed tissue, expressing higher level of BMP genes and lower levels of genes involved in osteoblast maturation - bone sialoprotein and osteopontin. Differences in the levels of expression of IL-1[alpha] and TNF-[alpha] were observed only at day 12 after rhBMP-2 implantation. These results suggest that gld mice have an increased recruitment of cells of mesenchymal origin and an abnormal pattern of differentiation and maturation of the newly formed mesenchymal tissues.

Kishimoto, Y., K. Wada, et al. (1997). "Quantitative analysis of cyclooxygenase-2 gene expression on acute gastric injury induced by ischemia-reperfusion in rats." Life Sciences **60**(8): PL127.

<http://www.sciencedirect.com/science/article/B6T99-3RHNJK5->

15/2/8852d99180513dfe8666b1461fa88f5b

The rat model of acute gastric damage induced by ischemia-reperfusion (I-R) has been used to evaluate the protective effect of various drugs on gastric injury. However, the quantitative expression state of cyclooxygenase-2 (COX-2), a protein which induces cytoprotective prostaglandins during inflammation, is still unknown in acute gastric injury induced by I-R. Therefore, we have quantitatively investigated the level of expression of COX-2 mRNA in injured gastric tissue of this model using the reverse transcription-competitive polymerase chain reaction method. The mRNA for COX-2 was expressed at low or undetectable levels in the normal gastric tissues in control rats, which were fasted for 18 hrs without I-R. The mRNA levels of COX-2 in injured gastric tissues were higher than those of control tissues between 6 hrs and 48 hrs after I-R. The maximum expression of COX-2 mRNA was recorded at 24 hrs (approximately a 200-fold increase). The expression state of COX-2, which has been ascertained in this study, should be useful in evaluating the effect of various drugs on the expression of COX-2 in acute gastric damage.

Liu, Z.-J., M. Maekawa, et al. (2003). "The multiple promoter methylation profile of PR gene and ER[alpha] gene in tumor cell lines." *Life Sciences* **73**(15): 1963.

<http://www.sciencedirect.com/science/article/B6T99-490H4NG-1/2/792c1e218beb6e76ecf29256ce844f35>

The changes of methylation status of various gene promoters are a common feature of malignant cells and these changes can occur early in the progression process. Therefore, abnormal methylation can be used as cancer marker. Such studies will first require the development of a panel of methylated markers that are methylated in cancer tissues but unmethylated in normal tissues or methylation status is different between cancer tissues and normal tissues. By using methylation-specific PCR (MSP) assay method, we observed alterations in DNA methylation at the double promoter regions of the progesterone receptor (PR) gene and estrogen receptor (ER[alpha]) gene in various tumor cell lines. Compared with normal white blood cell, the methylation status of PRA promoter in various cancer cell lines changed from unmethylation pattern to methylation pattern. That of PRB promoter changed from both unmethylated and methylated alleles to only methylated allele. The methylation status of ER[alpha]-A and ER[alpha]-B promoter in various cancer cell lines are cell -specific. This study indicates that PR promoter methylation may be a molecular marker in various cancer detections. And the methylation status of ER[alpha]-A and ER[alpha]-B is cell-specific.

Mao, T. K., J. Powell, et al. (2000). "The effect of cocoa procyanidins on the transcription and secretion of interleukin 1[beta] in peripheral blood mononuclear cells." *Life Sciences* **66**(15): 1377.

<http://www.sciencedirect.com/science/article/B6T99-3YXB9SD-1/2/71a41c13f5ebaf4156571a6df0ac2a89>

Recent data has demonstrated that cacao liquor polyphenols (procyanidins) have antioxidant activity, inhibit mRNA expression of interleukin-2 and are potent inhibitors of acute inflammation. Given the widespread ingestion of cocoa in many cultures, we investigated whether cocoa, in its isolated procyanidin fractions (monomer through decamer), would modulate synthesis of the pro-inflammatory cytokine, interleukin-1[beta]. Both resting and phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMC) were investigated at the levels of transcription and protein secretion. Individual cocoa fractions were shown to augment constitutive IL-1[beta] gene expression, although values varied between subjects. Interestingly, the smaller fractions of cocoa

(monomer-tetramer) consistently reduced IL-1[β] expression of PHA-stimulated cells by 1-15%, while the larger oligomers (pentamer-decamer) increased expression by 4-52%. These data, observed at the transcription level, were reflected in protein levels in PHA-induced PBMC. The presence or absence of PHA did not alter the effects of the cocoa procyanidins with the exception of the pentamer. This study offers additional data for the consideration of the health-benefits of dietary polyphenols from a wide variety of foods, including those benefits associated specifically with cocoa and chocolate consumption.

Mengheri, E., L. Ciapponi, et al. (1996). "Cytokine gene expression in intestine of rat during the postnatal developmental period: Increased IL-1 expression at weaning." *Life Sciences* **59**(15): 1227.

<http://www.sciencedirect.com/science/article/B6T99-3W2T831-6/2/2f8bf146ca53c502e0d6302462c6e5ac>

In the present study we have investigate whether cytokines are constitutively and differently expressed in intestine during the differentiative processes that take place at weaning. We have analyzed the expression of IL-1[β], IL-2, IL-4 and IFN[γ] by polymerase chain reaction in Peyer's patches (PP) and in intestine deprived of PP (I-PP) of rats from 16 to 30 days of age. The results showed a constitutive and marked expression of the cytokines already before weaning, with the exception of IL-2 in PP and IFN[γ] in I-PP. IL-[β] was the only cytokine to show a different expression at various ages with an initial increase at 19 days and a further elevation at 21 days when intestinal epithelium passes through major differentiative stages, suggesting an involvement of this cytokine in intestinal development. We have also tested whether treatment of rats with the immunosuppressor cyclosporin A (CsA) could affect intestinal differentiation. The results showed that only some markers of differentiation were affected (proliferation of staminal crypt cells and length of crypts). This was probably due to a direct effect rather than an immunomediated effect of CsA, since treatment of three intestinal cell lines (Caco-2, HT-29, FRIC) with CsA indicated that this drug can exert a cytostatic activity on intestinal cells.

Miyamoto, A., Y. Yamazaki, et al. (2003). "Enhancement of endotoxin-induced vascular hyporeactivity to phenylephrine in the thoracic aortas of Mg-deficient rats ex vivo." *Life Sciences* **73**(21): 2713.

<http://www.sciencedirect.com/science/article/B6T99-49H6BGJ-3/2/731d1f2fc76fbed391d32945c0e9ae05>

Since endotoxin lethality is enhanced by Mg deficiency in animals, we determined whether endotoxin-induced vascular hyporeactivity to phenylephrine (PE) is enhanced in Mg-deficient rats. Normal and Mg-deficient adult male Wistar rats were injected with *Escherichia coli* 011: B4 lipopolysaccharide (1 or 5 mg/kg, i.p.). Six h later, rings prepared from their thoracic aortas showed severe hyporeactivity to PE. This was more pronounced in the Mg-deficient rats, and was reversed by in vitro treatment with a highly selective inducible nitric oxide (NO) synthase inhibitor, 1400 W, or a highly selective soluble guanylyl cyclase inhibitor, ODQ. However, reversal required high doses of both inhibitors in Mg-deficient rats. Endotoxemia for 6 h was associated with elevated serum interleukin (IL)-1[β] and tumor necrosis factor (TNF)-[α] levels, and strong TNF receptor mRNA expression in the abdominal aortas, which were significantly greater in the Mg-deficient rats. Treatment of the thoracic aortas, isolated from control and Mg-deficient rats before endotoxic challenge, with IL-1[β] or TNF-[α] for 6 h in vitro caused hyporeactivity to PE, but its severity did not differ significantly between the two groups. These results suggest that high serum IL-1[β] and TNF-[α] levels, and increased TNF receptor production in the vascular tissue, contribute to vascular hyporeactivity to PE in endotoxemia, and to its enhancement in Mg-deficient rats, via NO/cGMP signaling.

Nara, M., T. Kanda, et al. (1999). "Running exercise increases tumor necrosis factor-[alpha] secreting from mesenteric fat in insulin-resistant rats." Life Sciences **65**(3): 237.

<http://www.sciencedirect.com/science/article/B6T99-3X05HTX-11/2/e914656134de1d8140d842a2cce532c8>

Tumor necrosis factor-[alpha] (TNF-[alpha]) is an important mediator of insulin resistance in obese subjects, through its overexpression in fat tissue. However, how exercise can modify the expression of TNF-[alpha] is controversial. We examined TNF-[alpha] in adipose tissue using an animal model of insulin resistance that was produced by feeding rats a diet high in sucrose. The rats were allocated to one of three groups: those receiving a starch-based diet (control group): those fed a high-sucrose diet (sucrose-fed group): and those fed a high-sucrose diet and given wheel exercise (exercised group). The animals were allowed to eat and drink ad lib for 4 or 12 weeks (4 wk: control N = 7, sucrose-fed N = 7, exercised N = 10; 12 wk: control N = 5, sucrose-fed N = 5, exercised N = 9). The voluntary wheel exercise was initiated with the feeding of the high-sucrose diet. The rats in the exercise groups ran 15 +/- 3 km/week. We showed that 12-week voluntary running exercise significantly (P Eq/L vs 141 +/- 11 Eq/L) and free fatty acid (0.98 +/- 0.07 Eq/L vs 1.4 +/- 0.05 mEq/L) concentrating in portal vein blood were reduced compared to sucrose-fed group. The amounts of fatty tissue both in mesenteric and subcutaneous tissues were significantly (P < 0.05) decreased through running exercise. We consider that up-regulation of TNF-[alpha] in mesenteric fat may be a compensatory mechanism for the reduction of fatty acid in adipose tissues and this change could control metabolic homeostasis during exercise to modulate a hyperinsulinemic state.

Ohashi, S.-i., H. Kaji, et al. (1995). "Effect of fasting and growth hormone (GH) administration on gh receptor (GHR) messenger ribonucleic acid (mRNA) and GH-binding protein (GHBP) mRNA levels in male rats." Life Sciences **57**(18): 1655.

<http://www.sciencedirect.com/science/article/B6T99-3YYTH6C-3F/2/edc23366ae0c793fbaa6989260c5f20c>

To elucidate whether GHR and GHBP are coordinately regulated or not, we studied the effect of fasting with or without GH administration on the GHR and GHBP mRNAs in the liver as well as in extrahepatic tissues in rats. Tissues were collected from 7-week-old male rats by decapitation 1, 3, and 7 days after the start of fasting. Liver GHR mRNA levels were not affected 1 day after the start of fasting but progressively decreased for the subsequent 3 and 7 days of fasting as compared with those in control rats fed ad libitum. In contrast, liver GHBP mRNA levels significantly rose after 1 day fasting, returned to the control level after 3 days and further reduced after 7 days of fasting. Changes in GHBP mRNA level after fasting were different among the tissues. A transient increase in GHBP mRNA levels was observed in muscle and heart as well as liver, while the GHBP mRNA levels in fat tissues did not change throughout 7 days of fasting. Next, bovine GH(bGH) was administered ip to the fasted rats and control fed rats for either 1 day(100mg, tid) or 5 days(150mg, daily). In fed rats, liver GHR mRNA level was significantly increased by 1 day bGH treatment, but after 5 days treatment with bGH it was not different from the level in saline-injected control. Accordingly, net increment of plasma IGF-I was 296.0 ng/ml with 1 day bGH treatment and 234.2 rig/ml with bGH administration for 5 days. In fasted rats, liver GHR mRNA level did not changed after 1 day treatment with bGH, but markedly decreased 5 days after bGH administration. Net increment of plasma IGF-I was slightly reduced to 284 ng/ml with 1 day treatment with bGH, and markedly decreased to 37.0 with bGH administration for 5 days. The effect of GH administration on liver GHBP mRNA level was virtually absent in either fasting or fed state. These findings suggest that GHR and GHBP mRNAs in the liver are expressed in different ways and that expression of GHBP mRNA is differently regulated among

tissues.

Pietruck, C., G.-X. Xie, et al. (1999). "Alternative exon splicing of cyclic AMP response element-binding protein in peripheral sensory and sympathetic ganglia of the rat." Life Sciences **65**(21): 2205.

<http://www.sciencedirect.com/science/article/B6T99-3XX6KNH-5/2/e72d01f6881ea6e50ce27b975671c1d8>

Alternative splicing patterns of cyclic AMP response element-binding protein (CREB) in dorsal root ganglia, lumbar sympathetic ganglia and several peripheral tissues of the rat have been investigated by an exon-flanking polymerase chain reaction strategy. A series of RT-PCR with primer pairs flanking all possible alternative splicing sites (corresponding to a genomic region with at least one full exon and two flanking introns) has revealed multiple tissue specific splice variants. These include some novel transcripts that lack the phosphorylation site and part of the leucine zipper region which is crucial for dimerization and DNA binding. Some isoforms previously reported as testis-specific were also detected in rat peripheral ganglia and other tissues. Notably, splicing patterns are specific for some regions. Some of the splice variants indicate inhibitory functions due to lacking phosphorylation sites or partially missing DNA-binding or leucine zipper domains. These findings suggest a complex expression and functional regulation of CREB in peripheral tissues including dorsal root and sympathetic ganglia.

Sango, K., H. Horie, et al. (2002). "Diabetes is not a potent inducer of neuronal cell death in mouse sensory ganglia, but it enhances neurite regeneration in vitro." Life Sciences **71**(20): 2351.

<http://www.sciencedirect.com/science/article/B6T99-46R0BWM-4/2/f467496d5906f8373aff8b0e50f81e08>

We examined the effects of diabetes on the morphological features and regenerative capabilities of adult mouse nodose ganglia (NG) and dorsal root ganglia (DRG). By light and electron microscopy, no apoptotic cell death was detected in the ganglia obtained from either streptozotocin (STZ)-induced diabetic or normal C57BL/6J mice in vivo. Neurite regeneration from transected nerve terminals of NG and DRG explants in culture at normal (10 mM) and high (30 mM) glucose concentrations was significantly enhanced in the diabetic mice. Chromatolytic changes (i.e. swelling and migration of the nucleus to an eccentric position in the neurons, and a loss of Nissl substance in the neuronal perikarya) and apoptotic cell death (less than one-fifth of the neurons) in the cultured ganglia were present, but neither hyperglycemia in vivo nor high glucose conditions in vitro altered the morphological features of the ganglia or the ratios of apoptotic cells at 3 days in culture. By semiquantitative RT-PCR analysis, the mRNA expressions of ciliary neurotrophic factor (CNTF) in DRG from both mice were down-regulated at 1 day in culture. The expression in diabetic DRG, but not in control DRG, was significantly up-regulated at later stages (3 and 7 days) in culture. In summary, hyperglycemia is unlikely to induce cell death in the sensory ganglia, but enhances the regenerative capability of vagal and spinal sensory nerves in vitro. The up-regulation of CNTF mRNA expression during the culture of diabetic DRG may play a role in the enhanced neurite regeneration.

Takahata, T., N. Yasui-Furukori, et al. (2003). "Nucleotide changes in the translated region of SCN5A from Japanese patients with Brugada syndrome and control subjects." Life Sciences **72**(21): 2391.

<http://www.sciencedirect.com/science/article/B6T99-481N39K-4/2/4e19138efc777137fcbd5aa218911e2c>

The mutations of the SCN5A gene have been implicated to play a pathogenetic role in Brugada syndrome, which causes ventricular fibrillation. To determine the Brugada-associated mutations in Japanese patients, facilitate pre-symptomatic diagnosis, and allow genotype-phenotype studies, we screened unrelated patients with Brugada syndrome for mutations. DNAs from 6 Japanese patients were obtained and the sequence in the translated region of SCN5A was determined. We could not find the mutations reported previously, but found 17 sites of nucleotide change, consisting of 7 synonymous and 10 non-synonymous nucleotide changes in our patients. Among them, two non-synonymous nucleotide changes (G1663A and G5227A) are specific to our patients and these changes were not found in 53 healthy controls. In 4 patients out of 6, no specific nucleotide change for Brugada syndrome could be detected. Our findings demonstrating no patient-specific change in the translated region of the SCN5A gene among two thirds of the small number of patients examined here imply that another gene other than the SCN5A may be associated with this disease, supporting previous investigations in Japan and other countries.

Takase, S., K. Suruga, et al. (1995). "Relationship between perinatal appearance of cellular retinol-binding protein, type II and retinal reductase activity in chick liver." *Life Sciences* **58**(2): 135.

<http://www.sciencedirect.com/science/article/B6T99-3VX9XS9-J/2/9ab373902d2a0d9bb11ee3e3eb6587ee>

To explore a role of the transiently appearing cellular retinol-binding protein, type II (CRBP(II)) in perinatal chick liver, we have examined whether the relationships exist among the perinatal changes in hepatic CRBP(II) protein and mRNA levels, retinal reductase activity and [beta]-carotene levels in liver and serum. Northern blot analysis for hepatic CRBP(II) revealed a transient expression of CRBP(II) mRNA around hatching. The protein of CRBP(II) was also expressed transiently and the highest levels of CRBP(II) were found in the livers 1-3 days after birth. The retinal reductase activity was very low at embryonic age, but its activity rapidly rose at hatching, peaking at 1 day after birth, followed by a gradual decrease to a lower level in 7-day-old chicks. This perinatal pattern of the retinal reductase activities was similar to the pattern of transient appearance of the hepatic CRBP(II), and was also paralleled to the developmental changes in serum and liver [beta]-carotene concentrations. These findings suggest that hepatic CRBP(II) transiently appearing during the perinatal period may involve in metabolizing hepatic [beta]-carotene, directing the retinal to the retinal reductase and leading further to the subsequent esterification of the converted retinol.

Tsukiji, J., K. Sango, et al. (2004). "Long-term induction of [beta]-CGRP mRNA in rat lungs by allergic inflammation." *Life Sciences* **76**(2): 163.

<http://www.sciencedirect.com/science/article/B6T99-4DN8G64-2/2/01b21ab2616ec51174cf28114fcd3aef>

Calcitonin gene-related peptide (CGRP) is one of the major neuropeptides released from sensory nerve endings and neuroendocrine cells of the lung. Two CGRP isoforms, [alpha]- and [beta]-CGRP, have been identified in rats and humans, but no studies have attempted to reveal direct evidence of differences in action or location of these isoforms in allergic inflammation (AI). We investigated mRNA expressions of [alpha]- and [beta]-CGRP in lungs, nodose ganglia (NG), and dorsal root ganglia (DRG) of an animal model for AI of the airways, utilizing a model created by sensitizing Brown Norway (BN) rats with ovalbumin (OVA). By semiquantitative RT-PCR analysis,

long-lasting enhanced expression of the [beta]-CGRP mRNA was shown in the lungs of the AI rats (14.5-fold enhancement at 6 hr, 8.1-fold at 24 hr, and 3.7-fold at 120 hr after OVA-challenge compared to the level in the lungs of phosphate-buffered saline (PBS)-challenged control rats). In contrast, the mRNA expression of the [alpha]-CGRP in AI lungs showed only a transient increase after OVA-challenge (2.7-fold at 6 hr) followed by a lower level of expression (0.5-fold at 48 hr and 0.6-fold at 120 hr). The mRNA expressions of both isoforms in NG, but not in DRG, were transiently up-regulated at 6 hr after antigen challenge. In situ RT-PCR in combination with immunohistochemical analysis revealed that [beta]-CGRP was expressed in neuroendocrine cells in clusters (termed neuroepithelial bodies [NEBs]) in AI lungs. These results indicate that the long-term induction of [beta]-CGRP in NEBs may play an important role in pulmonary AI such as bronchial asthma.

Vrana, K. E., P. J. Rucker, et al. (1994). "Recombinant rabbit tryptophan hydroxylase is a substrate for cAMP-dependent protein kinase." *Life Sciences* **55**(13): 1045.

<http://www.sciencedirect.com/science/article/B6T99-47548Y8-J9/2/dc9a0f329fed636149e1588fa867f2a6>

A full-length cDNA clone for rabbit tryptophan hydroxylase (TPH) was modified and subcloned into a bacterial expression vector. Expression of this gene in the protease-deficient strain of bacteria, BL21[DE3], produced TPH immunoreactive protein which exhibited enzyme activity. Treatment of the recombinant enzyme (in bacterial extracts) with the purified catalytic subunit of the cAMP-dependent protein kinase and [γ -³²P]-ATP resulted in specific phosphorylation of TPH. This expression system provides a means of generating and purifying large amounts of this important enzyme. Moreover, these experiments establish that TPH will serve as an in vitro substrate for cAMP-dependent protein kinase.

Xie, G.-X., T. Meuser, et al. (1999). "Presence of opioid receptor-like (ORL1) receptor mRNA splice variants in peripheral sensory and sympathetic neuronal ganglia." *Life Sciences* **64**(22): 2029.

<http://www.sciencedirect.com/science/article/B6T99-3WWTD97-7/2/acfaf4d19afdbd2d008092822c9c1353>

The expression of ORL1 receptor mRNA splice variants is determined in peripheral sensory and sympathetic ganglia and compared to mRNA expression for the three classic opioid receptor subtypes (mu, delta, and kappa) using the method of reverse transcription-polymerase chain reaction. ORL1, mu, delta and kappa receptor subtype mRNAs are present in human dorsal root ganglia (DRG) and trigeminal ganglia and rat DRG. ORL1, mu and delta receptor subtype mRNAs are present in rat superior cervical ganglia and only ORL1 and delta receptor mRNAs are present in rat lumbar sympathetic ganglia. Both the ORL1 mRNA splice variants are present in sensory and sympathetic ganglia, however, expression of the shorter ORL1 receptor mRNA dominates over expression of the longer splice variant in rat brain and DRG, whereas, expression of the longer splice variant is dominant in sympathetic ganglia.

Xu, Z., B. Dai, et al. (1994). "Gastrin gene expression in human colon cancer cells measured by a simple competitive PCR method." *Life Sciences* **54**(10): 671.

<http://www.sciencedirect.com/science/article/B6T99-474YKY4-1DP/2/2be6dbe850711eed068bb3dbae6a7f12>

Gastrin is mitogenic for several colon cancers and is postulated as an autocrine growth factor for colon cancer cells. In the present study we report the development of a simple competitive polymerase chain reaction (PCR) method for measuring relative abundance of gastrin gene expression in colon cancer cells. Primers flanking exons 2 and 3 of the gastrin gene were utilized for co-amplification of cDNA and genomic DNA. The amplification of genomic DNA was distinguished from that of cDNA by the presence of the 130 bp intron sequence which was resolved by electrophoresis on agarose gels. A standard reaction of competitive PCR, using known concentrations of genomic DNA and cDNA, was first established. The steady state levels of gastrin mRNA were next quantitated in three human colon cancer cell lines (HCT-116, Colo-205 and DLD-1) by competitive PCR. Gastrin mRNA levels in these cell lines ranged from ~0.1 to 1.0 fmoles/mg total RNA (~2-25 copies of gastrin mRNA per cell). This low to moderate levels of gastrin were expressed by human colon cancer cell lines which may function as autocrine growth factors for colon cancers.

Yamamoto, J., K. Ihara, et al. (2004). "Characteristic expression of aryl hydrocarbon receptor repressor gene in human tissues: Organ-specific distribution and variable induction patterns in mononuclear cells." *Life Sciences* **74**(8): 1039.

<http://www.sciencedirect.com/science/article/B6T99-4B0T00G-3/2/532bf5586dc34fd737653c127c049dac>

To investigate the expression of aryl hydrocarbon receptor repressor (AhRR) and related molecules in various tissues and the effects of aromatic hydrocarbons (AHs) on their expression, we developed a reliable technique of quantification of human AhRR as well as aryl hydrocarbon receptor (AhR), AhR nuclear translocator (ARNT) and cytochrome P450 1A1 (CYP1A1) mRNA by real-time TaqMan PCR method. First, we examined the expression of these genes in human adult or fetal tissues. The levels of AhRR expression were extremely high in testis, very high in lung, ovary, spleen and pancreas from adults, whereas those were low in those from fetuses. On the other hand, CYP1A1 expression was extremely high in lung, and AhR and ARNT were ubiquitously expressed in almost all tissues. Second, we compared the expression levels of these genes in mononuclear cells (MNCs) from various sources. Comparison of the basal expression levels of these genes in MNCs demonstrated that MNCs from umbilical cord blood showed higher AhRR or CYP1A1 expression than those from adults. The induction of AhRR or CYP1A1 expression by 3-methylcholanthrene (3-MC) was observed in MNCs from adults but not from umbilical cord blood. Consequently, there existed characteristic differences in the basal levels of AhRR and CYP1A1 expression in MNCs, as well as in their inducibility by 3-MC among MNCs from various types of human bloods. These results will provide basic information for a possible application of AhRR and CYP1A1 measurements to evaluate AH exposure in vivo.

Ying, S.-Y., S.-Q. Li, et al. (1995). "Expression and localization of inhibin [alpha]-subunit in rat retinal photoreceptor cells." *Life Sciences* **57**(1): 45.

<http://www.sciencedirect.com/science/article/B6T99-3YYTHNJ-BP/2/53a8f632190c92b468921a2ecb1717b1>

To determine whether rat retinal photoreceptor cells produce inhibin, a molecule closely related to activin, a multifunctional growth factor in the transforming growth factor [beta] superfamily (TGF[beta]), we have conducted immunohistochemistry using specific antibodies for inhibin which were raised against a synthetic N-terminal fragment of the [alpha]-subunit of inhibin. The mature inhibin molecule was identified at both the inner and outer segments of photoreceptor cells. To determine if messenger RNA for the [alpha]-subunit of inhibin is expressed in the retinal cells, both in situ hybridization with a specific probe and the reverse transcription-polymerase chain

reaction (RT-PCR) technique with primers specific for the [alpha]-subunit of inhibin were used. Messenger RNA expression of the [alpha]-subunit of inhibin was detected by RT-PCR and localized in the photoreceptor cells as determined by in situ hybridization. In addition, the identity of the cDNA product of RT-PCR was verified with Southern analysis and DNA sequencing. The localization of mature inhibin protein and its corresponding message to photoreceptor cells suggest that inhibin may have a paracrine function in the retina, perhaps in the photoreceptor cells themselves.

Lung Cancer (15)

Breuer, R. H. J., P. J. F. Snijders, et al. "Expression of the p16INK4a gene product, methylation of the p16INK4a promoter region and expression of the polycomb-group gene BMI-1 in squamous cell lung carcinoma and premalignant endobronchial lesions." *Lung Cancer In Press, Corrected Proof* <http://www.sciencedirect.com/science/article/B6T9C-4FCG28Y-3/2/de413e8a15c8585ba1ca3ef9e593ab31>

SummaryIt is generally assumed that squamous cell carcinoma develops in a stepwise manner from normal bronchial epithelium towards cancer by the accumulation of (epi)genetic alterations. Several mechanisms including mutations and homozygous deletions or hypermethylation of the p16INK4a promoter region can cause loss of p16 expression. Recent studies suggest overexpression of the polycomb-group gene BMI-1 might also down-regulate p16 expression. In this study, we analyzed the p16 expression in relation to the methylation status of the p16 promoter region of the p16INK4a gene and the expression of BMI-1 in bronchial squamous cell carcinomas (SCC) and its premalignant lesions. Nine (69%) SCC showed loss of p16 expression and 10 (77%) showed expression of BMI-1. Of four p16 positive samples two (50%) were BMI-1 positive, whereas among nine p16 negative samples, eight (89%) revealed BMI-1 staining. Four (44%) p16 negative samples were hypermethylated at the p16INK4a promoter region; the other p16 negative tumors that showed no hypermethylation revealed BMI-1 staining. Only two premalignant lesions showed absence of p16 expression, of which one (carcinoma in situ) was hypermethylated at the p16INK4a promoter region and the other (severe dysplasia) showed BMI-1 expression. In total, 11 precursor lesions (48%) revealed BMI-1 expression. In conclusion, the results of this study suggest that loss of p16 expression by promoter hypermethylation is inconsistently and occurs late in the carcinogenic process at the level of severe dysplasia. To what extent overexpression of the polycomb-group protein BMI-1 attributes to down regulating of p16 expression remains unclear.

Gao, W.-M., H. H. Mady, et al. (2003). "Comparison of p53 mutations between adenocarcinoma and squamous cell carcinoma of the lung: unique spectra involving G to A transitions and G to T transversions in both histologic types." *Lung Cancer* **40**(2): 141.

<http://www.sciencedirect.com/science/article/B6T9C-480CRM1-2/2/cf958b9032120f2627c75f528fb15014>

The p53 gene is frequently mutated in lung tumors, and mutations may be caused by both polycyclic aromatic hydrocarbons (PAHs) and nitrosamines found in tobacco smoke. The two major forms of lung cancer, adenocarcinoma (AC) and squamous cell carcinoma (SCC), are known to differ in the proportion of tumors exhibiting p53 mutation, and may also differ in the

mutational spectra produced. Previous studies comparing p53 mutational spectra between AC and SCC of the lung have been limited by small sample size. We examined p53 mutations in exons 5-8 in 202 cases of AC and 82 cases of SCC from smoking lung cancer patients in the Western Pennsylvania region. The percent of cases with p53 mutation was significantly lower in ACs (40/202, 20%) compared to SCCs (29/82, 35%, $P=0.006$). The proportion of mutations present that were G to T transversions was not significantly different between the two tumor types (52% of p53 mutations in AC compared to 32% in SCC). G to A transitions either did not differ in frequency in the two types of lung cancer (20% of mutations in AC and 24% of mutations in SCC). A distinct spectrum was observed, however, in the p53 mutation pattern in the two types of lung cancer. ACs showed a strong preference for a mutational hotspot at codons 248 and 249, while squamous cell tumors showed mutational events spread throughout exons 5-8, with a preference for codon 267. Mutations at codon 267 in SCC were all C to T transitions that occurred at CpG sites. Both tumor types demonstrated preferential mutation of the non-transcribed strand (100% of all G to T transversions and 55% of the G to A transitions). These results suggest that p53 mutations in both types of lung tumors may arise from adduction by both PAHs and nitrosamines. Mutations arising in ACs appear selectively in regions of p53 that produce more rigid proteins, suggesting a drastic change in p53 function is needed to result in ACs, while less constrained changes in p53 function can result in SCCs. Mutation in p53 was not found to be related to patient survival in this group of patients, while tumor size and degree of differentiation were poor survival predictors.

Gessner, C., H. Kuhn, et al. (2004). "Detection of p53 gene mutations in exhaled breath condensate of non-small cell lung cancer patients." *Lung Cancer* **43**(2): 215.

<http://www.sciencedirect.com/science/article/B6T9C-4B4VPNH-2/2/92db0969ccb19672e3d02e5b779c1463>

Early diagnosis of lung carcinoma is greatly desired. A potential source of early information regarding the process of cancerisation in the airways is exhaled breath condensate (EBC). The direct approach to detecting cancerisation is examining DNA from the area of chronic damage, i.e. airways and lung parenchyma. We therefore investigated DNA in EBC of patients with NSCLC and healthy volunteers. Human DNA was amplified by PCR in exhaled breath condensate and used to detect p53 mutations. A PCR of the [beta]-actin gene fragment was used to detect human DNA in each of the EBC samples. In 65.7% of the samples, the [beta]-actin gene was found. Extracted DNA as well as native EBC were equally suited as starting material for amplification. Mutations of the p53 gene were investigated in all EBC samples of NSCLC patients. p53 exons 5-8 were amplified using nested PCR and subsequently sequenced. Mutations were found in four of the patients ($n=11$; 36.4%) while no mutation was found in volunteers ($n=10$). Mutations detected in EBC were also compared with those of corresponding tumor tissue. Different point mutations in EBC and tumor tissue were revealed in all cases. Our findings demonstrate that exhaled breath condensate may be used for analysis of somatic gene mutations in an area of direct tobacco-related DNA damage.

Gotoh, K., Y. Yatabe, et al. (1998). "Frequency of MAGE-3 gene expression in HLA-A2 positive patients with non-small cell lung cancer." *Lung Cancer* **20**(2): 117.

<http://www.sciencedirect.com/science/article/B6T9C-3VCKRM0-6/2/2eaa0a27e5a02d1bf2f81b7179db520e>

Melanoma tumor antigens, MAGE-1 and -3 are presented on HLA-A1 and -Cw*1601, or -A1 and -A2, respectively, to the corresponding cytotoxic T lymphocytes (CTL). If CTL recognizing these antigens were generated in patients, clones of positive tumor cells should be eliminated. To

ascertain whether such an immunological response is active in patients with lung cancer and to determine what fraction of lung cancer patients are candidates for MAGE oriented immunotherapy, we assessed the relationship between HLA-A1 or -A2 expression and MAGE-1 or -3 gene expression in their tumors. MAGE-1 and -3 were detected in 18/55 (33%) and 23/55 (42%), respectively, by reverse transcriptase (RT)-polymerase chain reaction (PCR). Allele specific PCR revealed HLA-A1 and -A2 alleles to be expressed in 0/55 (0%) and 22/55 (40%) of our cohort, respectively. Among the 22 patients with HLA-A2 genotype, expression of HLA class I antigens detectable by immunohistochemistry was lost in five (23%) cases. The frequency of MAGE-3 expression in HLA-A2 patients was 5/17 (29%), somewhat lower than that of patients without HLA-A2 expression, 18/38 (47%), although the difference was not statistically significant ($P=0.17$). Neither was there a significant association between HLA-A2/MAGE-3 co-expression and survival ($P=0.15$, logrank test). We conclude that there is no clear evidence for elimination of lung cancers co-expressing HLA-A2 and MAGE-3 in vivo. Approximately 10% (5/55) of Japanese lung cancer patients are potential candidates for MAGE-3-based immunotherapy.

Junker, N., J. S. Johansen, et al. "Expression of YKL-40 by peritumoral macrophages in human small cell lung cancer." Lung Cancer **In Press, Corrected Proof**

<http://www.sciencedirect.com/science/article/B6T9C-4F6MDHH-2/2/be2ce66bcd2f12a7a0217340b75d56b>

Summary YKL-40 is a 40 kDa protein with possible involvement in tissue remodeling, cell proliferation and angiogenesis. Elevated serum YKL-40 levels in patients with metastatic cancers (including small cell lung cancer (SCLC)) are associated with poor prognosis. The aim of this study was to identify the cellular source of YKL-40 in SCLC patient biopsies and in a panel of 20 human SCLC lines cultured in vitro and in vivo in nude mice. In general, the SCLC cell lines had no or very limited (human) YKL-40 expression, whereas, by RT-PCR a pronounced murine (i.e., stromal) YKL-40 expression was present in all tumors. YKL-40 mRNA transcripts were detected by in situ hybridization in 9 of 10 biopsies from SCLC patients, and in each case the signal was localized in the peritumoral stroma in cells of typical macrophage morphology (confirmed by a CD68 macrophage specific stain). No YKL-40 mRNA expression was found in the cancer cells, in macrophages infiltrating the solid tumor areas, or in non-malignant tissue. In conclusion, the predominant source of elevated serum YKL-40 in SCLC is peritumoral macrophages.

Kawai, H., K. Kiura, et al. (2002). "Characterization of non-small-cell lung cancer cell lines established before and after chemotherapy." Lung Cancer **35**(3): 305.

<http://www.sciencedirect.com/science/article/B6T9C-453598H-6/2/281ca568028ee048134c6feb3c89922>

We established several in vitro drug-resistant cell lines after continuous, long-term exposure of each drug to elucidate mechanisms of drug resistance. Whether drug resistance in these in vitro resistant cell lines reflects clinical drug resistance still remains unanswered. In this study, a pair of lung cancer cell lines was established from one patient with squamous cell carcinoma of the lung, with one line being established before and one line after combination chemotherapy (cisplatin/ifosfamide/vindesine). Combination chemotherapy selected resistant EBC-2/R cells, which showed cross-resistance to 4-hydroxyifosfamide (3.2-fold), cisplatin (2.3-fold), and methotrexate (3.7-fold) and collateral sensitivity to vindesine (0.77-fold) compared with parent EBC-2 cells. EBC-2/R cells showed decrease in intracellular accumulation of cisplatin, increase in intracellular concentration of glutathione (GSH), and overexpression of multidrug resistance-associated protein (MRP) 3 when compared with EBC-2 cells. A single cycle of chemotherapy was not sufficient to select other mechanisms of drug resistance, such as multidrug resistance-1/P-glycoprotein, MRPs 1, 2, 4, and 5, lung resistance-related protein, metallothionein IIa,

glutathione S-transferase [pi], [gamma]-glutamylcysteine synthetase (light and heavy chain), and excision repair cross complementing 1. Sequentially we established two cell lines, which cell lines showed the differences of the cisplatin resistance, expression level of MRP3, intracellular GSH level and intracellular accumulation of cisplatin. A pair of cell lines will be useful to elucidate resistant mechanisms of cisplatin in heterogeneous lung cancer cells.

Koty, P. P., H. Zhang, et al. (1999). "Antisense bcl-2 treatment increases programmed cell death in non-small cell lung cancer cell lines." Lung Cancer **23**(2): 115.

<http://www.sciencedirect.com/science/article/B6T9C-3W19GP9-2/2/467e50c723c5e3b4732a532ffa01b9e6>

Programmed cell death (PCD) is a genetically regulated pathway that is altered in many cancers. This process is, in part, regulated by the ratio of PCD inducers (Bax) or inhibitors (Bcl-2). An abnormally high ratio of Bcl-2 to Bax prevents PCD, thus contributing to resistance to chemotherapeutic agents, many of which are capable of inducing PCD. Non-small cell lung cancer (NSCLC) cells demonstrate resistance to these PCD-inducing agents. If Bcl-2 prevents NSCLC cells from entering the PCD pathway, then reducing the amount of endogenous Bcl-2 product may allow these cells to spontaneously enter the PCD pathway. Our purpose was to determine the effects of bcl-2 antisense treatment on the levels of programmed cell death in NSCLC cells. First, we determined whether bcl-2 and bax mRNA were expressed in three morphologically distinct NSCLC cell lines: NCI-H226 (squamous), NCI-H358 (adenocarcinoma), and NCI-H596 (adenosquamous). Cells were then exposed to synthetic antisense bcl-2 oligonucleotide treatment, after which programmed cell death was determined, as evidenced by DNA fragmentation. Bcl-2 protein expression was detected immunohistochemically. All three NSCLC cell lines expressed both bcl-2 and bax mRNA and had functional PCD pathways. Synthetic antisense bcl-2 oligonucleotide treatment resulted in decreased Bcl-2 levels, reduced cell proliferation, decreased cell viability, and increased levels of spontaneous PCD. This represents the first evidence that decreasing Bcl-2 in three morphologically distinct NSCLC cell lines allows the cells to spontaneously enter a PCD pathway. It also indicates the potential therapeutic use of antisense bcl-2 in the treatment of NSCLC.

Merrick, D. T., J. Haney, et al. (2005). "Overexpression of vascular endothelial growth factor and its receptors in bronchial dysplasia demonstrated by quantitative RT-PCR analysis." Lung Cancer **48**(1): 31.

<http://www.sciencedirect.com/science/article/B6T9C-4DYW52N-1/2/56eba500fd3aabbf03160f605bf42fb84>

SummaryNeoangiogenesis is required for the growth of invasive lung carcinoma, however, the role of angiogenesis in the progression of premalignant changes to carcinoma of the lung is less clear. We have evaluated vascular endothelial growth factor (VEGF) expression and microvessel densities (MVDs) in 62 bronchoscopic biopsies from normal, reactive (basal cell hyperplasia (BCH)) and dysplastic bronchial epithelium and in tissue from twenty-seven invasive lung carcinomas in an effort to demonstrate angiogenic activity in these preneoplastic lesions and determine whether it is associated with increased bronchial epithelial VEGF expression. MVDs and VEGF RNA expression measured by quantitative RT-PCR were found to be elevated in comparison to normal bronchial tissue in bronchial dysplasias and invasive lung carcinomas but not in basal cell hyperplasias. Immunohistochemical (IHC) analyses revealed that expression of VEGF arose predominantly from bronchial epithelium. ELISA analysis of lung tumor tissue showed that elevated VEGF protein expression correlated with VEGF RNA levels ($r = 0.59$, $p = 0.004$). Increased expression of VEGF RNA was also found in histologically normal bronchial

mucosa from patients with either dysplasia at other sites or a history of heavy tobacco use suggesting a possible field effect in regard to the elaboration of VEGF. Furthermore, analysis of VEGF isoforms and VEGF receptors by semi-quantitative RT-PCR in dysplastic and invasive lesions revealed characteristic altered patterns of expression in dysplasia and early cancer as compared to normal tissue. These results indicate that angiogenesis develops early in lung carcinogenesis and is associated with overexpression of VEGF.

Oyanagi, H., K. Takenaka, et al. (2004). "Expression of LUN gene that encodes a novel RING finger protein is correlated with development and progression of non-small cell lung cancer." Lung Cancer **46**(1): 21.

<http://www.sciencedirect.com/science/article/B6T9C-4CGNT0T-1/2/777a75c799a0ee1355c8e552e63b0637>

LUN is a novel RING finger protein that is highly expressed in the lung and might be a transcriptional regulator of E-cadherin [J. Biol. Chem. 276 (2001) 14004]. It might be possible that LUN plays important roles in the development and progression of lung cancer through regulating expression of E-cadherin, but no clinical study on LUN expression has been reported. In the present study, we quantitatively examined gene expression of the LUN in surgical specimens resected from non-small cell lung cancer (NSCLC) patients. In normal lung tissues, the LUN gene expression was down-regulated in smokers (the mean LUN/GAPDH ratios, 0.222 for non-smokers and 0.144 for smokers; $P=0.030$). In addition, the mean LUN/GAPDH ratio in lung cancer tissues was significantly lower than that in normal lung tissues (0.072 versus 0.162; PLUN gene expression was slightly down-regulated along with progression of primary tumors, and strongly down-regulated along with nodal metastases (the mean LUN/GAPDH ratios, 0.091 for pN0, 0.073 for pN1, and 0.034 for pN2 diseases; $P=0.001$). These results suggested that LUN might play important roles in inhibition of nodal metastases as well as in suppression of smoking-related oncogenesis in NSCLC.

Seike, M., A. Gemma, et al. (2002). "The promoter region of the human BUBR1 gene and its expression analysis in lung cancer." Lung Cancer **38**(3): 229.

<http://www.sciencedirect.com/science/article/B6T9C-46WWBBY-B/2/96b7574d05564c6148d6af763269b85b>

Mitotic checkpoint impairment is present in human lung cancers with chromosomal instability (CIN). Spindle-checkpoint genes have been reported to be mutated in several human cancers, but these mutations are infrequent. Recent reports suggest that the hBUBR1 gene may play an important role in mitotic checkpoint control and in mitotic checkpoint impairment in human cancers. We analyzed the expression of hBUBR1 in lung cancer cell lines using real time quantitative RT-PCR. The expression of BUBR1 was found to be up-regulated in all of these cell lines. In addition, we cloned and characterized the promoter region of hBUBR1 and determined its genomic structure, which includes 23 exons. The open reading frame (ORF) of the hBUBR1 gene comprises exons 1 through 23. There are GC-rich regions located at the flanking region and about 150 bp upstream from exon 1. The promoter region (424 bp upstream from exon 1) showed promoter activity and includes multiple transcription factor consensus binding motifs, including those for Sp1, Nkx-2, CdxA, SRY, MyoD, Ik-2, HNF-3b, Staf, Oct-1, Nkx-2, v-Myb, and AML 1a. Multiple pathways leading to activation of those binding factors may contribute to hBUBR1 gene transcription. Knowledge of the genomic structure and the promoter region of the hBUBR1 gene will facilitate investigation of its role in mitotic checkpoint control and tumor progression in human cancers.

Tajima, K., Y. Obata, et al. (2003). "Expression of cancer/testis (CT) antigens in lung cancer." Lung Cancer **42**(1): 23.

<http://www.sciencedirect.com/science/article/B6T9C-48VTJFX-3/2/46b22f84e8b2a04c070e5efbe0f41f23>

Cancer/testis (CT) antigens are considered promising candidates for vaccine-based immunotherapy. The aim of this study was to investigate which CT antigens should be targeted in immunotherapy of Japanese lung cancer. To determine the expression of 12 CT antigens in Japanese primary lung cancers and cell lines, a reverse-transcription polymerase chain reaction (RT-PCR) analysis was performed. Among 46 primary lung cancers, high expression rates were found for MAGE-3 (41%, 19/46), and SSX-4 (35%, 16/46). A similar pattern of CT antigen expression was observed in 29 lung cancer cell lines. The expression frequency of a certain CT antigen, namely NY-ESO-1, in Japanese cases was drastically different from that in Caucasians. Polyvalent CT antigen vaccine may be effective to increase the number of lung cancer patients eligible for cancer-specific immunotherapy. Vaccination with MAGE-3 and SSX-1 would cover 57% of all patients, with three antigens, MAGE-3, SSX-1, and MAGE-4, would cover 65%, and with four antigens, MAGE-3, SSX-1, MAGE-4 and SSX-4, would cover 70%. Simultaneous expression of two or more CT antigens was observed in 25/46 (54%) primary lung cancers and 18/29 (62%) lung cancer cell lines. Polyvalent CT antigen vaccines may be also effective to reduce a chance of emergence of antigen loss variants, thus preventing tumors from escaping from the immune system. For this purpose, vaccination with combinations of MAGE-3 with MAGE-6, SSX-4, MAGE-1 or BAGE may be effective for a quarter of Japanese lung cancer patients. In addition, in silico surveys of dbEST database were used for identification of new CT antigens. We identified a novel gene, TES101RP, expressed only in some small cell lung cancers (SCLC) and in testis, as confirmed by RT-PCR analysis.

Tsai, Y.-Y., K. A. McGlynn, et al. (2003). "Genetic susceptibility and dietary patterns in lung cancer." Lung Cancer **41**(3): 269.

<http://www.sciencedirect.com/science/article/B6T9C-48V964D-1/2/9a761b0002ab1d455068a5ddf9e7b28e>

Cigarette smoking is the dominant risk factor for lung cancer, but only a minority of smokers ever develops tumors. Though genetic susceptibility is likely to explain some of the variability in risk, results from previous studies of genetic polymorphisms have been inconclusive. As diet may also affect the risk of lung cancer, it is possible that the degree of risk produced by smoking and genetic susceptibility varies, depending on diet. To assess this hypothesis, we conducted a case-control study to examine the effect of cigarette smoking, dietary patterns and variation in genes involved in phase II metabolism. A total of 254 individuals with lung cancer and 184 healthy controls were recruited for the study. To identify persons with similar dietary patterns, cluster analysis was performed using nutrient densities of four major dietary constituents: protein, carbohydrate, animal fat, and dietary fiber. Two groups of individuals were identified with distinct dietary patterns: (1) a group (n=2241) with a high intake of animal fat and protein and a low intake of carbohydrates and dietary fiber (the 'healthy' pattern) (2) a group (n=197) with a high intake of fiber and carbohydrate and a low intake of protein and animal fat (the 'unhealthy' pattern). On stratified analysis, several genotype/dietary pattern combinations were found to affect risk of lung cancer. Smokers who were not homozygous for the most common GSTP1 allele and had a healthy dietary pattern were at significantly lower risk than smokers who were homozygous for the GSTP1 common allele and who had an unhealthy dietary pattern (OR=0.16, 95%CI: 0.04-0.57). Among smokers who were GSTM1 null, persons with a healthy dietary pattern were at lower risk than persons with an unhealthy dietary pattern (OR: 0.46, 95%CI: 0.21-1.01). Among

smokers with an unhealthy dietary patterns, persons with a His/His genotype in the exon 3 polymorphism of EPHX1 were at significantly lower risk than persons who were not homozygous. These data suggest that dietary factors may affect the risk imposed by genetic susceptibility at detoxification loci. Adjustments using dietary pattern may be useful in elucidating the effects of polymorphisms in genes responsible for carcinogen metabolism.

Yang, H.-K., F. M. Scott, et al. (1998). "Correlation of expression of bombesin-like peptides and receptors with growth inhibition by an anti-bombesin antibody in small-cell lung cancer cell lines." Lung Cancer **21**(3): 165.

<http://www.sciencedirect.com/science/article/B6T9C-3TYVX73-2/2/db3e6557c518d36d35e95a50f6509d97>

The murine anti-bombesin monoclonal antibody, 2A11, has been demonstrated to inhibit growth of some small-cell lung cancer (SCLC) cells in nude mice xenografts and in a clinical trial. To determine if the expression of bombesin-like peptides (BLP) and their receptors (GRP-R and NMB-R) correlate with an in vitro response to 2A11, we measured these parameters in seven SCLC cell lines. Gastrin releasing peptide (GRP) mRNA was detected in three of seven cell lines (NCI-H69, NCI-H345, NCI-H510) and neuromedin B (NMB) mRNA was detected in all seven lines using an RNase protection assay (RPA). Immunoreactive BLP was detected in the cell pellets of all lines (range 0.11-59.90 pmol/mg protein) by a solid phase GRP radioimmunoassay (RIA) using ¹²⁵I-labeled 2A11. RPA detected GRP-receptor mRNA in two cell lines (NCI-H69 and NCI-H345) and NMB-receptor in three lines (NCI-H345, NCI-H510, and NCI-H660). Reverse transcriptase-PCR confirmed the presence of receptor mRNA in these lines and detected NMB-receptor in an additional three lines (NCI-H69, NCI-H82, and NCI-H187). Calcium mobilization in response to BLP stimulation was detected in the six cell lines expressing either GRP-R or NMB-R mRNA but not in NCI-N417, which had no detectable BLP-receptor. 2A11 (5 [μg/ml]) inhibited colony formation by 26-61% after 2 weeks in all cell lines except NCI-N417. Thus, growth inhibition by 2A11 requires the presence of at least one BLP-receptor. These findings may be useful in selecting patients with SCLC for treatment with 2A11.

Yano, T., K. Sugio, et al. (2000). "Direct IFN influence of interferon-[gamma] on proliferation and cell-surface antigen expression of non-small cell lung cancer cells." Lung Cancer **30**(3): 169.

<http://www.sciencedirect.com/science/article/B6T9C-41Y86HW-3/2/5aa88284cb7d8d2e94c14691fb729f7d>

In order to clarify the anti-tumor activity of IFN-[gamma], we investigated the direct IFN influence of IFN-[gamma] on both the growth and cell-surface antigen expression of tumor cells. In the present study, four human lung cancer cell lines were used; two squamous cell lines (QG-56, QG-95) and two adenocarcinoma cell lines (PC-9, PC-12). In all four tumor cell lines, mutations were detected in exon 7 of the p53 gene by a PCR-FSSCP analysis. The proliferation of QG-56 or QG-95 was inhibited by IFN-[gamma] in a dose-dependent manner with about 70% inhibition at 1000 JRU/ml while that of PC-9 was slightly inhibited with maximally 25% inhibition at 1000 JRU/ml. The growth of PC-12 was not inhibited at all. In QG-56, QG-95 and PC-9, the fraction of cells in G1 phase increased while the fractions of cells in both S and G2/M phases decreased after exposure to IFN-[gamma] (200 JRU/ml) for 72 h. The growth inhibition by long-term exposure to IFN-[gamma] was irreversible in QG-56. After culture in the presence of IFN-[gamma] (200 JRU/ml) for 14-16 days, tumor cells were examined for expression of various antigens, including HLA-class I, HLA-class II, and CEA. In all cell lines but PC-12, 100% of cells expressed HLA-class I after incubation with IFN-[gamma]. Both HLA-class II and CEA were also induced in those cell lines. The proportion of HLA-class II-positive cells or that of CEA-positive cells varied

among the cell lines. Of the three antigens, the degree of HLA-class II expression paralleled that of growth inhibition by IFN-[gamma] treatment. These results suggested that in various function of IFN-[gamma] against tumor cells, the anti-proliferative effect might be closely linked with the induction of HLA-class II probably through a similar posttranscriptional process, independent of the function of p53 gene.

Zhou, J. X., G. A. Niehans, et al. (2001). "Mechanisms of G1 checkpoint loss in resected early stage non-small cell lung cancer." Lung Cancer **32**(1): 27.

<http://www.sciencedirect.com/science/article/B6T9C-42MW5KD-5/2/780af80a7813174545e5a37f0469d857>

Loss of the G1 checkpoint appears to be extremely common among virtually all neoplasms. A variety of genetic and epigenetic mechanisms have been demonstrated to play significant roles in this process. In a consecutive series of early stage non-small cell lung cancer (NSCLC), we have established the loss of expression of the G1 Cdk inhibitors p15INK4b and p16INK4a by DNA methylation is very common (37%), and methylation of p16INK4a is tightly correlated with loss of expression of p16INK4a protein ($P=0.0018$). Furthermore, methylation of p15INK4b and p16INK4a appear inversely correlated, although methylation of p15INK4b is an infrequent event in this cohort (4%). Methylation was detected in all stages of NSCLC equally, and did not correlate with survival in these patients. Evidence for methylation was more frequent in squamous cell carcinomas in comparison to other tumor histologies ($P=0.0156$). In addition, over-expression of cyclin D1 was found to be tightly restricted ($P=0.0032$) to those tumors that had retained wild-type expression of pRB, and did not correlate with methylation or expression of p16INK4a gene product. Although loss of p16INK4a function remains tightly correlated with pRB expression, loss of other regulatory elements in NSCLC such as p53 mutation and cyclin D1 over-expression appear independent of loss of the p16INK4a gene product.

Mammalian Biology - Zeitschrift fur Saugetierkunde (1)

Lorenzini, R., R. Fico, et al. "Mitochondrial DNA evidence for a genetic distinction of the native red deer of Mesola, northern Italy, from the Alpine populations and the Sardinian subspecies." Mammalian Biology - Zeitschrift fur Saugetierkunde **In Press, Corrected Proof**

<http://www.sciencedirect.com/science/article/B7GX2-4FG898J-4/2/78df556a535b4f9660eab24cad6deaad>

Native red deer of Mesola Wood, northern Italy, were compared with the Sardinian subspecies and with some populations from the Italian Alps and Austria using the analysis of restriction fragment length polymorphism (RELP) of mitochondrial (mt) DNA segments. The results highlight the existence of four main genetic lineages, and provide evidence for a structuring of populations according to their geographic occurrence. Two mitochondrial lineages, although highly distantly related, are shared between the populations from the centre-eastern Alps of Italy and Austria, while the other two lineages characterize the Sardinian and Mesola red deer, respectively. The exclusive haplotype found in the Mesola population appears as being an offshot of one of the two main Alpine lineages, suggesting a presumed origin of these deer from a panmictic population which dwelt in mid-southern Europe, prior to the fragmentation of populations caused by human activities and manipulations. Considering their distinctiveness in morphologic and genetic traits,

as well as their historical background and biogeographical value, these native deer should be regarded as a national conservation priority. The Sardinian red deer is highly divergent from both Mesola and Alpine populations. However, the controversial question of the phylogeographic origin of this subspecies remains unresolved. The utility of RFLP analyses of mtDNA segments as a tool to discriminate among red deer populations as well as to develop effective strategies for their conservation and management. Zusammenfassung Mitochondrien-DNA-Daten zur genetischen Unterscheidung der autochthonen Rothirsche von Mesola, Norditalien, von jenen aus alpinen Populationen und von der Unterart auf Sardinien. Autochthone Rothirsche aus dem Mesola-Waldgebiet in Norditalien wurden mittels Restriktionsfragmenteangelpolymorphismen (RFLPs) von Abschnitten der mitochondrialen (mt)DNA mit Rotwild der sardischen Unterart und Tieren aus alpinen Populationen verglichen. Die Ergebnisse weisen auf die Existenz von vier genetischen Hauptlinien hin und belegen auch eine Strukturierung der Populationen gemäß ihrer geographischen Herkunft. Zwei entfernt verwandte mtDNA-Linien wurden in Beständen aus dem Zentral- und Ostalpenbereich von Italien und Österreich gefunden. Die beiden anderen Linien waren jeweils charakteristisch für Rotwild auf Sardinien und Rotwild in Mesola. Der exklusive Haplotyp der Mesola-Population scheint ein Abkommling einer der beiden alpinen Hauptlinien zu sein. Dies legt eine Abstammung der Mesola-Rothirsche von einer großräumigen, panmiktischen Population nahe, wie sie vor der anthropogen bedingten Fragmentierung und Isolation von Beständen im Bereich von Mittel- und Südeuropa existiert haben sollte. Sowohl nach der Eigenständigkeit in morphologischen und molekularen Merkmalen als auch im Hinblick auf die Bestandsgeschichte und den biogeographischen Wert sollte der letzten autochthonen Rotwildpopulation in Mesola eine nationale Priorität im Rahmen von Arterhaltungsprogrammen zugewilligt werden. Das sardinische Rotwild unterschied sich in hohem Maße von den alpinen Beständen und der Mesola-Population. Die phylogeographische Herkunft bleibt allerdings unklar. Die Brauchbarkeit von mtDNA-RFLPs zur Abgrenzung von Rothirschbeständen sowie zur Entwicklung von Arterhaltungs- und Management-Konzepten wird diskutiert.

Matrix Biology (15)

Asuncion, L., B. Fogelgren, et al. (2001). "A novel human lysyl oxidase-like gene (LOXL4) on chromosome 10q24 has an altered scavenger receptor cysteine rich domain." Matrix Biology **20**(7): 487.

<http://www.sciencedirect.com/science/article/B6VPM-44B213D-9/2/64a14940501849e3d4af5a8c8e8d5050>

We have identified a novel 14-exon human lysyl oxidase-like gene, LOXL4, on chromosome 10q24. The cDNA and derived amino acid sequence of LOXL4 demonstrates a conserved C-terminal region including the characteristic copper-binding site, lysyl and tyrosyl residues and a cytokine receptor-like domain. One of the four N-terminal SRCR domains contains a 13 amino acid insertion encoded by a short exon not present within the closely homologous LOXL2 and LOXL3 genes. The 3.5-kb LOXL4 mRNA is present in pancreas and testis and at lower levels in several other tissues. Fibroblasts, smooth muscle and osteosarcoma (HOS) cells express LOXL4. No expression was detected in HCT-116 and DLD-1 colon, MCF-7 breast and DU-145 prostate cancer cell lines.

Azizan, A., J. U. Gaw, et al. (2000). "Chondromodulin I and pleiotrophin gene expression in bovine

cartilage and epiphysis." Matrix Biology **19**(6): 521.

<http://www.sciencedirect.com/science/article/B6VPM-41JTP8W-8/2/b7c91962c0e234eb8a9de9474362d01e>

Pleiotrophin and chondromodulin-I are low molecular weight proteins that are abundant (20 [mu]g/g tissue) in fetal cartilage and difficult to detect in adult cartilage. We characterized their gene and protein expression patterns to gain a better understanding of their roles in the regulation of limb development and growth. In order to compare and contrast the relative amounts of the respective mRNA species within the developing epiphysis, a competitive PCR assay was developed. The results showed that the mRNAs for both proteins were abundant in fetal cartilage and while present in adult cartilage, were at 20-60-fold lower levels. Northern blotting revealed gradients of mRNA for both of these proteins in growth plate cartilage, with the highest levels in the resting zone, and the lowest in the hypertrophic zone. In contrast to pleiotrophin, chondromodulin-1 is down-regulated by retinoic acid with a pattern of expression similar to collagen type II and link protein, and may play a more specific role than pleiotrophin in modulating the chondrocyte phenotype.

Botella, L. M., F. Sanz-Rodriguez, et al. (2004). "Lumican is down-regulated in cells expressing endoglin. Evidence for an inverse correlation between Endoglin and Lumican expression." Matrix Biology **22**(7): 561.

<http://www.sciencedirect.com/science/article/B6VPM-4BSW8KC-5/2/ce9b85ce637808f11e5fc5a99d1ca51a>

Endoglin (CD105) is a homodimeric membrane glycoprotein, which acts as a TGF-[beta] coreceptor in the vasculature and plays an important role in cardiovascular development and vascular remodelling. To isolate putative genes regulated by endoglin expression, a PCR-based RNA fingerprinting technique was carried out. Myoblasts stably transfected with endoglin showed a decrease in the expression of lumican both at the RNA and protein levels. Lumican is a proteoglycan of the extracellular matrix, belonging to the SLRP (Small Leucine-Rich Repeat Proteoglycans) family. Lumican down-regulation by endoglin appeared to be controlled, at least in part, at the transcriptional level, as indicated by RT-PCR, and transient transfection experiments using a lumican promoter reporter based vector. This inverse correlation between endoglin and lumican expression was substantiated by immunohistochemical staining of vessels from human tissues. Thus, cells belonging to the high endothelia, such as tonsil, express a large amount of endoglin, and the lumican content of their matrix is considerably reduced. Conversely, in resting endothelia, such as that of large vessels, the expression of endoglin is reduced whereas the amount of lumican is greatly increased. The inverse regulation in the expression of endoglin and lumican was also evident after TGF-[beta] treatments since endoglin was up-regulated, whereas lumican was down-regulated by this cytokine. This report describes for the first time a relationship between endoglin and lumican expression.

Clark, A. G., A. L. Rohrbach, et al. (2002). "The effects of ascorbic acid on cartilage metabolism in guinea pig articular cartilage explants." Matrix Biology **21**(2): 175.

<http://www.sciencedirect.com/science/article/B6VPM-44PVT88-1/2/1dab29c60aa570ed11d39fc00c27f674>

Ascorbic acid has been associated with the slowing of osteoarthritis progression in guinea pig and man. The goal of this study was to evaluate transcriptional and translational regulation of

cartilage matrix components by ascorbic acid. Guinea pig articular cartilage explants were grown in the presence of -ascorbic acid (-Asc), -isoascorbic acid (-Asc), sodium -ascorbate (Na -Asc), sodium -isoascorbate (Na -Asc), or ascorbyl-2-phosphate (A2P) to isolate and analyze the acidic and nutrient effects of ascorbic acid. Transcription of type II collagen, prolyl 4-hydroxylase (alpha subunit), and aggrecan increased in response to the antiscorbutic forms of ascorbic acid (-Asc, Na -Asc, and A2P) and was stereospecific to the -forms. Collagen and aggrecan synthesis also increased in response to the antiscorbutic forms but only in the absence of acidity. All ascorbic acid forms tended to increase oxidative damage over control. This was especially true for the non-nutrient -forms and the high dose -Asc. Finally, we investigated the ability of chondrocytes to express the newly described sodium-dependent vitamin C transporters (SVCTs). We identified transcripts for SVCT2 but not SVCT1 in guinea pig cartilage explants. This represents the first characterization of SVCTs in chondrocytes. This study confirms that ascorbic acid stimulates collagen synthesis and in addition modestly stimulates aggrecan synthesis. These effects are exerted at both transcriptional and post-transcriptional levels. The stereospecificity of these effects is consistent with chondrocyte expression of SVCT2, shown previously to transport -Asc more efficiently than -Asc. Therefore, this transporter may be the primary mechanism by which the -forms of ascorbic acid enter the chondrocyte to control matrix gene activity.

Ilic, M. Z., M. N. Vankemmelbeke, et al. (2000). "Bovine joint capsule and fibroblasts derived from joint capsule express aggrecanase activity." Matrix Biology **19**(3): 257.

<http://www.sciencedirect.com/science/article/B6VPM-40WDVPV-8/2/d2f1992f0b009049ad0caaa4773f63fc>

Bovine joint capsule was maintained in explant culture in the presence of bovine aggrecan monomer and it was shown that the aggrecan monomer was degraded. Amino-terminal sequence analysis of the resulting aggrecan core protein fragments revealed that the core protein was cleaved at five specific sites attributed to glutamyl endopeptidases referred to as aggrecanase activity. Fibroblast cultures were established from explant cultures of joint capsule and when these cells were exposed to aggrecan, cleavage of the core protein of aggrecan at the aggrecanase sites was observed. Inclusion of either retinoic acid or interleukin-1[alpha] in medium of either joint capsule explant cultures or fibroblast cultures did not increase the rate of cleavage of exogenous aggrecan present in the culture medium. When aggrecan monomer was incubated with conditioned medium from explant cultures of joint capsule maintained in medium, degradation could be detected after 10 min. After a 6-h incubation period the same fragments of aggrecan core protein were observed as those for tissue or cells incubated directly with aggrecan monomer. RT-PCR analysis of mRNA extracted from joint capsule fibroblasts showed that these cells express both aggrecanase-1 and -2 [ADAMTS-2 (Tang) and ADAMTS-5].

Kerkvliet, E. H. M., I. C. Jansen, et al. (2003). "Collagen type I, III and V differently modulate synthesis and activation of matrix metalloproteinases by cultured rabbit periosteal fibroblasts." Matrix Biology **22**(3): 217.

<http://www.sciencedirect.com/science/article/B6VPM-48N3JC5-2/2/09b5c676bc243cfa7f1c7add918040b1>

In the present study we investigated whether the collagen types I, III and V affect the activity of fibroblasts obtained from rabbit periosteum. The cells were cultured on plates either or not coated with different amounts of collagen type I, III or V and analyzed for their attachment, DNA synthesis and the expression and activity of matrix metalloproteinases (MMPs). Our data show that the three collagen types promoted attachment and spreading of the cells and stimulated DNA synthesis when used in relatively low concentrations. High concentrations of type V--but not of

type I or III--proved to inhibit thymidine incorporation. The expression and activity of matrix metalloproteinase 1 (MMP-1; interstitial collagenase) decreased under the influence of relatively low amounts of collagen (1-integrin or echistatin increased the level of MMP-1 but had no effect on MMP-2. All parameters tested were similarly affected by type I and III collagen, whereas the effect of type V was always less. We conclude that the collagen types I, III and V provide different sets of signals for fibroblasts that differently modulate their proliferation and MMP expression.

Konttinen, Y., S. Halinen, et al. (1998). "Matrix metalloproteinase (MMP)-9 type IV collagenase/gelatinase implicated in the pathogenesis of Sjogren's syndrome." Matrix Biology **17**(5): 335.

<http://www.sciencedirect.com/science/article/B6VPM-46YXFH8-9/2/20e3abd241f27ab103ecef45e8742c53>

Type IV collagenases/gelatinases (matrix metalloproteinases MMP-2 and MMP-9) in labial salivary glands (LSG) and saliva in Sjogren's syndrome (SS) and healthy controls were studied. Zymograms and Western blots disclosed that SS saliva contained 92/82 kD MMP-9/type IV collagenase duplex. Specific activity measurement disclosed 53.1 +/- 9.8 U/mg protein MMP-9 in SS compared to 16.5 +/- 2.6 U/mg in healthy controls (p = 0.01). MMP-2 did not differ between SS and controls. In SS salivary glands, MMP-2 and MMP-9 were also expressed, in addition to stromal fibroblasts and occasional infiltrating neutrophils, respectively, in acinar end piece cells. In addition, an effective proMMP-9 activator, human trypsin-2 (also known as tumor-associated trypsin-2 or TAT-2), was found in acinar end piece cells and in saliva. Interestingly, proteolytically processed MMP-9 was found in saliva (vide supra), and in vivo activated MMP-9 was significantly higher in SS than in controls (p = 0.002). LSGs, particularly in SS, were characterized ultrastructurally by areas containing small cytoplasmic vesicles in the basal parts of the epithelial cells associated with areas of disordered and thickened basal lamina. Based on our results, we conclude here that SS saliva contains increased concentrations of MMP-9, which is of glandular origin in part. Pro MMP-9 is to a large extent proteolytically activated. This is probably mediated by the most potent pro MMP-9 activator found in vivo thus far, namely trypsin-2. Therefore, the MMP-9/trypsin-2 cascade may be responsible for the increased remodelling and/or structural destruction of the basement membrane scaffolding in salivary glands in SS. Due to the role of basal lamina as an important molecular sieve and extracellular matrix-cell signal, these pathological changes may contribute to the pathogenesis of the syndrome.

Lorenzo, P., P. Aman, et al. (1999). "The human CILP gene: exon/intron organization and chromosomal mapping." Matrix Biology **18**(5): 445.

<http://www.sciencedirect.com/science/article/B6VPM-3Y3XR41-4/2/29b7d42200c08062426066ea150a7481>

The human cDNA for cartilage intermediate layer protein (CILP) codes for a larger precursor protein that consists of CILP and a homologue to porcine Nucleotide pyrophosphohydrolase (NTPPHase) [Lorenzo et al. 1998a. J. Biol. Chem. 273, 23469-23475]. The human gene has now been isolated and characterized. Southern blot analysis indicated a single copy of the CILP gene in the human genome. The gene spans approximately 15.3 kbp of genomic DNA, and is organized in nine exons. The 5' flanking region contains a putative promoter region with a TATA-like box localized from -29 to -23 bp upstream of the transcription start site. Analysis of the putative promoter region revealed potentially cis-regulatory eukaryotic elements such as GATA-1, MyoD, MZF1, and CdxA. The protein coding region begins in exon 2 with the putative signal peptide. CILP is encoded from exon 3 to exon 9. In addition, exon 9 also codes for the entire NTPPHase homologue and contains the 3' untranslated region of the gene. All the introns follow the 'gt-ag' rule, except the last intron, intron 8, that belongs to the minor class of pre-mRNA

introns that contain 'at-ac' at their 5' and 3' ends, respectively. The CILP gene was mapped to human chromosome 15q22.

Mates, L., E. Korpos, et al. (2002). "Comparative analysis of the mouse and human genes (Matn2 and MATN2) for matrilin-2, a filament-forming protein widely distributed in extracellular matrices." Matrix Biology **21**(2): 163.

<http://www.sciencedirect.com/science/article/B6VPM-44V2101-1/2/98d11c77df0765fe3767c67d2dd15ab0>

We previously identified matrilin-2 (MATN2), the largest member of the novel family of matrilins. These filament-forming adapter proteins expressed in a distinct, but partially overlapping, pattern in all tissues were implicated in the organization of the extracellular matrix. Matrilin-2 functions in a great variety of tissues. Here, we present the genomic organization of the highly conserved mouse and human MATN2 loci, which cover >100 kb and 167.167 kb genomic regions, respectively, and are composed of 19 exons. RT-PCR analysis revealed that alternative transcripts with identical protein coding regions are transcribed from two promoters in both species. The upstream, housekeeping type promoter is functional in all tissues and cell types tested. The activity of the downstream, TATA-like promoter preceded with putative motifs for the homeobox transcription factor PRRX2 is restricted to embryonic fibroblasts and certain cell lines. The oligomerization module is split by an U12-type AT-AC intron found in conserved position in all four matrilin genes. We assigned Matn2 to mouse chromosome 15, linked to Trhr and Sntb1 in a region syntenic to human chromosome 8q22-24.

Mates, L., C. Nicolae, et al. (2004). "Mice lacking the extracellular matrix adaptor protein matrilin-2 develop without obvious abnormalities." Matrix Biology **23**(3): 195.

<http://www.sciencedirect.com/science/article/B6VPM-4CPDFTP-5/2/e61391f65a1ed6f4c4943fa2fbebcb9>

Matrilins are putative adaptor proteins of the extracellular matrix (ECM) which can form both collagen-dependent and collagen-independent filamentous networks. While all known matrilins (matrilin-1, -2, -3, and -4) are expressed in cartilage, only matrilin-2 and matrilin-4 are abundant in non-skeletal tissues. To clarify the biological role of matrilin-2, we have developed a matrilin-2-deficient mouse strain. Matrilin-2 null mice show no gross abnormalities during embryonic or adult development, are fertile, and have a normal lifespan. Histological and ultrastructural analyses indicate apparently normal structure of all organs and tissues where matrilin-2 is expressed. Although matrilin-2 co-localizes with matrilin-4 in many tissues, Northern hybridization, semiquantitative RT-PCR, immunohistochemistry and biochemical analysis reveal no significant alteration in the steady-state level of matrilin-4 expression in homozygous mutant mice. Immunostaining of wild-type and mutant skin samples indicate no detectable differences in the expression and deposition of matrilin-2 binding partners including collagen I, laminin-nidogen complexes, fibrillin-2 and fibronectin. In addition, electron microscopy reveals an intact basement membrane at the epidermal-dermal junction and normal organization of the dermal collagen fibrils in mutant skin. These data suggest that either matrilin-2 and matrilin-2-mediated matrix-matrix interactions are dispensable for proper ECM assembly and function, or that they are efficiently compensated by other matrix components including wild-type levels of matrilin-4.

Sztrolovics, R., M. Van Der Rest, et al. (1994). "Identification of type I collagen gene polymorphisms:

Tolerance of sequence variation at an $[\alpha]2(I)$ Helix Y position." *Matrix Biology* **14**(1): 9.

<http://www.sciencedirect.com/science/article/B6VPM-47T8XVT-3/2/09c76fbc8fd8f57337779f517b6f9721>

This study has examined the frequency and distribution of polymorphisms in the type 1 collagen coding sequences. RNA from a group of human skin fibroblast cell lines, was analyzed by the chemical cleavage mismatch detection method using hydroxylamine, a reagent specific for C base mismatches, and overlapping cDNA probes covering the entire prepro $[\alpha]1(I)$ and prepro $[\alpha]2(I)$ coding regions. Mismatches were detected at only two nucleotide positions, one in each of the type I collagen sequences, suggesting that polymorphisms are relatively rare within these cDNAs. cDNA sequence analysis demonstrated that the prepro $[\alpha]1(I)$ mismatch, detected in only one cell line, was due to a sequence polymorphism involving the wobble position of the codon for arginine residue 59 within the amino-propeptide globular subdomain of the pro $[\alpha]1(I)$ chain and not resulting in a change in the polypeptide primary structure. In contrast, the prepro $[\alpha]2(I)$ mismatch, detected in 6 of the 16 cell lines, was shown to arise from a sequence polymorphism affecting the identity of Y-position residue 459 of the $[\alpha]2(I)$ triple helical domain, resulting in an alanine/proline dimorphism at this position. This study is the first to identify a type I collagen coding sequence polymorphism resulting in an alteration at the level of the amino acid sequence. The data suggest that at least some $[\alpha]1(I)$ and $[\alpha]2(I)$ helix Y positions may be tolerant of sequence variation, particularly if the replacing amino acid is proline, a residue involved in stabilizing the collagen triple helix.

Sztrolovicz, R., D. L. Rimoin, et al. (1994). "Single-strand conformation polymorphism analysis of human decorin, biglycan and fibromodulin cDNAs." *Matrix Biology* **14**(4): 307.

<http://www.sciencedirect.com/science/article/B6VPM-47GH9YC-5/2/6d785b226dfcb2d2c6a39f04cef75db0>

The coding regions of the human decorin, biglycan and fibromodulin cDNAs have been examined utilizing the method of single-strand conformation polymorphism analysis. Analysis of total RNA from a group of eight human skin fibroblast cell lines did not detect any sequence variations in the decorin cDNA. In contrast, the analysis detected three sequence variations in the biglycan cDNA and one in the fibromodulin cDNA from the same group of cell lines. For the biglycan cDNA, one variation involved a position in the 5'-untranslated region, while the other two affected the wobble bases of triplets encoding serine residues 10 and 143 of the mature core protein. For the fibromodulin cDNA, the variation involved the wobble position of the codon for glutamic acid residue 61 of the putative mature core protein. Single-strand conformation polymorphism analysis of these proteoglycan cDNAs was also applied to study patients exhibiting a variety of connective tissue pathologies, including chondrodysplasia punctata, Desbuquois syndrome, Dyggve-Melchior-Clausen syndrome, dyssegmental dysplasia, Ehlers-Danlos syndrome types I and III, Ellis van Creveld syndrome and thanatophoric dysplasia, though no additional sequence variations were detected.

Walker, L. C., M. A. Overstreet, et al. (2005). "Tissue-specific expression and regulation of the alternatively-spliced forms of lysyl hydroxylase 2 (LH2) in human kidney cells and skin fibroblasts." *Matrix Biology* **23**(8): 515.

<http://www.sciencedirect.com/science/article/B6VPM-4F0GR57-1/2/b93bfdf39d962c515c0244713b5e9d57>

Lysyl hydroxylases 1, 2, and 3 catalyse the hydroxylation of specific lysines in collagen. A small percentage of these hydroxylysine residues are precursors for the cross-link formation essential for the tensile strength of collagen. Lysyl hydroxylase 2 (LH2) exists as two alternatively-spliced forms; the long transcript (the major ubiquitously-expressed form) includes a 63 bp exon (13A) that is spliced out in the short form (expressed, together with the long form, in human kidney, spleen, liver, and placenta). This study shows that this alternative splicing event can be regulated by both cell density and cycloheximide (CHX). Although only the long form of LH2 is detected in untreated confluent human skin fibroblasts, after 24 h treatment with CHX the short LH2 transcript is also expressed. In kidney cells, in which both LH2 transcripts are equally expressed, the long LH2 transcript is significantly decreased after 24 h CHX treatment, whereas expression of the short transcript is slightly increased. This suggests that, in kidney cells, the splicing mechanism for the inclusion of exon 13A in LH2 requires a newly-synthesized protein factor that is suppressed by CHX, whereas, in skin fibroblasts in which levels of LH2 (long) are unaffected, CHX appears to suppress a factor that inhibits exclusion of exon 13A, thereby promoting expression of LH2 (short). As these alternate transcripts of LH2 may have specificity for hydroxylation of lysines in either telopeptide or helical collagen domains, their relative expression determines the type of cross-links formed, thereby affecting collagen strength. Therefore, any perturbation of the regulation of LH2 splicing could influence the stability of the extracellular matrix and contribute to specific connective tissue disorders.

Yao, L. Y., C. Moody, et al. (1994). "Identification of the proteoglycan versican in aorta and smooth muscle cells by DNA sequence analysis, in situ hybridization and immunohistochemistry." Matrix Biology **14**(3): 213.

<http://www.sciencedirect.com/science/article/B6VPM-47T2M7S-3/2/49c7a02a82d775d3b05fe4fea36e0b23>

Versican is a large chondroitin sulfate proteoglycan (CSPG) initially identified in cultured human fibroblasts. Previous studies have shown that there is a versican-like molecule in cultured monkey smooth muscle cells. In this study, we have cloned and sequenced the large CSPG from cultured monkey smooth muscle cells, fetal and juvenile monkey aorta, and human fetal aorta. The cDNA sequence from human fetal aorta is completely homologous to the human fibroblast versican. We obtained 2.5 kb of cDNA sequence from monkey aortic RNA and cultured monkey smooth muscle cell RNA. This sequence covers three distinct domains of versican (hyaluronic acid binding domain, glycosaminoglycan attachment domain and protein binding domain) and demonstrates over 90% homology to the human versican sequence. In situ hybridization histochemistry indicates that the versican RNA transcript is located in the epithelium throughout the tunica media of the aorta. Western blot analysis and immunohistochemistry also confirm the presence of versican in human and monkey aorta.

Yoon, S., H. Kuivaniemi, et al. (2002). "MMP13 promoter polymorphism is associated with atherosclerosis in the abdominal aorta of young black males." Matrix Biology **21**(6): 487.

<http://www.sciencedirect.com/science/article/B6VPM-470KM47-4/2/52aa52578ad341bd4c2a9e7b974c6211>

Previous studies suggested that remodeling of connective tissue is important in progression of atherosclerosis. We investigated the importance of matrix metalloproteinase 13 (MMP13), in the pathogenesis of atherosclerosis using 995 samples from the Pathobiological Determinants of Atherosclerosis in Youth collection in an association study. We identified two new MMP13 promoter polymorphisms. The genotype for one of the MMP13 polymorphisms was associated with fibrous plaque ($P=0.024$) in black males. Immunohistochemistry using antibodies for MMP13

showed that MMP13 is expressed in all layers of the aorta. In-vitro transfection experiments with reporter gene constructs and electrophoretic mobility-shift assays showed that the MMP13 polymorphism was a functional variant. MMP13 is therefore, a genetic risk factor for extent of fibrous plaque in the abdominal aorta in young black males. Elucidation of the currently unknown mechanism of the MMP13 polymorphism's action may provide for pharmacological intervention to reduce the severity of atherosclerotic changes in susceptible individuals.

Maturitas (5)

Arko, B., J. Prezelj, et al. "Association of the osteoprotegerin gene polymorphisms with bone mineral density in postmenopausal women." **Maturitas In Press, Corrected Proof**
<http://www.sciencedirect.com/science/article/B6T9F-4DF4D3G-1/2/0bed3adc5adb1ce693b7eda24819ba76>

Objectives: Osteoprotegerin (OPG) is a recently discovered member of the tumour necrosis factor receptor superfamily. It plays a crucial role in the control of bone resorption and its gene could therefore be a good candidate gene for osteoporosis. The aim of our work was to find polymorphisms in the OPG gene and to investigate their possible contribution to the genetic susceptibility to osteoporosis by testing for their association with bone mineral density (BMD). **Methods:** The whole OPG gene coding region was screened for the presence of polymorphisms in a group of 60 osteoporotic women by single-strand conformation polymorphism analysis (SSCP) approach. Association of the discovered polymorphisms with bone mineral density was investigated in 136 Slovenian postmenopausal women. **Results:** We detected eight OPG gene polymorphisms that were confirmed by direct DNA sequencing, deletion 4752_4753delCT and nucleotide substitutions 1181G > C, 1217C > T, 1284G > A, 4501C > T, 6893A > G, 6950A > C and 8738T > A. Nucleotide substitutions 1284G > A and 8738T > A have not been previously described. Polymorphisms 4752_4753delCT, 6893A > G and 6950A > C were in complete linkage and the same was true for 1217C > T and 4501C > T. The association with BMD was found only for polymorphism 1181G > C. Subjects with genotype 1181GG had significantly lower lumbar spine BMD than subjects displaying 1181GC. **Conclusions:** By our approach we detected eight polymorphisms in the OPG gene. According to our analysis polymorphism 1181G > C is associated with BMD and could therefore be considered as an element of genetic susceptibility to osteoporosis.

Chen, H.-Y., W.-C. Chen, et al. (2001). "Relation of the estrogen receptor [alpha] gene microsatellite polymorphism to bone mineral density and the susceptibility to osteoporosis in postmenopausal Chinese women in Taiwan." **Maturitas** **40**(2): 143.

<http://www.sciencedirect.com/science/article/B6T9F-44F6SB1-4/2/43554eca3c2a947ad222558cba6da90a>

Objective: Osteoporosis is a common disorder with a strong genetic component. Our aim was to investigate the correlation of the estrogen receptor [alpha] gene microsatellite polymorphism (TA dinucleotide repeat polymorphism 5' upstream of exon 1) with bone mineral density and their relationship to osteoporosis. **Methods:** We determined the estrogen receptor [alpha] gene microsatellite polymorphism using polymerase chain reaction-based microsatellite analysis in postmenopausal Chinese women in Taiwan. Bone mineral density of the lumbar spine and

proximal femur were measured using dual-energy X-ray absorptiometry. Results: The ER[alpha] genotype was classified into '12' through '27' according to the number of TA dinucleotide repeats they contained, as a 'signpost'. After adjustment for potential confounding factors including age, height, and weight, subjects with genotype 18+ (n=4) had lower bone mineral density values and a 54.5 times greater risk for osteoporosis when compared with subjects with genotype 18- (n=170) at the lumbar spine. This should be interpreted with caution because of the small number of subjects with the unfavorable genotype 18+. According to mean number of TA dinucleotide repeats, women with a high number of repeats (TA[gE]20) (n=38) had the lowest bone mineral density and a 6.1 times greater risk for osteoporosis than women with a low number of repeats (TA[lE]15) (n=61) at the femoral neck, after adjustment for potential confounding factors such as age, height, and weight. Conclusion: The present study suggests that the estrogen receptor [alpha] gene microsatellite polymorphism may be a candidate genetic marker for risk of osteoporosis in postmenopausal Chinese women in Taiwan.

Chen, H.-Y., W.-C. Chen, et al. (2003). "Interleukin-1[beta] and interleukin-1 receptor antagonist gene polymorphism in postmenopausal women: correlation to bone mineral density and susceptibility to osteoporosis." *Maturitas* **44**(1): 49.

<http://www.sciencedirect.com/science/article/B6T9F-47FDMSJ-2/2/b48f6a5d87faf376308ea0ba64999eb6>

Objective: Osteoporosis is a common disorder with a strong genetic component. Our aim was to investigate the correlations of the interleukin-1[beta] (IL-1[beta]) and interleukin-1 receptor antagonist (IL-1Ra) gene polymorphisms with bone mineral density (BMD) and their relationship to osteoporosis. Methods: The IL-1[beta] (promoter and exon 5) and IL-1Ra (intron 2) gene polymorphisms were determined using polymerase chain reaction. BMD of the lumbar spine and proximal femur were measured using dual-energy X-ray absorptiometry. Results: The prevalence of each genotype of the interleukin-1 related genes in the study population was: (1) 14% C/C, 71.5% C/T, and 14.5% T/T in IL-1[beta] promoter; (2) 95.3% E1/E1 and 4.7% E1/E2 in IL-1[beta] exon 5; (3) 92.4% I/I, 6.4% I/II, and 1.2% II/II in IL-1Ra intron 2. After adjustment for potential confounding factors such as age, height, weight, years since menopause, and daily calcium intake, subjects with genotype E1/E2 (n=8) in IL-1[beta] exon 5 had lower BMD values and a significantly greater risk for osteoporosis (OR 10.6, 95% CI 1.3-83.8) at the lumbar spine when compared with subjects with genotype E1/E1 (n=164) in IL-1[beta] exon 5. Conclusion: The Taq I IL-1[beta] exon 5 gene polymorphism is associated with reduced BMD and predisposes women to osteoporosis at the lumbar spine, but our results should be interpreted with caution because of the small number of subjects with the unfavorable E1/E2 genotype.

O'Toole, S. A., E. Dunn, et al. "Oestrogen regulated gene expression in normal and malignant endometrial tissue." *Maturitas In Press, Corrected Proof*

<http://www.sciencedirect.com/science/article/B6T9F-4D7K6Y9-1/2/c3bd3a050e898a062cb3eeed473d507b>

Objectives: The aim of this study was to examine the expression of oestrogen regulated genes in premenopausal and postmenopausal normal and malignant endometrial specimens. The molecular mechanisms and the role of these genes in endometrial carcinogenesis are poorly understood. Methods: Normal and malignant endometrial specimens were collected from patients undergoing hysterectomy. Real time TaqMan PCR was used to examine the mRNA expression levels of oestrogen receptor a (ERa) and b (ERb), progesterone receptor (PR), insulin like growth factor 1 (IGF-1) and vascular endothelial growth factor (VEGF). Results: Expression analysis was carried out on 60 patients. ERa was more predominantly expressed in the endometrial samples than ERb, 28% of the specimens did not express ER. Normal pre and postmenopausal tissue

expressed higher levels of ER α , PR and IGF-1 than malignant tissue. ER α and PR expression was significantly higher in the proliferative phase endometrium compared to the secretory phase (P Conclusions:ER α expression may play an important role in the regulation of PR in normal and malignant endometrium. Further work is needed to establish if IGF-1 plays a role in a subset of endometrial cancers and if isoforms of VEGF play a role in endometrial cancer.

Seidlova-Wuttke, D., H. Jarry, et al. "Effects of estradiol-17[beta], testosterone and a black cohosh preparation on bone and prostate in orchidectomized rats." *Maturitas In Press, Corrected Proof*
<http://www.sciencedirect.com/science/article/B6T9F-4D5JY24-4/2/4287cd3858fad5f64b24ec2b9ff3df39>

Estradiol (E2) and testosterone (T) effectively prevent orchidectomy (orx) induced osteoporosis. T, however, stimulates prostate proliferation which may lead to malignancy. We showed that a *Cimicifuga racemosa* (CR) preparation had bone-sparing effects without exerting estrogenic effects in the uterus. We studied therefore whether a CR preparation has also antiosteoporotic effects in orx rats substituted with E2, T or CR via pelleted food over a period of 3 months. Average daily intake per animal was: T: 25 mg; E2: 0.325 mg, CR low dose: 33 mg; CR high dose: 133 mg. E2, T and CR at the high dose partially prevented development of osteoporosis as measured by quantitative computer tomography in the metaphysis of the tibia. E2, but not T or CR reduced serum osteocalcin and the metabolic products of collagen-1[α 1]. Gene expression of collagen-1[α 1] and tartrate-resistant acid phosphatase was decreased by E2 and the higher dose of the CR extract but increased in the T-treated animals. In the prostate T inhibited androgen receptor, estrogen receptor [α] and insulin-like growth factor-1 gene expression but stimulated the expression of the ER[beta] gene. These effects were not shared by E2 or both doses of the CR extract. It is concluded that E2, T and CR exert antiosteoporotic effects in the metaphysis of the tibia of orx rats. T has profound effects in the prostate which were not seen in the E2- and CR-treated animals. Therefore, the *Cimicifuga racemosa* extract BNO 1055 may be useful to prevent osteoporosis in aged male patients with reduced testosterone production.

Mechanisms of Ageing and Development (12)

Bloom, E. T., W. C. Thompson, et al. (1995). "Differential effects of interleukin-12 treatment on gene expression by allostimulated T cells from young and aged mice." *Mechanisms of Ageing and Development* **85**(2-3): 109.

<http://www.sciencedirect.com/science/article/B6T31-4031VR5-5/2/288ee226303e28d562da033ec310a6cd>

Alloantigen stimulation was used to examine the effect of interleukin (IL-12) treatment of stimulated cells from young and aged mice on the expression of mRNAs for perforin and granzyme B, two proteins known to be intimately involved in an important lytic pathway used by CTL, and mRNA for interferon (IFN)-[γ], production of which is highly stimulated by IL-12. As reported previously, IL-12 augmented the lytic activity by cells from both young and aged mice, although the relative increase was greater for the latter. The mRNAs encoding perforin and granzyme B were both marginally enhanced at early time points (for cells from young mice) or throughout the stimulation (for cells from aged mice) following allo-stimulation in the presence of

IL-12. The levels of augmentation of these mRNAs was consistent with the augmentation of lytic activity. In contrast, mRNA encoding IFN-[gamma] was markedly enhanced throughout stimulation in cells from animals of both age groups, corresponding to the more substantial increase in interferon protein in response to IL-12.

Frasca, D., S. Pucci, et al. (1997). "Regulation of cytokine production in aging: use of recombinant cytokines to upregulate mitogen-stimulated spleen cells." Mechanisms of Ageing and Development **93**(1-3): 157.

<http://www.sciencedirect.com/science/article/B6T31-3RM6SHJ-J/2/34dfc926c705218b8370a1d5e4cf7d63>

We investigated the production of IL-2 and IFN-[gamma] (Th1 type) and IL-4 (Th2 type) cytokines by mitogen-activated spleen cells from young, adult and old mice. Cytokine production was evaluated in culture supernatants by CTLL proliferation (IL-2), ELISA (IFN-[gamma]), CT4.S proliferation (IL-4) and in mRNA extracted from activated CD4+ cells by RT-PCR (IL-2, IFN-[gamma] and IL-4). Results show that the production of IL-2, as protein and mRNA, is profoundly depressed by aging, whereas that of IFN-[gamma], as protein and mRNA, firstly declines and then increases with age. The production of IL-4, as protein, monotonically declines with aging whereas, as mRNA, firstly decreases and then increases above the level in young mice. Spleen cells in culture were also incubated with mitogens and with a recombinant cytokine (IL-1[beta], IL-2, IL-3, IL-4, IL-12 or IFN-[gamma]) at various concentrations. It was found that recombinant cytokines by and large enhance cytokine production when the level induced by mitogens only is low. This conclusion applies to IL-2 and IFN-[gamma] production as protein and mRNA. The addition of recombinant cytokines also increases the production of IL-4 at the protein level in spleen cells from old mice but, at the mRNA level, only in spleen cells from young mice. This finding suggests age-related changes in IL-4-specific mRNA transcription rate and post-transcriptional half-life as well as translation kinetics.

Goyns, M. H., M. A. Charlton, et al. (1998). "Differential display analysis of gene expression indicates that age-related changes are restricted to a small cohort of genes." Mechanisms of Ageing and Development **101**(1-2): 73.

<http://www.sciencedirect.com/science/article/B6T31-3SY3GPD-6/2/883d564bc38f272e91bf524c8a5bf326>

It is clear that there is a genetic component associated with the ageing process. Although evolutionary theory has suggested that the activity of certain genes may facilitate ageing by favouring resource utilisation by the germ cells at the expense of somatic cells, there is reason to believe that the senescent phenotype, which is the endpoint of the ageing process, may be due to alterations in the levels of expression of other genes. To investigate this situation we have used the differential display technique to survey gene expression during ageing of the rat brain, heart and liver. By optimising this technique it is possible to identify up to 10000-14000 PCR products, which represent genes expressed in the tissue under study. Interestingly, only a relatively small cohort ([ap]2%) of these genes appear to show significant changes in their levels of expression during ageing. Characterisation of the latter has so far revealed certain genes, such as glial fibrillary acidic protein, which are associated with the senescent phenotype. It has also revealed that the level of fos, a component of the AP-1 transcription factor, decreases with age, which has implications for AP-1 regulated genes. The differential display technique has also revealed an increase in mitochondrial RNA during ageing of the heart, which may be due to a gene dosage effect caused by the presence of increased numbers of mitochondrial genomes in myocytes in old age. The differential display technique therefore appears to offer a powerful tool for identifying

genes which contribute to the emergence of a senescent phenotype.

Hardouin, S., F. Bourgeois, et al. (1998). "[beta]-Adrenergic and muscarinic receptor mRNA accumulation in the sinoatrial node area of adult and senescent rat hearts." Mechanisms of Ageing and Development **100**(3): 277.

<http://www.sciencedirect.com/science/article/B6T31-3SBVW1W-7/2/a25302ff79b2094b024744160b6d7978>

The sinoatrial (SA) node is the cardiac pacemaker and changes in its adrenergic-muscarinic phenotype have been postulated as a determinant of age-associated modifications in heart rate variability. To address this question, right atria were microdissected, the SA node area was identified by acetylcholinesterase staining, and, using a RT-PCR method, the accumulation of mRNA molecules encoding [beta]1- and [beta]2-adrenergic ([beta]1- and [beta]2-AR) and muscarinic (M2-R) receptor was quantified to define the proportion between [beta]-AR and M2-R mRNAs within the sinoatrial area of adult (3 months) and senescent (24 months) individual rat hearts. In adult hearts, the highest M2-R/[beta]-AR mRNA ratio was observed within the sinoatrial area compared with adjacent atrial myocardium, while in the senescent hearts, no difference was observed between sinoatrial and adjacent areas. This change was specific of the sinoatrial area since adult and senescent whole atrial or ventricular myocardium did not differ in their M2-R/[beta]-AR mRNA ratio, and was associated with a fragmentation of acetylcholinesterase staining of the senescent SA node. Quantitative changes in the expression of genes encoding proteins involved in heart rate regulation specifically affect the sinoatrial area of the senescent heart.

Iida, R., T. Yasuda, et al. (2000). "Age-related changes of gene expression in mouse kidney: fluorescence differential display -- PCR analyses." Mechanisms of Ageing and Development **113**(2): 135.

<http://www.sciencedirect.com/science/article/B6T31-3YGD54P-5/2/264dc89b51bc9e2fe07cbb458a3009bd>

We used a fluorescence differential display -- PCR (FDD-PCR) technique to analyze the genes expressed in mouse kidneys collected at nine different developmental stages ranging from 3 days to 15 months after birth. We found ten genes that were age-dependent and differentially-expressed in the kidneys during our experimental period. We confirmed by comparative RT-PCR that of the ten cDNAs, seven showed reproducible age-dependent expression. Four of the nucleotide sequences of these cDNA clones, had high homology with known genes (fibronectin, soluble guanylyl cyclase [alpha]-1 subunit, cytosolic aldehyde dehydrogenase and mitochondrial DNA), and three with expressed sequence tags of unknown genes. The FDD-PCR method was very useful for detecting new age-related genes expressed differentially in the mouse kidney.

Janapati, V., A. Wu, et al. (1995). "Post-transcriptional regulation of the Na⁺/Ca²⁺ exchanger in aging rat heart." Mechanisms of Ageing and Development **84**(3): 195.

<http://www.sciencedirect.com/science/article/B6T31-4031SF0-B/2/58f921810951f50305607fef030aa006>

Altered calcium homeostasis in the senescent heart appears to be the result, at least in part, of decreased Na⁺/Ca²⁺ exchange activity. To further investigate the basis of the decrease in

Na⁺/Ca²⁺ exchange activity, Na⁺/Ca²⁺ exchanger gene expression in the heart was compared in 3 and 24 month old male Fischer 344 rats. Sarcolemmal vesicles prepared from left ventricle and septum showed reduced Na⁺-dependent Ca²⁺ uptake in 24 month old animals when compared to 3 month old animals (0.156 ± 0.005 and 0.135 ± 0.008 nmol Ca²⁺/mg/10 s; mean ± S.E. for 3 month and 24 month old animals, respectively). Western analysis showed immunodetectable Na⁺/Ca²⁺ exchanger protein levels were decreased by 19% in 24 month old animals when compared to 3 month old animals. Poly(A⁺) RNA was purified from left and right ventricle and left and right atria and subjected to Northern analysis using digoxin labeled cDNA probes for the Na⁺/Ca²⁺ exchanger and actin. The Na⁺/Ca²⁺ exchanger probe labeled a 7 kb message in both ventricle and atria, while the actin probe labeled both [β]-actin (2.2 kb) and [α]-actin (1.4 kb). The steady state level of expression of Na⁺/Ca²⁺ exchanger Poly(A⁺) RNA when normalized to [β]-actin, was similar when ventricle and atria were compared. There were no observable differences in Na⁺/Ca²⁺ exchanger or [α]-actin Poly(A⁺) RNA steady state levels when comparing 3 and 24 month old animals. The results suggest that reduced Na⁺/Ca²⁺ exchange activity in the left ventricle of 24 month old animals was most likely the result of post-transcriptional modification of the protein that was detectable by Western analysis.

Jung, K.-Y., D. Dean, et al. (2004). "Loss of N-cadherin and [α]-catenin in the proximal tubules of aging male Fischer 344 rats." Mechanisms of Ageing and Development **125**(6): 445.

<http://www.sciencedirect.com/science/article/B6T31-4C8DD85-1/2/f64e1fcd3b3c2f00a4b40a6ae1b44c26>

Aging is associated with a loss of renal reserve, and increased sensitivity to either xenobiotic or physiologic insult. Given the critical role of the cadherin/catenin complex in establishing and maintaining the integrity and polarity of tubular epithelial cells, it was hypothesized that aging was associated with alterations in renal cadherin/catenin complexes. Histological assessment of aged (24 months) kidneys harvested from male Fischer 344 rats demonstrates mild degeneration of proximal tubules, multifocal chronic lymphocytic infiltration, moderate development of protein casts inside tubules, and tubular dilatation or degeneration. Western blot analysis revealed that N-cadherin protein expression is not constant over 24 months. N-cadherin expression increased from 4 to 9 months, with peak levels at 9 and 13 months. A decrease in expression was seen at 19 months and an almost complete loss of expression was seen at 24 months. In contrast, the expression of E- and Ksp-cadherin was constant over 24 months. A loss of [α]-catenin was seen at 19 and 24 months in the absence of changes in [β]-, [γ]-, and p120-catenin. This pattern of N-cadherin expression (increase followed by decrease) was confirmed by real-time PCR analysis, which demonstrated a similar pattern as the Western blot, suggesting that the loss of N-cadherin protein was due to decreased gene expression. The loss of N-cadherin was specific for the kidney, as no changes in N-cadherin expression in the liver, brain, or testes were seen during aging. The conclusion that loss of N-cadherin expression is a critical component of the renal dysfunction associated with aging is supported by the finding that caloric restriction attenuates the loss of N-cadherin, as well as the finding that a significant loss of N-cadherin is seen in the kidneys of ZDF x SHHF rats, a genetic model of end-stage renal disease. Cadherin and catenin expression was further analyzed by immunofluorescence. A significant loss of staining of both N-cadherin and [α]-catenin was seen in the proximal tubules of rats at 24 months. Interestingly, this corresponded with delocalization of the [α]-1 subunit of the Na⁺K⁺-ATPase, i.e. aberrant staining on cell-cell borders and some indication of apical staining in proximal tubules. Taken together, these data suggest that aging is associated with decreased expression of N-cadherin and [α]-catenin and is associated with a loss of cell polarity.

Kuehl-Kovarik, M. C., K. R. Magnusson, et al. (2000). "Electrophysiological analysis of NMDA receptor subunit changes in the aging mouse cortex." Mechanisms of Ageing and Development **115**(1-2):

<http://www.sciencedirect.com/science/article/B6T31-40GJ21G-3/2/f86c4ba64eed016f60591936696026a9>

NMDA receptors play an important role in memory processes and plasticity in the brain. We have previously demonstrated a significant decrease in NMDAR[ϵ]2 subunit mRNA and protein with increasing age in the C57Bl/6 mouse frontal cortex. In the present study, two-electrode voltage clamp electrophysiology on *Xenopus* oocytes injected with total RNA harvested from the frontal cortex of young and old C57Bl mice was used to detect changes in receptor composition during aging. Ifenprodil concentration-response curves, magnesium current-voltage curves, and single channel conductances were determined for native receptors. In addition, ifenprodil and magnesium curves were generated for recombinant NMDA receptors of varying subunit ratios. Ifenprodil dose-response curves for all receptors were biphasic. The low affinity component of the curve increased slightly with age, while the high affinity population decreased, mimicking recombinant receptors with decreasing levels of [ϵ]2. A decrease in maximal current was also observed in aged animals with decreased levels of [ϵ]2, although single channel conductances were identical between young and old mice. In addition, an increase in sensitivity to magnesium was observed for receptors from older animals. Results are consistent with the interpretation that the [ϵ]2 subunit is reduced in older mouse frontal cortex. A change in NMDA receptor subunit composition could influence memory processes during aging.

Nemani, M., M. Sahbatou, et al. (2000). "The efficiency of genetic analysis of DNA from aged siblings to detect chromosomal regions implicated in longevity." Mechanisms of Ageing and Development **119**(1-2): 25.

<http://www.sciencedirect.com/science/article/B6T31-41F62WP-4/2/dbd2b07103f96fe873b2355545c0b2f7>

Studies of the frequencies of different alleles in young adults and aged individuals have implicated several genes, such as ApoE and ACE, in longevity. However such association studies can easily give rise to spurious results through unsuspected population subdivision, and an approach making use of genetic relationships among relatives is desirable. We have studied the effectiveness of non-parametric genetic analysis to detect different types of loci affecting longevity. The non-parametric method has high statistical power to detect infrequent recessive alleles that are required for, or significantly increase the probability of, survival to advanced age. Statistical power is reduced if a proportion of carriers of the alternative allele is allowed to survive. The method is least effective in detecting alleles that occur at low frequency in young individuals and that subsequently experience high mortality, as is the case for carriers of the [ϵ]4 allele of ApoE. Genotyping errors will also reduce the value of the NPL statistic in a linear fashion with the error rate and the number of loci genotyped. We have also used the method to analyse genotypes of seven highly polymorphic markers near the ApoE gene in a sample of 188 sibships of nonagenarians and centenarians (n=434) and their children (n=124), however no excess sharing of alleles was detected.

O'Brien, M. E., S. Jensen, et al. (1998). "Hutchinson-Gilford progeria: faithful DNA maintenance, inheritance and allelic transcription of [beta](1-4) galactosyltransferase." Mechanisms of Ageing and Development **101**(1-2): 43.

<http://www.sciencedirect.com/science/article/B6T31-3SY3GPD-4/2/a543014d6fe9a83108ad0b1174a4aa5e>

Hutchinson-Gilford progeria syndrome (HGPS) is a fatal segmental aging disorder affecting children. There is a paucity of prior data at the nucleotide level on DNA maintenance in HGPS. We have examined the specific nucleotide sequences and production of allelic transcripts from the locus GGTB2 encoding [beta](1-4) galactosyltransferase. Quantitative Northern blots of mRNA from HGPS and control fibroblasts indicated identical mature [beta](1-4) galactosyltransferase transcript sizes and amounts, regardless of their altered glycosylation status. DNA sequencing of cDNA derived from HGPS [beta](1-4) galactosyltransferase mRNA populations confirmed the encoded amino acid sequence was unaffected. Population studies of 41 unrelated individuals provided allelic frequency estimates for a novel FokI polymorphism, which was identified in two of six progeria cell strains. The polymorphism was faithfully inherited in a progeria pedigree in a Mendelian manner. Furthermore, the polymorphism provided direct evidence through sequencing of reverse transcription polymerase chain reaction products that both alleles were transcribed and generated mature mRNA. Any defects in transcripts were below detectable levels over the lengths of coding sequences examined, despite multiple replication events from conception leading to the production and maintenance of patient-derived cells. These results indicate faithful transcription in HGPS.

Ohzeki, K., M. Yamaguchi, et al. (1999). "Effect of cellular aging on the induction of cyclooxygenase-2 by mechanical stress in human periodontal ligament cells." Mechanisms of Ageing and Development **108**(2): 151.

<http://www.sciencedirect.com/science/article/B6T31-3WHKRFH-5/2/9d39ccaac1423ba891584062951de9a3>

The production of prostaglandin (PGE₂) in human periodontal ligament fibroblast (hPLF) cells is increased by mechanical stress, however, the age-related changes in the susceptibility of hPLF cells in response to mechanical stress remain unclear. The purpose of this study was to examine the influence of in vitro cellular aging on PGE₂ production and the gene expression of cyclooxygenase (COX) in mechanically stressed hPLF cells. In vitro cellular aged hPLF cells were prepared by sequential subcultivations of hPLF cells from young healthy periodontal ligaments. In vitro cellular aged hPLF cells produced a significantly higher amount of PGE₂, as compared with young hPLF cells, when the cells were exposed to cyclic tension force in a time- and magnitude-dependent manner. The COX-2 mRNA level in aged cells was higher than that in young cells, whereas COX-1 mRNA remained unchanged. Since PGE₂ from hPLF cells was stimulated by in vitro aging as presented here, aging of hPLF cells may affect the severity of inflammation and bone resorption in the aged through the production of a large amount of PGE₂ in response to an excessive force such as a traumatic occlusion.

Shimizu, Y., J. Suzuki, et al. (2003). "In vitro aging of macaque adherent cells: similar pattern of cellular aging between human and macaque." Mechanisms of Ageing and Development **124**(2): 237.

<http://www.sciencedirect.com/science/article/B6T31-47F750C-1/2/fabed59b1c3d8a624db946ccc0d5bb52>

To explore new models for human cellular aging as well as to evaluate aging of the macaques, profiles of cellular aging in macaques were studied. Adherent cells were obtained from five Japanese macaques (*Macaca fuscata*), 14 long-tailed macaques (*Macaca fascicularis*), two bonnet monkeys (*Macaca radiata*) and a rhesus monkey (*Macaca mulatta*). A total of 35 cultures were performed and cell morphology, doubling time, telomere length and telomerase activity were studied. They were classified into three groups; group I: cell strains with a definite replicative life-span (<41 PDLs) (presence of M1), group II: cell strains with a limited extension of replicative life-span (79-106 PDLs) with p53 mutation(s) (presence of M2), and group III: a cell strain with an

indefinite replicative life-span (>150 PDLs) with characteristics of transformation. Except for the last group, telomerase activity was not observed. Macaque cells demonstrated three chronological patterns comprising both human and rodent patterns, however, presence of the two limits of proliferation in vitro grants macaque cells to be more appropriate than rodents in both studying human aging and oncogenesis.

Mechanisms of Development (17)

Bragg, A. D., H. L. Moses, et al. (2001). "Signaling to the epithelium is not sufficient to mediate all of the effects of transforming growth factor [beta] and bone morphogenetic protein 4 on murine embryonic lung development." Mechanisms of Development **109**(1): 13.

<http://www.sciencedirect.com/science/article/B6T9H-43VSC56-1/2/4ed51d02554b38d46c8b5c9ebc938efd>

Many studies have suggested that transforming growth factor [beta] (TGF-[beta]) and bone morphogenetic protein 4 (Bmp4) regulate early development of the lung. In this study, administration of growth factors directly into the lumen of lungs grown in organ culture was used to limit their activity to the epithelium and test the hypothesis that signaling to the epithelium is sufficient to mediate the known effects of TGF-[beta] and BMP-4 on early lung development. Addition of TGF-[beta]1, [beta]2, or [beta]3 to the medium surrounding lungs grown in organ culture resulted in decreased branching, reduced cell proliferation, accumulation of [alpha]-smooth muscle actin protein ([alpha]-SMA) in the mesenchyme, and decreased expression of a marker for respiratory epithelium, surfactant protein-C (Sp-C). When TGF-[beta]1 was restricted to the epithelium, accumulation of [alpha]-SMA and inhibition of Sp-C expression were not observed but branching and proliferation were inhibited. In contrast, branching was not inhibited in lungs where TGF-[beta]2 or TGF-[beta]3 were restricted to the epithelium suggesting differences in the mechanism of signaling by TGF-[beta]1, TGF-[beta]2 or TGF-[beta]3 in lung. Addition of Bmp4 to the medium surrounding lungs grown in organ culture stimulated cell proliferation and branching morphogenesis; however, direct injection of Bmp4 into the lung lumen had no effect on proliferation or branching. Based on these data and data from mesenchyme-free cultures, we propose that the mesenchyme influences growth factor signaling in the lung.

Groger, H., P. Callaerts, et al. (2000). "Characterization and expression analysis of an ancestor-type Pax gene in the hydrozoan jellyfish *Podocoryne carnea*." Mechanisms of Development **94**(1-2): 157.

<http://www.sciencedirect.com/science/article/B6T9H-40D61D7-G/2/244cdf18fa8047c379b96ea5bc8337e7>

We characterized a Pax gene from the hydrozoan *Podocoryne carnea*. It is most similar to cnidarian Pax-B genes and encodes a paired domain, a homeodomain and an octapeptide. Expression analysis demonstrates the presence of Pax-B transcripts in eggs, the ectoderm of the planula larva and in a few scattered cells in the apical polyp ectoderm. In developing and mature medusae, Pax-B is localized in particular endodermal cells, oriented toward the outside. Pax-B is not expressed in muscle cells. However, if isolated striated muscle tissue is activated for transdifferentiation, the gene is expressed within 1 h, before new cell types, such as smooth muscle and nerve cells, have formed. The expression data indicate that Pax-B is involved in

nerve cell differentiation.

Hinman, V. F., A. T. Nguyen, et al. (2003). "Expression and function of a starfish Otx ortholog, AmOtx: a conserved role for Otx proteins in endoderm development that predates divergence of the eleutherozoa." Mechanisms of Development **120**(10): 1165.

<http://www.sciencedirect.com/science/article/B6T9H-49H1FN9-1/2/4f079e3f8aa2d08249e03ff39ce3f7da>

The sea urchin orthodenticle (Otx)-related transcription factor is an early activator of other endomesodermally expressed transcription factors. Its normal function is required for the development of the archenteron and to lock cells into endomesodermal fate. To determine if this is a basal Otx function in echinoderms we have studied the role of an Otx ortholog in a starfish, *Asterina miniata*. The patterns of AmOtx expression are found to be similar, in many details, to those reported for other indirectly developing echinoderms and hemichordates, suggestive of a conserved function both in endoderm development and ciliary band formation in these two phyla. When downstream targets of the AmOtx protein are suppressed using a dominant engrailed repressor strategy, embryos fail to develop the endodermal component of the archenteron, though initial phases of mesoderm development proceed normally. The function of Otx proteins in endodermal development at least predated the evolution of the free-living echinoderms (Eleutherozoa).

Hodges, D. and S. I. Bernstein (1992). "Suboptimal 5' and 3' splice sites regulate alternative splicing of *Drosophila melanogaster* myosin heavy chain transcripts in vitro." Mechanisms of Development **37**(3): 127.

<http://www.sciencedirect.com/science/article/B6T9H-491TYSM-4/2/f6e5e1416a117f6511bc61acb0534f85>

Using a *Drosophila* cell-free system, we have analyzed the regulation of alternative splicing of *Drosophila* muscle myosin heavy chain (MHC) transcripts. Splicing of MHC 3' end transcripts results in exclusion of adult-specific alternative exon 18, as is observed in embryonic and larval muscle in vivo. Mutations that strengthen either the 5' or the 3' splice sites of exon 18 do not promote inclusion of this exon. However, strengthening both splice junctions results in efficient removal of both introns and completely inhibits skip splicing. Our data suggest that the affinity of exons 17 and 19, as well as failure of constitutive splicing factors to recognize exon 18 splice sites, causes the exclusion of exon 18 in wild-type transcripts processed in vitro.

Joos, T. O., R. David, et al. (1996). "xGCNF, a nuclear orphan receptor is expressed during neurulation in *Xenopus laevis*." Mechanisms of Development **60**(1): 45.

<http://www.sciencedirect.com/science/article/B6T9H-3W3158V-4/2/1689d08366ea0ab755840bcdd59b8794>

Nuclear orphan receptors are DNA binding proteins that share the domain structure of the nuclear hormone receptor superfamily, although ligands are unknown. We have identified an orphan receptor in *Xenopus laevis* and named it xGCNF based on its high degree of sequence homology to the previously described murine germ cell nuclear factor (mGCNF). In gel-electrophoresis mobility shift analysis experiments in vitro translated xGCNF and mGCNF proteins both bind

specifically as homodimers to the same response element, a direct repeat of the half-site consensus AGGTCA with zero spacing (DR0). Transcripts of xGCNF are found in oocytes and in much smaller amounts in the testes. In developmental Northern blots and RNase protection using RNA from different embryonic stages, zygotic expression of xGCNF peaks at midneurula. From late gastrula to midneurula stages, an anterior to posterior concentration gradient of the RNA was observed in whole mount in situ analysis. This antero-posterior gradient of expression was also observed in exogastrulae, both in the ectoderm and mesoderm. In the midneurula embryo, the mRNA was predominantly found in the neural plate and neural crest. Transcription of xGCNF in animal cap explants occurred independent of mesoderm induction.

Krovel, A. V. and L. C. Olsen (2002). "Expression of a vas:EGFP transgene in primordial germ cells of the zebrafish." Mechanisms of Development **116**(1-2): 141.

<http://www.sciencedirect.com/science/article/B6T9H-4604280-6/2/7eb545b5a8d192a047b2c6214e288393>

In zebrafish, maternally produced vasa (vas) transcripts become targeted to the cleavage planes of early embryos and subsequently incorporated into the primordial germ cells (PGCs). Zygotic vas transcription occurs from the onset of gastrulation. Here, we report on the characterisation of the zebrafish vas locus. The gene consists of 27 exons, spans about 25 kb, and contains two CpG-rich regions. We have used vas regulatory regions to establish transgenic zebrafish lines expressing enhanced green fluorescent protein (EGFP) in their PGCs. Maternally encoded vas:EGFP transcripts and VAS:EGFP protein segregate with the PGCs during embryogenesis. We find that the maternally deposited vas:EGFP transcripts are stable during embryogenesis at least up to 50 h of development. Vas:EGFP transcripts could not be detected in embryos that inherit the transgene from males, most likely due to the lack of one or more regulatory elements required for early zygotic expression. We show that vas:EGFP transcripts become enriched to the cleavage planes in early embryos, a finding that supported an RNA localisation signal localised within the vas region of these transcripts.

Panzer, Y., M. R. Esteban, et al. (2001). "Meics, a novel zinc-finger protein which relocates from nuclei to the central meiotic spindle during Drosophila spermatogenesis." Mechanisms of Development **106**(1-2): 151.

<http://www.sciencedirect.com/science/article/B6T9H-43J0R4T-J/2/752eb87171bac6f54629ed701a5cada7>

A Drosophila gene encoding a novel zinc-finger protein, Meics, was cloned using a monoclonal antibody. The predicted amino acid sequence contains 12 zinc-finger motifs of the C2H2-type. During spermatogenesis, Meics distributes intranuclearly at pre- and post-meiotic stages whereas it relocates to central-spindle microtubules at both meiotic divisions.

Pesce, M., X. Wang, et al. (1998). "Differential expression of the Oct-4 transcription factor during mouse germ cell differentiation." Mechanisms of Development **71**(1-2): 89.

<http://www.sciencedirect.com/science/article/B6T9H-3S7XFX8-7/2/442d7cc1db1a11fa82adc5c13c3a9d0c>

The POU transcription factor Oct-4 is expressed in early mouse embryogenesis and in pluripotent

embryonal stem (ES) and embryonal carcinoma (EC) stem cell lines. After gastrulation in the embryo, Oct-4 expression is confined to the germline. The present study provides evidence that Oct-4 undergoes downregulation during oogenesis and spermatogenesis, coincident with entry into meiosis. Furthermore, analysis of maturation stages of oocytes showed that Oct-4 is upregulated de novo in the final stages of meiotic prophase I in female germ cells. These data suggest that Oct-4 downregulation in germ cells in both sexes might represent one of the molecular triggers involved in the commitment to meiosis. The upregulation of Oct-4 in oocytes at the completion of the prophase I of meiotic division further suggests a specific involvement of this transcription factor in oocyte growth or the acquisition of meiotic competence.

Pitera, J. E., P. J. Milla, et al. (2001). "Cloning of HOXD1 from unfertilised human oocytes and expression analyses during murine oogenesis and embryogenesis." Mechanisms of Development **109**(2): 377.

<http://www.sciencedirect.com/science/article/B6T9H-442HVW8-7/2/03317bce14226ec767e32a210f8f35bd>

We describe the cloning of HOXD1 in human unfertilised oocytes and detailed expression analyses during mouse oogenesis and embryogenesis. The cDNA of 1991 bp has an open reading frame of 987 bp encoding a protein of 329 amino acids. A comparison of the amino acid sequence with the mouse homologue revealed an overall homology of 85.5% with 99% identity within the homeodomain. Expression was detected in unfertilised human oocytes and 2-, 4-, 8-cell and blastocyst stage embryos. Expression analyses in mature mouse ovaries, early embryos and isolated gut revealed expression in the oocytes of the primary and secondary ovarian follicles, and in embryonal mesodermal derivatives such as dermatomes, urogenital tubercle, tail bud, kidney, ovaries, testes and enteric mesoderm adjacent to the caecum where expression was up-regulated in vitro in response to increasing doses of retinoic acid. Our observations indicate a possible role for HOXD1/Hoxd1 in the ovarian oocytes and the establishment of mesodermal derivatives during embryogenesis.

Ritvos, O., T. Tuuri, et al. (1995). "Activin disrupts epithelial branching morphogenesis in developing glandular organs of the mouse." Mechanisms of Development **50**(2-3): 229.

<http://www.sciencedirect.com/science/article/B6T9H-3YVM6H9-C/2/e25af882159dbec2fc406420442d1f18>

We report that activin profoundly alters epithelial branching morphogenesis of embryonic mouse salivary gland, pancreas and kidney rudiments in culture, indicating that it may play a role as a morphogen during mammalian organogenesis. In developing pancreas and salivary gland rudiments, activin causes severe disruption of normal lobulation patterns of the epithelium whereas follistatin, an activin-binding protein, counteracts the effect of activin. In the kidney, activin delays branching of the ureter bud and reduces the number of secondary branches. TGF- β induces a pattern of aberrant branching in the ureter bud derived epithelium distinct from that seen for activin. Reverse-transcriptase polymerase chain reaction, Northern hybridization and in situ hybridization analyses indicate that these developing tissues express the mRNA transcripts for activin subunits, follistatin or activin receptors. Our results are suggestive of a potential role for the activin-follistatin system as an intrinsic regulator of epithelial branching morphogenesis during mammalian organogenesis.

Rizzoti, K., L. Paquereau, et al. (1998). "A constitutively activated mutant of G[alpha]q down-regulates EP-cadherin expression and decreases adhesion between ectodermal cells at gastrulation." Mechanisms of Development **76**(1-2): 19.

<http://www.sciencedirect.com/science/article/B6T9H-3TVXPY2-2/2/eb15a9ff7e6d023a3ed50957deabe16d>

We have examined the expression and function of the heterotrimeric GTP-binding protein Gq during early *Xenopus* embryogenesis. Abundant XG[alpha]q transcripts were detected in oocytes and early embryos by Northern blot analysis. In situ hybridization revealed that these transcripts are confined to the animal hemisphere of the mature oocyte and to the presumptive ectoderm of cleaving embryos. Microinjection at the two-cell stage of [alpha]q and Q209L[alpha]q, a constitutively activated mutant, causes a disruption in ectodermal cell adhesion at late gastrulation. Dissociation/reaggregation experiments performed on animal cap explants clearly demonstrate that the Q209L[alpha]q-induced phenotype occurs after reaggregation of the explants with a time-course similar to that observed in whole embryos. RT-PCR experiments performed on the explants from Q209L[alpha]q-injected embryos revealed a selective decrease in the amount of EP-cadherin mRNA. Co-injection of EP-cadherin RNA, but also E-cadherin RNA, rescued the disaggregated phenotype. These data emphasize the functional link between Gq protein-coupled signalling pathways and cadherin molecules in the ectodermal layer during the morphogenetic movements of gastrulation.

Sadek, C. M., S. Jalaguier, et al. (2000). "Isolation and characterization of AINT: a novel ARNT interacting protein expressed during murine embryonic development." Mechanisms of Development **97**(1-2): 13.

<http://www.sciencedirect.com/science/article/B6T9H-41WC91G-2/2/cfb86c4d1a056d349d6affabb5b07c4b>

Basic helix-loop-helix-PER-ARNT-SIM (bHLH-PAS) proteins form dimeric transcription factors to mediate diverse biological functions including xenobiotic metabolism, hypoxic response, circadian rhythm and central nervous system midline development. The Ah receptor nuclear translocator protein (ARNT) plays a central role as a common heterodimerization partner. Herein, we describe a novel, embryonically expressed, ARNT interacting protein (AINT) that may be a member of a larger coiled-coil PAS interacting protein family. The AINT C-terminus mediates interaction with the PAS domain of ARNT in yeast and interacts in vitro with ARNT and ARNT2 specifically. AINT localizes to the cytoplasm and overexpression leads to non-nuclear localization of ARNT. A dynamic pattern of AINT mRNA expression during embryogenesis and cerebellum ontogeny supports a role for AINT in development.

Shiraishi, E., H. Imazato, et al. (2004). "Identification of two teleost homologs of the *Drosophila* sex determination factor, transformer-2 in medaka (*Oryzias latipes*)." Mechanisms of Development **121**(7-8): 991.

<http://www.sciencedirect.com/science/article/B6T9H-4CNCT3Y-15/2/01aba50ed0f08aa1fdee8b4d47681b0c>

Transformer-2 (Tra2), an RNA-binding protein, is an important regulator in *Drosophila* sex determination. In vertebrates, however, the role of Tra2 homologues is not known. We identified two teleost homologues of Tra2, which we named Tra2a and Tra2b, in medaka (*Oryzias latipes*). Furthermore, we demonstrated that both Tra2 mRNAs were predominantly expressed in germ

cells of both sexes before the onset of sex differentiation, suggesting that both Tra2 homologues might be involved in the sex differentiation in medaka.

Su, M.-T., T. V. Venkatesh, et al. (1999). "The pioneer gene, *apontic*, is required for morphogenesis and function of the *Drosophila* heart." Mechanisms of Development **80**(2): 125.

<http://www.sciencedirect.com/science/article/B6T9H-3VYXXTM-1/2/de2653189cf412699c6dced32a9e7064>

In an effort to isolate genes required for heart development and to further our understanding of cardiac specification at the molecular level, we screened PlacZ enhancer trap lines for expression in the *Drosophila* heart. One of the lines generated in this screen, designated B2-2-15, was particularly interesting because of its early pattern of expression in cardiac precursor cells, which is dependent on the homeobox gene *tinman*, a key determinant of heart development in *Drosophila*. We isolated and characterized a gene in the vicinity of B2-2-15 that exhibits an identical expression pattern than the reporter gene of the enhancer trap. The product of this gene, *apontic* (*apt*; see also Gellon et al., 1997), does not appear to have any homology with known genes. *apt* mutant embryos show distinct abnormalities in heart morphology as early as mid-embryonic stages when the heart tube assembles, in that segments of heart cells (those of myocardial and pericardial identity) are often missing. Most strikingly, however, *apt* mutant embryos or larvae only develop a much reduced heart rate, perhaps because of defects in the assembly of an intact heart tube and/or because of defects in the function or physiological control of the myocardial cells, which normally mediate heart contractions. These cardiac defects may be the cause of death of these mutants during late embryonic or early larval stages.

Teel, A. L. and H. J. Yost (1996). "Embryonic expression patterns of *Xenopus* syndecans." Mechanisms of Development **59**(2): 115.

<http://www.sciencedirect.com/science/article/B6T9H-3W2TBCW-2/2/427b27f79a11de50f30f8b32786159a2>

Syndecans are a family of heparan sulfate proteoglycans implicated in cell-cell and cell-matrix interactions. To investigate the roles of syndecans in early development, we identified three syndecan family members in *Xenopus laevis*: Xsyn-1, Xsyn-2, and Xsyn-3. Xsyn-1 and Xsyn-2 are maternal mRNAs localized to the animal pole in blastulae, and are expressed in the ectoderm of gastrulae. In neurulae, Xsyn-1 is restricted to non-neural ectoderm and Xsyn-2 is restricted to neural ectoderm. In tailbud embryos, the three syndecans are expressed in adjacent, non-overlapping patterns. Xsyn-2 is expressed in the heart while Xsyn-1 is expressed in the underlying anterior endoderm. Xsyn-3 is expressed in the hindbrain, midbrain, and forebrain, while Xsyn-2 is expressed in the intervening regions. These results suggest that different members of the syndecan family have distinct developmental roles, perhaps acting as barriers to define tissue boundaries.

Therond, P., D. Busson, et al. (1993). "Molecular organisation and expression pattern of the segment polarity gene *fused* of *Drosophila melanogaster*." Mechanisms of Development **44**(1): 65.

<http://www.sciencedirect.com/science/article/B6T9H-491V0TK-BS/2/4ce61c3c52d835e9cff9c24f5c44d846>

The *Drosophila* segment-polarity gene *fused* (*fu*) is required for pattern formation within embryonic segments and imaginal discs. We previously reported that the 5' part of the *fused* gene is homologous to the catalytic domain of serine/threonine kinases. We present here the sequence of the complete transcription unit, which predicts a 805 amino acid long protein. The kinase domain actually corresponds to 268 amino acids in the N-terminal part, and no known function can be attributed to the rest of the putative FUSED protein. Transcripts from the *fused* gene have been characterized: a unique 3.2 kb *fused* transcript is produced in nurse cells, in low abundance, from stage 8 of oogenesis, and persistently through the rest of oogenesis. In embryos, this transcript is evenly distributed in all embryonic cells until the extended germ band stage, after which its amount strongly decreases. Ubiquitous expression is detected later in imaginal wing and leg discs. Possible roles of the FUSED protein in signal transduction pathways required for intercellular communication at different stages of development are discussed.

Wibrand, K. and L. C. Olsen (2002). "Linker histone H1M transcripts mark the developing germ line in zebrafish." Mechanisms of Development **117**(1-2): 249.

<http://www.sciencedirect.com/science/article/B6T9H-460M9MW-2/2/9c0f506d29c7142bb60bd4fca58fc2ae>

Maternally synthesised factors contribute to the establishment of the germ cell lineage in lower vertebrates. In zebrafish, germ-soma segregation appears to be completed by the late blastula stage of development. To search for new germ cell factors in the zebrafish, we have used subtractive cDNA cloning. Here we report that linker histone H1M transcripts mark the germ line from the early gastrulation up to 18 h post-fertilisation.

Metabolic Engineering (2)

Tao, L., R. E. Jackson, et al. (2005). "Directed evolution of copy number of a broad host range plasmid for metabolic engineering." Metabolic Engineering **7**(1): 10.

<http://www.sciencedirect.com/science/article/B6WN3-4F6CRCC-4/2/43a817de209efe98b03e17b2f861ae6e>

Random mutagenesis and directed evolution has been successfully used to improve desired properties of enzymes for biocatalysis and metabolic engineering. Here we employ the method to increase copy number of a pBBR-based broad host range plasmid, which can be used to express desired enzymes in a variety of microbial hosts. Localized random mutagenesis was performed in the replication control region of a pBBR-derived plasmid containing a [beta]-carotene reporter. Mutant plasmids were isolated that showed increased [beta]-carotene production. Real-time PCR analysis confirmed that the copy number of the mutant plasmids increased 3-7 fold. Sequence of the 10 mutant plasmids indicated that each plasmid contained single or multiple mutations in the *rep* gene or the flanking regions. Single amino acid change of serine to leucine at codon 100 of the replication protein and single nucleotide change of C to T at 46 bp upstream of the *rep* gene caused the increase of plasmid copy number. The utility of the mutant plasmids for metabolic engineering were further demonstrated by increased [beta]-carotene production, when an isoprenoid pathway gene (*dxs*) was co-expressed on a compatible plasmid. The mutant plasmids were tested in *Agrobacterium tumefaciens*. Increase of plasmid copy number and [beta]-carotene

production was also observed in the non-Escherichia coli host.

Van Eenennaam, A. L., G. Li, et al. (2004). "Elevation of seed [alpha]-tocopherol levels using plant-based transcription factors targeted to an endogenous locus." *Metabolic Engineering* **6**(2): 101.

<http://www.sciencedirect.com/science/article/B6WN3-4B66812-1/2/d85f7d659795e1b56e6472c256990fbd>

Synthetic zinc finger transcription factors (ZFP-TFs) were designed to upregulate the expression of the endogenous Arabidopsis [gamma]-tocopherol methyltransferase (GMT) gene. This gene encodes the enzyme responsible for the conversion of [gamma]-tocopherol to [alpha]-tocopherol, the tocopherol species with the highest vitamin E activity. Five three-finger zinc finger protein (ZFP) DNA binding domains were constructed and proven to bind tightly to 9 bp DNA sequences located in either the promoter or coding region of the GMT gene. When these ZFPs were fused to a nuclear localization signal and the maize C1 activation domain, four of the five resulting ZFP-TFs were able to upregulate the expression of the GMT gene in leaf protoplast transient assays. Seed-specific expression of these ZFP-TFs in transgenic Arabidopsis produced several lines with a heritable elevation in seed [alpha]-tocopherol. These results demonstrate that engineered ZFP-TFs comprised of plant-derived elements are capable of modulating the expression of endogenous genes in plants.

Metabolism (6)

Hallman, D. M., S. R. Srinivasan, et al. (2004). "The [beta]2-adrenergic receptor Arg16-gly polymorphism and interactions involving [beta]2- and [beta]3-adrenergic receptor polymorphisms are associated with variations in longitudinal serum lipid profiles: The Bogalusa Heart Study." *Metabolism* **53**(9): 1184.

<http://www.sciencedirect.com/science/article/B6WN4-4D4T0WJ-R/2/730400e89fda7a2349546e4dab4e90c1>

We examined the effects of combined genotypes of the [beta]2-adrenergic receptor (AR) Arg16-Gly and [beta]3-AR Trp64-Arg polymorphisms on longitudinal serum total (T-C) and low-density lipoprotein cholesterol (LDL-C) profiles in 1,198 subjects examined multiple times (6,488 observations) from 1973 to 1996 in the Bogalusa Heart Study, at ages from 4.5 to 38 years. Within 5-year age groups, T-C was significantly (P 2-AR Arg16/Arg16 homozygotes than in Gly16 carriers among those 4 to 8 (171.4 +/- 30.0 v 161.5 +/- 27.7 mg/dL), 9 to 13 (167.7 +/- 28.6 v 162.4 +/- 27.4 mg/dL), and 14 to 18 (158.8 +/- 29.6 v 154.7 +/- 27.5 mg/dL) years of age, but not in those 19 to 23, 24 to 28, 29 to 33, or 34 to 38 years of age. The [beta]3-AR polymorphism was not associated with variation in either T-C or LDL-C. In multilevel polynomial growth curve models, the combination of the [beta]2-AR Arg16/Arg16 genotype with either the [beta]3-AR Arg64/Arg64 or Trp64/Arg64 genotypes, denoted AA/AX, was associated with variation in longitudinal T-C (P 2/[beta]3-AR genotype and lipid profiles differed among race/sex groups, being most marked in black females, in whom the AA/AX combination was associated with higher T-C and LDL-C profiles across all ages. In White males, the AA/AX combination was most strongly associated with higher lipids in adults. In black males and white females, lipid profiles differed little between genotype groups. Our findings suggest that the [beta]2-AR Arg16-Gly

genotype influences T-C and LDL-C levels in an age-specific manner, that it may interact with [beta]3-AR Trp64-Arg genotypes to influence longitudinal T-C and LDL-C profiles, and that the effect of combined [beta]2/[beta]3-AR genotypes on T-C and LDL-C profiles may differ among race/sex groups.

Kristensen, K., S. B. Pedersen, et al. (1999). "Regulation of leptin by thyroid hormone in humans: Studies in vivo and in vitro." Metabolism **48**(12): 1603.

<http://www.sciencedirect.com/science/article/B6WN4-4D6XM8X-P/2/71e015d9814feeb4834df33c865f83e8>

The influence of thyroid hormones on human adipose tissue leptin production and leptin gene expression was investigated in vitro and in vivo. Twelve women received 60 [mu]g triiodothyronine (T3) per day for 7 days, which increased total T3 by 195% (1.78 +/- 0.07 to 5.25 +/- 0.39 mU/L, P P P v 8.90 +/- 1.3 [mu]g/L, nonsignificant). Human subcutaneous adipose tissue biopsies from eight healthy women were incubated in vitro as small fragments with T3 in concentrations from 1 to 50 nmol/L. Leptin production was inhibited dose-dependently. After 24 hours of incubation, a T3 concentration of 50 nmol/L reduced basal leptin production by 42% (P P 3 decreased basal leptin mRNA expression by 47% compared with controls (P 3 (> 20 nmol/L) inhibited leptin production and leptin gene expression in vitro, whereas an elevation of T3 corresponding to a moderate thyrotoxic state (T3 5.25 +/- 0.39 nmol/L) was without any impact on serum leptin levels in vivo.

Sbraccia, P., A. Giaccari, et al. (1998). "Expression of the two insulin receptor isoforms is not altered in the skeletal muscle and liver of diabetic rats." Metabolism **47**(2): 129.

<http://www.sciencedirect.com/science/article/B6WN4-4D6XKW1-BG/2/6ecac3d43fc9168afdcbacd4302ae04c>

Alternative splicing of the 36--base pair exon 11 of the human insulin receptor (IR) gene and of the corresponding domain of the rat IR gene results in the synthesis of two IR isoforms with distinct functional characteristics. Altered expression of these IR isoforms has been previously demonstrated in the skeletal muscle of patients with non--insulin-dependent diabetes mellitus (NIDDM); however, this observation was not confirmed by other studies and is still a matter of debate. To assess whether the reported altered isoform expression is due to the secondary metabolic derangement of diabetes, we examined alternative splicing of IR mRNAs (IR36+ and IR36-, corresponding to human Ex11+ and Ex11-) in the skeletal muscle and liver of 6-hour fasting 90% pancreatectomized insulin-resistant diabetic and control Sprague-Dawley rats, using the reverse transcriptase--polymerase chain reaction (PCR) technique. Both diabetic and control rats showed the same pattern of IR mRNA expression: the liver exclusively expressed IR36+ mRNA, whereas only IR36- mRNA was detected in muscle. In conclusion, diabetes mellitus per se does not alter the expression of IR isoforms in the liver and skeletal muscle, and therefore, at least in this animal model of NIDDM, impaired insulin action develops independently from a relative increase in IR36+ mRNA expression in skeletal muscle.

Srinivasan, S. R., S. Li, et al. (2003). "R219K polymorphism of the ABCA1 gene and its modulation of the variations in serum high-density lipoprotein cholesterol and triglycerides related to age and adiposity in white versus black young adults. The bogalusa heart study." Metabolism **52**(7): 930.

<http://www.sciencedirect.com/science/article/B6WN4-490H4VJ-X/2/c8b9f4173d4dcfe1cfd1461f5473b4ce>

Mutations in adenosine triphosphate (ATP)-binding cassette transporter 1 (ABCA1) gene have been established as the molecular defect in Tangier disease and familial hypoalphalipoproteinemia, uncommon genetic disorders characterized by deficient or depressed high-density lipoprotein (HDL) cholesterol and increased triglycerides. However, information regarding the frequency of common variants, including Arg219Lys (R219K) within the coding region of the ABCA1 gene and their effect on these phenotypes in the general population is limited. This study examined the frequency and phenotypic effect of R219K variant in a community-based sample of 887 white and 390 black young adults aged 20 to 38 years. The frequency of the variant allele (K219) was higher in blacks than in whites (0.595 v 0.262, $P = .029$) were found in whites. Carriers (KK+RK), unlike noncarriers (RR) showed a positive relationship between age and HDL cholesterol (regression coefficient $[\beta] = 0.28$, $P = .029$ for carriers v $[\beta] = -0.18$, $P = .112$ for noncarriers). In addition, the variant allele attenuated the adverse positive relationship between BMI and triglycerides ($[\beta] = 0.032$, $P < .001$ for noncarriers). These results indicate that the K219 allele frequency differs markedly between blacks and whites, and that the variant-allele modulates the association between age and HDL cholesterol, as well as body fatness and triglycerides in a beneficial manner only in whites.

Yokoyama, H., H. Tahara, et al. (2005). "The K469E polymorphism of the intercellular adhesion molecule-1 gene is associated with plasma fibrinogen level in type 2 diabetes." *Metabolism* **54**(3): 381.

<http://www.sciencedirect.com/science/article/B6WN4-4FHJ9TP-P/2/1d74028a8ffe955a62d3eb0d8835d4a9>

Intercellular adhesion molecule-1 (ICAM-1) is involved in inflammation and development of atherosclerotic change of vascular endothelium. The aim of the present study is to investigate whether K469E polymorphism of the ICAM-1 gene is associated with various clinical factors including plasma fibrinogen in patients with type 2 diabetes. ICAM-1 gene polymorphism was examined using polymerase chain reaction and restriction enzyme analysis in 360 type 2 diabetic patients. Plasma fibrinogen levels and other clinical variables were measured as well as circulating soluble ICAM-1 (sICAM-1) levels by enzyme-linked immunosorbent assay. The distribution of ICAM-1 genotypes, EE, EK, and KK, was not significantly different between type 2 diabetes and 152 healthy control subjects. Among 3 groups according to ICAM-1 genotypes in type 2 diabetes, no difference was found in adiposity, glycemic control, lipid profile, insulin sensitivity evaluated by homeostasis model assessment, or sICAM-1. Regarding fibrinogen, the patients with E allele showed significantly lower plasma fibrinogen levels in a dose-dependent manner ($P = .033$). Spearman rank correlation analyses revealed that ICAM-1 genotype showed significant correlation with plasma fibrinogen level ($P R^2 = 0.148$, $P < .001$). In conclusion, K469E polymorphism of the ICAM-1 gene had impact on plasma fibrinogen level independently of other clinical factors in 360 type 2 diabetic patients, suggesting that fibrinogen is a candidate which links the ICAM-1 gene polymorphism to atherosclerosis.

Yuan, X., K. Yamada, et al. (1999). "Analysis of trinucleotide-repeat combination polymorphism at the rad gene in patients with type 2 diabetes mellitus." *Metabolism* **48**(2): 173.

<http://www.sciencedirect.com/science/article/B6WN4-4D6XKP9-92/2/8d44da8220b4da43689ae4c75f751676>

A combined (GTT)_n (ATT)_n trinucleotide-repeat polymorphism designated as RAD1 has been

identified at intron 2 of the rad gene on chromosome 16q. An association between the total length of the RAD1 locus and type 2 diabetes has been shown in white American subjects, but not in Finns. We genotyped 115 Japanese patients with type 2 diabetes and 114 nondiabetic control subjects at the RAD1 locus by the direct sequencing method, and found 16 RAD1 alleles composed of various combinations of GTTs and ATTs. Allele 14 consisting of four GTTs and seven ATTs accounted for the majority in both control subjects and diabetic patients, suggesting that RAD1 polymorphism is not a major genetic component for susceptibility to common forms of diabetes in the Japanese. There was no significant association between total repeat length and diabetes. However, the frequency of minor alleles containing five GTTs or three GTTs was significantly higher in diabetic patients versus nondiabetic subjects (4.8% v 0.9%, $P = .012$). Thus, genetic variability at the rad gene in linkage disequilibrium with RAD1 could be associated with a predisposition to type 2 diabetes in the Japanese population.

Methods (5)

DeRyckere, D. and J. DeGregori (2002). "Identification and characterization of transcription factor target genes using gene-targeted mice." Methods **26**(1): 57.

<http://www.sciencedirect.com/science/article/B6WN5-45MGN48-8/2/54e4ae6759fa9a01acc55552df5daaae>

Dobrovic, A., T. Bianco, et al. (2002). "Screening for and analysis of methylation differences using methylation-sensitive single-strand conformation analysis." Methods **27**(2): 134.

<http://www.sciencedirect.com/science/article/B6WN5-466CGKS-7/2/1a73cc2765a421ab59ca5065cce8a845>

Millar, D. S., P. M. Warnecke, et al. (2002). "Methylation sequencing from limiting DNA: embryonic, fixed, and microdissected cells." Methods **27**(2): 108.

<http://www.sciencedirect.com/science/article/B6WN5-466CGKS-3/2/4ad9183769098660b3bdfd8695ec9f26>

Niizeki, H., H. Inoko, et al. (2002). "Polymorphisms in the TNF region confer susceptibility to UVB-induced impairment of contact hypersensitivity induction in mice and humans." Methods **28**(1): 46.

<http://www.sciencedirect.com/science/article/B6WN5-46T3743-9/2/c0a7161677e1f3e5fccb1eaef15b6d3c>

Saito, S., R. Matoba, et al. (2003). "Adapter-tagged competitive PCR (ATAC-PCR) - a high-throughput quantitative PCR method for microarray validation." Methods **31**(4): 326.

<http://www.sciencedirect.com/science/article/B6WN5-49W60TF-B/2/2c5249679dfe760060dee9ead782ba50>

Adapter-tagged competitive PCR (ATAC-PCR) is an advanced version of competitive quantitative PCR that is characterized by the addition of unique adapters to cDNA derived from each sample RNA. Using multiple adapters, we can accurately measure the relative expression ratios of many samples, with a calibration curve obtained from internal standards included in the same reaction. ATAC-PCR can identify differences in gene expression as small as twofold, even from very small amounts of sample RNA. This technique is suitable for confirming results obtained with cDNA microarrays or differential display, and it can process more than a thousand of genes per day when used in conjunction with a capillary DNA sequencer.

Mol. Biol. Cell (10)

Askjaer, P., V. Galy, et al. (2002). "Ran GTPase Cycle and Importins alpha and beta Are Essential for Spindle Formation and Nuclear Envelope Assembly in Living *Caenorhabditis elegans* Embryos." Mol. Biol. Cell **13**(12): 4355-4370.

<http://www.molbiolcell.org/cgi/content/abstract/13/12/4355>

The small GTPase Ran has been found to play pivotal roles in several aspects of cell function. We have investigated the role of the Ran GTPase cycle in spindle formation and nuclear envelope assembly in dividing *Caenorhabditis elegans* embryos in real time. We found that Ran and its cofactors RanBP2, RanGAP, and RCC1 are all essential for reformation of the nuclear envelope after cell division. Reducing the expression of any of these components of the Ran GTPase cycle by RNAi leads to strong extranuclear clustering of integral nuclear envelope proteins and nucleoporins. Ran, RanBP2, and RanGAP are also required for building a mitotic spindle, whereas astral microtubules are normal in the absence of these proteins. RCC1(RNAi) embryos have similar abnormalities in the initial phase of spindle formation but eventually recover to form a bipolar spindle. Irregular chromatin structures and chromatin bridges due to spindle failure were frequently observed in embryos where the Ran cycle was perturbed. In addition, connection between the centrosomes and the male pronucleus, and thus centrosome positioning, depends upon the Ran cycle components. Finally, we have demonstrated that both IMA-2 and IMB-1, the homologues of vertebrate importin [alpha] and [beta], are essential for both spindle assembly and nuclear formation in early embryos.

Chou, W.-C., V. Prokova, et al. (2003). "Mechanism of a Transcriptional Cross Talk between Transforming Growth Factor-beta -regulated Smad3 and Smad4 Proteins and Orphan Nuclear Receptor Hepatocyte Nuclear Factor-4." Mol. Biol. Cell **14**(3): 1279-1294.

<http://www.molbiolcell.org/cgi/content/abstract/14/3/1279>

We have shown previously that the transforming growth factor-[beta] (TGF[beta])-regulated Smad-Mad (Smad) protein 3 and Smad4 proteins transactivate the apolipoprotein C-III promoter in hepatic cells via a hormone response element that binds the nuclear receptor hepatocyte nuclear factor 4 (HNF-4). In the present study, we show that Smad3 and Smad4 but not Smad2 physically interact with HNF-4 via their Mad homology 1 domains both in vitro and in vivo. The synergistic

transactivation of target promoters by Smads and HNF-4 was shown to depend on the specific promoter context and did not require an intact [beta]-hairpin/DNA binding domain of the Smads. Using glutathione S-transferase interaction assays, we established that two regions of HNF-4, the N-terminal activation function 1 (AF-1) domain (aa 1-24) and the C-terminal F domain (aa 388-455) can mediate physical Smad3/HNF-4 interactions in vitro. In vivo, Smad3 and Smad4 proteins enhanced the transactivation function of various GAL4-HNF-4 fusion proteins via the AF-1 and the adjacent DNA binding domain, whereas a single tyrosine to alanine substitution in AF-1 abolished coactivation by Smads. The findings suggest that the transcriptional cross talk between the TGF[beta]-regulated Smads and HNF-4 is mediated by specific functional domains in the two types of transcription factors. Furthermore, the specificity of this interaction for certain target promoters may play an important role in various hepatocyte functions, which are regulated by TGF[beta] and the Smads.

Eisenkolb, M., C. Zenzmaier, et al. (2002). "A Specific Structural Requirement for Ergosterol in Long-chain Fatty Acid Synthesis Mutants Important for Maintaining Raft Domains in Yeast." Mol. Biol. Cell **13**(12): 4414-4428.

<http://www.molbiolcell.org/cgi/content/abstract/13/12/4414>

Fungal sphingolipids contain ceramide with a very-long-chain fatty acid (C26). To investigate the physiological significance of the C26-substitution on this lipid, we performed a screen for mutants that are synthetically lethal with ELO3. Elo3p is a component of the ER-associated fatty acid elongase and is required for the final elongation cycle to produce C26 from C22/C24 fatty acids. elo3[Delta] mutant cells thus contain C22/C24- instead of the natural C26-substituted ceramide. We now report that under these conditions, an otherwise nonessential, but also fungal-specific, structural modification of the major sterol of yeast, ergosterol, becomes essential, because mutations in ELO3 are synthetically lethal with mutations in ERG6. Erg6p catalyzes the methylation of carbon atom 24 in the aliphatic side chain of sterol. The lethality of an elo3[Delta] erg6[Delta] double mutant is rescued by supplementation with ergosterol but not with cholesterol, indicating a vital structural requirement for the ergosterol-specific methyl group. To characterize this structural requirement in more detail, we generated a strain that is temperature sensitive for the function of Erg6p in an elo3[Delta] mutant background. Examination of raft association of the GPI-anchored Gas1p and plasma membrane ATPase, Pma1p, in the conditional elo3[Delta] erg6ts double mutant, revealed a specific defect of the mutant to maintain raft association of preexisting Pma1p. Interestingly, in an elo3[Delta] mutant at 37{degrees}C, newly synthesized Pma1p failed to enter raft domains early in the biosynthetic pathway, and upon arrival at the plasma membrane was rerouted to the vacuole for degradation. These observations indicate that the C26 fatty acid substitution on lipids is important for establishing raft association of Pma1p and stabilizing the protein at the cell surface. Analysis of raft lipids in the conditional mutant strain revealed a selective enrichment of ergosterol in detergent-resistant membrane domains, indicating that specific structural determinants on both sterols and sphingolipids are required for their association into raft domains.

Lee, K. M., I. Miklos, et al. (2005). "Impairment of the TFIIF-associated CDK-activating Kinase Selectively Affects Cell Cycle-regulated Gene Expression in Fission Yeast." Mol. Biol. Cell: E04-11-0982.

<http://www.molbiolcell.org/cgi/content/abstract/E04-11-0982v1>

Monitoring Editor: Tim Stearns
The fission yeast Mcs6-Mcs2-Pmh1 complex, homologous to metazoan Cdk7-cyclin H-Mat1, has dual functions in cell division and transcription: as a partially redundant CDK-activating kinase (CAK) that phosphorylates the major cell-cycle CDK, Cdc2, on Thr-167; and as the RNA polymerase (Pol) II carboxyl-terminal domain (CTD) kinase associated

with transcription factor (TF) IIH. We analyzed conditional mutants of *mcs6* and *pmh1*, which activate Cdc2 normally but cannot complete cell division at restrictive temperature and arrest with decreased CTD phosphorylation. Transcriptional profiling by microarray hybridization revealed only modest effects on global gene expression: a one-third reduction in a severe *mcs6* mutant after prolonged incubation at 36°C. In contrast, a small subset of transcripts (~5%) decreased by >2-fold after Mcs6-complex function was compromised. The signature of repressed genes overlapped significantly with those of cell-separation mutants *sep10* and *sep15*. *Sep10*, a component of the Pol II Mediator complex, becomes essential in *mcs6* or *pmh1* mutant backgrounds. Moreover, transcripts dependent on the forkhead transcription factor *Sep1*, which are expressed coordinately during mitosis, were repressed in Mcs6-complex mutants, and Mcs6 also interacts genetically with *Sep1*. Thus the Mcs6 complex, a direct activator of Cdc2, also influences the cell-cycle transcriptional program, possibly through its TFIH-associated kinase function.

Park, E. K., N. Warner, et al. (2004). "Ectopic EphA4 Receptor Induces Posterior Protrusions via FGF Signaling in *Xenopus* Embryos." *Mol. Biol. Cell* **15**(4): 1647-1655.

<http://www.molbiolcell.org/cgi/content/abstract/15/4/1647>

The Eph family of receptor tyrosine kinases regulates numerous biological processes. To examine the biochemical and developmental contributions of specific structural motifs within Eph receptors, wild-type or mutant forms of the EphA4 receptor were ectopically expressed in developing *Xenopus* embryos. Wild-type EphA4 and a mutant lacking both the SAM domain and PDZ binding motif were constitutively tyrosine phosphorylated in vivo and catalytically active in vitro. EphA4 induced loss of cell adhesion, ventro-lateral protrusions, and severely expanded posterior structures in *Xenopus* embryos. Moreover, mutation of a conserved SAM domain tyrosine to phenylalanine (Y928F) enhanced the ability of EphA4 to induce these phenotypes, suggesting that the SAM domain may negatively regulate some aspects of EphA4 activity in *Xenopus*. Analysis of double mutants revealed that the Y928F EphA4 phenotypes were dependent on kinase activity; juxtamembrane sites of tyrosine phosphorylation and SH2 domain-binding were required for cell dissociation, but not for posterior protrusions. The induction of protrusions and expansion of posterior structures is similar to phenotypic effects observed in *Xenopus* embryos expressing activated FGFR1. Furthermore, the budding ectopic protrusions induced by EphA4 express FGF-8, FGFR1, and FGFR4a. In addition, antisense morpholino oligonucleotide-mediated loss of FGF-8 expression in vivo substantially reduced the phenotypic effects in EphA4Y928F expressing embryos, suggesting a connection between Eph and FGF signaling.

Rudge, S. A., D. M. Anderson, et al. (2004). "Vacuole Size Control: Regulation of PtdIns(3,5)P₂ Levels by the Vacuole-associated Vac14-Fig4 Complex, a PtdIns(3,5)P₂-specific Phosphatase." *Mol. Biol. Cell* **15**(1): 24-36.

<http://www.molbiolcell.org/cgi/content/abstract/15/1/24>

In the budding yeast *Saccharomyces cerevisiae*, phosphatidylinositol 3,5-bisphosphate (PtdIns(3,5)P₂) is synthesized by a single phosphatidylinositol 3-phosphate 5-kinase, Fab1. Cells deficient in PtdIns(3,5)P₂ synthesis exhibit a grossly enlarged vacuole morphology, whereas increased levels of PtdIns(3,5)P₂ provokes the formation of multiple small vacuoles, suggesting a specific role for PtdIns(3,5)P₂ in vacuole size control. Genetic studies have indicated that Fab1 kinase is positively regulated by Vac7 and Vac14; deletion of either gene results in ablation of PtdIns(3,5)P₂ synthesis and the formation of a grossly enlarged vacuole. More recently, a suppressor of *vac7*Δ mutants was identified and shown to encode a putative

phosphoinositide phosphatase, Fig4. We demonstrate that Fig4 is a magnesium-activated PtdIns(3,5)P₂-selective phosphoinositide phosphatase in vitro. Analysis of a Fig4-GFP fusion protein revealed that the Fig4 phosphatase is localized to the limiting membrane of the vacuole. Surprisingly, in the absence of Vac14, Fig4-GFP no longer localizes to the vacuole. However, Fig4-GFP remains localized to the grossly enlarged vacuoles of vac7 deletion mutants. Consistent with these observations, we found that Fig4 physically associates with Vac14 in a common membrane-associated complex. Our studies indicate that Vac14 both positively regulates Fab1 kinase activity and directs the localization/activation of the Fig4 PtdIns(3,5)P₂ phosphatase.

Sciorra, V. A., A. Audhya, et al. (2005). "Synthetic Genetic Array Analysis of the PtdIns 4-kinase Pik1p Identifies Components in a Golgi-specific Ypt31/rab-GTPase Signaling Pathway." Mol. Biol. Cell **16**(2): 776-793.

<http://www.molbiolcell.org/cgi/content/abstract/16/2/776>

Phosphorylated derivatives of phosphatidylinositol are essential regulators of both endocytic and exocytic trafficking in eukaryotic cells. In *Saccharomyces cerevisiae*, the phosphatidylinositol 4-kinase, Pik1p generates a distinct pool of PtdIns(4)P that is required for normal Golgi structure and secretory function. Here, we utilize a synthetic genetic array analysis of a conditional pik1 mutant to identify candidate components of the Pik1p/PtdIns(4)P signaling pathway at the Golgi. Our data suggest a mechanistic involvement for Pik1p with a specific subset of Golgi-associated proteins, including the Ypt31p rab-GTPase and the TRAPPII protein complex, to regulate protein trafficking through the secretory pathway. We further demonstrate that TRAPPII specifically functions in a Ypt31p-dependent pathway and identify Gyp2p as the first biologically relevant GTPase activating protein for Ypt31p. We propose that multiple stage-specific signals, which may include Pik1p/PtdIns(4)P, TRAPPII and Gyp2p, impinge upon Ypt31 signaling to regulate Golgi secretory function.

Stang, E., F. D. Blystad, et al. (2004). "Cbl-dependent Ubiquitination Is Required for Progression of EGF Receptors into Clathrin-coated Pits." Mol. Biol. Cell **15**(8): 3591-3604.

<http://www.molbiolcell.org/cgi/content/abstract/15/8/3591>

Ligand binding causes the EGF receptor (EGFR) to become ubiquitinated by Cbl upon association with the adaptor protein Grb2. We have investigated the role of ubiquitin and Grb2 in ligand-induced endocytosis of the EGFR. Incubation of cells with EGF on ice caused translocation of Grb2 and Cbl from the cytosol to the rim of coated pits. Grb2 with point mutations in both SH3 domains inhibited recruitment of the EGFR to clathrin-coated pits, in a Ras-independent manner. On overexpression of the Cbl-binding protein Sprouty, ubiquitination of the EGFR was inhibited, the EGFR was recruited only to the rim of coated pits, and endocytosis of the EGFR was inhibited. Conjugation-defective ubiquitin similarly inhibited recruitment of EGF-EGFR to clathrin-coated pits. Even though this does not prove that cargo must be ubiquitinated, this indicates the importance of interaction of ubiquitinated protein(s) with proteins harboring ubiquitin-interacting domains. We propose that Grb2 mediates transient anchoring of the EGFR to an Eps15-containing molecular complex at the rim of coated pits and that Cbl-induced ubiquitination of the EGFR allows relocation of EGFR from the rim to the center of clathrin-coated pits.

Tatzer, V., G. Zellnig, et al. (2002). "Lipid-dependent Subcellular Relocalization of the Acyl Chain Desaturase in Yeast." Mol. Biol. Cell **13**(12): 4429-4442.

<http://www.molbiolcell.org/cgi/content/abstract/13/12/4429>

The degree of acyl chain desaturation of membrane lipids is a critical determinant of membrane fluidity. Temperature-sensitive mutants of the single essential acyl chain desaturase, Ole1p, of yeast have previously been isolated in screens for mitochondrial inheritance mutants (Stewart, L.C., and Yaffe, M.P. (1991). *J. Cell Biol.* 115, 1249-1257). We now report that the mutant desaturase relocalizes from its uniform ER distribution to a more punctuate localization at the cell periphery upon inactivation of the enzyme. This relocalization takes place within minutes at nonpermissive conditions, a time scale at which mitochondrial morphology and inheritance is not yet affected. Relocalization of the desaturase is fully reversible and does not affect the steady state localization of other ER resident proteins or the kinetic and fidelity of the secretory pathway, indicating a high degree of selectivity for the desaturase. Relocalization of the desaturase is energy independent but is lipid dependent because it is rescued by supplementation with unsaturated fatty acids. Relocalization of the desaturase is also observed in cells treated with inhibitors of the enzyme, indicating that it is independent of temperature-induced alterations of the enzyme. In the absence of desaturase function, lipid synthesis continues, resulting in the generation of lipids with saturated acyl chains. A model is discussed in which the accumulation of saturated lipids in a microdomain around the desaturase could induce the observed segregation and relocalization of the enzyme.

Vedrenne, C., C. Giroud, et al. (2002). "Two Related Subpellicular Cytoskeleton-associated Proteins in *Trypanosoma brucei* Stabilize Microtubules." Mol. Biol. Cell **13**(3): 1058-1070.

<http://www.molbiolcell.org/cgi/content/abstract/13/3/1058>

The subpellicular microtubules of the trypanosome cytoskeleton are cross-linked to each other and the plasma membrane, creating a cage-like structure. We have isolated, from *Trypanosoma brucei*, two related low-molecular-weight cytoskeleton-associated proteins (15- and 17-kDa), called CAP15 and CAP17, which are differentially expressed during the life cycle. Immunolabeling shows a corset-like colocalization of both CAPs and tubulin. Western blot and electron microscope analyses show CAP15 and CAP17 labeling on detergent-extracted cytoskeletons. However, the localization of both proteins is restricted to the anterior, microtubule minus, and less dynamic half of the corset. CAP15 and CAP17 share properties of microtubule-associated proteins when expressed in heterologous cells (Chinese hamster ovary and HeLa), colocalization with their microtubules, induction of microtubule bundle formation, cold resistance, and insensitivity to nocodazole. When overexpressed in *T. brucei*, both CAP15 and CAP17 cover the whole subpellicular corset and induce morphological disorders, cell cycle-based abnormalities, and subsequent asymmetric cytokinesis.

Mol. Biol. Evol.(19)

Beck, N. R., M. C. Double, et al. (2003). "Microsatellite Evolution at Two Hypervariable Loci Revealed by Extensive Avian Pedigrees." Mol. Biol. Evol. **20**(1): 54-61.

<http://mbe.oupjournals.org/cgi/content/abstract/20/1/54>

Genealogies generated through a long-term study of superb fairy-wrens (*Malurus cyaneus*) were used to investigate mutation within two hypervariable microsatellite loci. Of 3,230 meioses examined at the tetranucleotide locus (Mcy{micro}8), 45 mutations were identified, giving a mutation rate of 1.4%. At the dinucleotide locus (Mcy{micro}4) 30 mutations were recorded from 2,750 meioses giving a mutation rate of 1.1%. Mutations at both loci primarily (80%; 60/75) involved the loss or gain of a single repeat unit. Unlike previous studies, there was no significant bias toward additions over deletions. The mutation rate at Mcy{micro}8 increased with allele size, and very long alleles (>70 repeats) mutated at a rate of almost 20%. The length of the mutating allele and allele span, however, were strongly correlated so it was not possible to isolate the causative factor. Allele size did not appear to affect mutation rate at Mcy{micro}4, but the repeat number was considerably lower at this locus. The gender of the mutating parent was significant only at Mcy{micro}8, where mutations occurred more frequently in maternal alleles. However, at both loci we found that alleles inherited from the mother were on average larger than those from the father, and this in part drove the higher mutation rate among maternally inherited alleles at Mcy{micro}8.

Beltran, M., C. D. Jiggins, et al. (2002). "Phylogenetic Discordance at the Species Boundary: Comparative Gene Genealogies Among Rapidly Radiating *Heliconius* Butterflies." Mol. Biol. Evol. **19**(12): 2176-2190.

<http://mbe.oupjournals.org/cgi/content/abstract/19/12/2176>

Recent adaptive radiations provide excellent model systems for understanding speciation, but rapid diversification can cause problems for phylogenetic inference. Here we use gene genealogies to investigate the phylogeny of recent speciation in the heliconiine butterflies. We sequenced three gene regions, intron 3 ({approx}550 bp) of sex-linked triose-phosphate isomerase (Tpi), intron 3 ({approx}450 bp) of autosomal mannose-phosphate isomerase (Mpi), and 1,603 bp of mitochondrial cytochrome oxidase subunits I and II (COI and COII), for 37 individuals from 25 species of *Heliconius* and related genera. The nuclear intron sequences evolved at rates similar to those of mitochondrial coding sequences, but the phylogenetic utility of introns was restricted to closely related geographic populations and species due to high levels of indel variation. For two sister species pairs, *Heliconius erato*-*Heliconius himera* and *Heliconius melpomene*-*Heliconius cydno*, there was highly significant discordance between the three genes. At mtDNA and Tpi, the hypotheses of reciprocal monophyly and paraphyly of at least one species with respect to its sister could not be distinguished. In contrast alleles sampled from the third locus, Mpi, showed polyphyletic relationships between both species pairs. In all cases, recent coalescence of mtDNA lineages within species suggests that polyphyly of nuclear genes is not unexpected. In addition, very similar alleles were shared between *melpomene* and *cydno*, implying recent gene flow. Our finding of discordant genealogies between genes is consistent with models of adaptive speciation with ongoing gene flow and highlights the need for multiple locus comparisons to resolve phylogeny among closely related species.

Bonneton, F., D. Zelus, et al. (2003). "Rapid Divergence of the Ecdysone Receptor in Diptera and Lepidoptera Suggests Coevolution Between ECR and USP-RXR." Mol. Biol. Evol. **20**(4): 541-553.

<http://mbe.oupjournals.org/cgi/content/abstract/20/4/541>

Ecdysteroid hormones are major regulators in reproduction and development of insects, including

larval molts and metamorphosis. The functional ecdysone receptor is a heterodimer of ECR (NR1H1) and USP-RXR (NR2B4), which is the orthologue of vertebrate retinoid X receptors (RXR {alpha}, {beta}, {gamma}). Both proteins belong to the superfamily of nuclear hormone receptors, ligand-dependent transcription factors that share two conserved domains: the DNA-binding domain (DBD) and the ligand-binding domain (LBD). In order to gain further insight into the evolution of metamorphosis and gene regulation by ecdysone in arthropods, we performed a phylogenetic analysis of both partners of the heterodimer ECR/USP-RXR. Overall, 38 USP-RXR and 19 ECR protein sequences, from 33 species, have been used for this analysis. Interestingly, sequence alignments and structural comparisons reveal high divergence rates, for both ECR and USP-RXR, specifically among Diptera and Lepidoptera. The most impressive differences affect the ligand-binding domain of USP-RXR. In addition, ECR sequences show variability in other domains, namely the DNA-binding and the carboxy-terminal F domains. Our data provide the first evidence that ECR and USP-RXR may have coevolved during holometabolous insect diversification, leading to a functional divergence of the ecdysone receptor. These results have general implications on fundamental aspects of insect development, evolution of nuclear receptors, and the design of specific insecticides.

Cohen, S. (2002). "Strong Positive Selection and Habitat-Specific Amino Acid Substitution Patterns in Mhc from an Estuarine Fish Under Intense Pollution Stress." *Mol. Biol. Evol.* **19**(11): 1870-1880.

<http://mbe.oupjournals.org/cgi/content/abstract/19/11/1870>

Population-level studies using the major histocompatibility complex (Mhc) have linked specific alleles with specific diseases, but data requirements are high and the power to detect disease association is low. A novel use of Mhc population surveys involves mapping allelic substitutions onto the inferred structural molecular model to show functional differentiation related to local selective pressures. In the estuarine fish *Fundulus heteroclitus*, populations experiencing strong differences in antigenic challenges show significant differences in amino acid substitution patterns that are reflected as variation in the structural location of changes between populations. Fish from a population genetically adapted to severe chemical pollution also show novel patterns of DNA substitution at a highly variable Mhc class II B locus including strong signals of positive selection at inferred antigen-binding sites and population-specific signatures of amino acid substitution. Heavily parasitized fish from an extreme PCB-contaminated (U.S. Environmental Protection Agency Superfund) site show enhanced population-specific substitutions in the α -helix portion of the inferred antigen-binding region. In contrast, fish from an unpolluted site show a significantly different pattern focused on the first strand of the B-pleated sheet. Whether Mhc population profile differences represent the direct effects of chemical toxicants or indirect parasite-mediated selection, the result is a composite habitat-specific signature of strong selection and evolution affecting the genetic repertoire of the major histocompatibility complex.

Cooper, J. L. and S. Henikoff (2004). "Adaptive Evolution of the Histone Fold Domain in Centromeric Histones." *Mol. Biol. Evol.* **21**(9): 1712-1718.

<http://mbe.oupjournals.org/cgi/content/abstract/21/9/1712>

Centromeric DNA, being highly repetitive, has been refractory to molecular analysis. However, centromeric structural proteins are encoded by single-copy genes, and these can be analyzed by using standard phylogenetic tools. The centromere-specific histone, CenH3, replaces histone H3 in centromeric nucleosomes, and is required for the proper distribution of chromosomes during cell division. Whereas histone H3s are nearly identical between species, CenH3s are divergent, with an N-terminal tail that is highly variable in length and sequence. Both the N-terminal tail and histone fold domain (HFD) are subject to adaptive evolution in *Drosophila*. Similarly, comparisons

between *Arabidopsis thaliana* and *Arabidopsis arenosa* detected adaptive evolution, but only in the N-terminal tail. We have extended our evolutionary analyses of CenH3s to other members of the Brassicaceae, which allowed the detection of positive selection in both the N-terminal tail and in the HFD. We find that adaptively evolving sites in the HFD can potentially interact with DNA, including sites in the loop 1 region of the HFD that are required for centromeric targeting in *Drosophila*. Other adaptively evolving sites in the HFD can be localized on the structure of the nucleosome core particle, revealing an extended surface in addition to loop 1 in which conformational changes might alter histone-DNA contacts or water bridges. The identification of adaptively evolving sites provides a structural basis for the interaction between centromeric DNA and the protein that is thought to underlie the evolution of centromeres and the accumulation of pericentric heterochromatin.

Dvornyk, V., A. Sirvio, et al. (2002). "Low Nucleotide Diversity at the *pal1* Locus in the Widely Distributed *Pinus sylvestris*." *Mol. Biol. Evol.* **19**(2): 179-188.

<http://mbe.oupjournals.org/cgi/content/abstract/19/2/179>

Nucleotide polymorphism in Scots pine (*Pinus sylvestris*) was studied in the gene encoding phenylalanine ammonia-lyase (Pal, EC 4.3.1.5). Scots pine, like many other pine species, has a large current population size. The observed levels of inbreeding depression suggest that Scots pine may have a high mutation rate to deleterious alleles. Many Scots pine markers such as isozymes, RFLPs, and microsatellites are highly variable. These observations suggest that the levels of nucleotide variation should be higher than those in other plant species. A 2,045-bp fragment of the *pal1* locus was sequenced from five megagametophytes each from a different individual from each of four populations, from northern and southern Finland, central Russia, and northern Spain. There were 12 segregating sites in the locus. The synonymous site overall nucleotide diversity was only 0.0049. In order to compare *pal1* with other pine genes, sequence was obtained from two alleles of 11 other loci (total length 4,606 bp). For these, the synonymous nucleotide diversity was 0.0056. These estimates are lower than those from other plants. This is most likely because of a low mutation rate, as estimated from between-pine species synonymous site divergence. In other respects, Scots pine has the characteristics of a species with a large effective population. There was no linkage disequilibrium even between closely linked sites. This resulted in high haplotype diversity (14 different haplotypes among 20 sequences). This could also give rise to high per locus diversity at the protein level. Divergence between populations in the main range was low, whereas an isolated Spanish population had slightly lower diversity and higher divergence than the remaining populations.

Fischer, A., V. Wiebe, et al. (2004). "Evidence for a Complex Demographic History of Chimpanzees." *Mol. Biol. Evol.* **21**(5): 799-808.

<http://mbe.oupjournals.org/cgi/content/abstract/21/5/799>

To characterize patterns of genomic variation in central chimpanzees (*Pan troglodytes troglodytes*) and gain insight into their evolution, we sequenced nine unlinked, intergenic regions, representing a total of 19,000 base pairs, in 14 individuals. When these DNA sequences are compared with homologous sequences previously collected in humans and in western chimpanzees (*Pan troglodytes verus*), nucleotide diversity is higher in central chimpanzees than in western chimpanzees or in humans. Consistent with a larger effective population size of central chimpanzees, levels of linkage disequilibrium are lower than in humans. Patterns of linkage disequilibrium further suggest that homologous gene conversion may be an important contributor to genetic exchange at short distances, in agreement with a previous study of the same DNA sequences in humans. In central chimpanzees, but not in western chimpanzees, the allele

frequency spectrum is significantly skewed towards rare alleles, pointing to population size changes or fine-scale population structure. Strikingly, the extent of genetic differentiation between western and central chimpanzees is much stronger than what is seen between human populations. This suggests that careful attention should be paid to geographic sampling in studies of chimpanzee genetic variation.

Gorinsek, B., F. Gubensek, et al. (2004). "Evolutionary Genomics of Chromoviruses in Eukaryotes." Mol. Biol. Evol. **21**(5): 781-798.

<http://mbe.oupjournals.org/cgi/content/abstract/21/5/781>

The diversity, origin, and evolution of chromoviruses in Eukaryota were examined using the massive amount of genome sequence data for different eukaryotic lineages. A surprisingly large number of novel full-length chromoviral elements were found, greatly exceeding the number of the known chromoviruses. These new elements are mostly structurally intact and highly conserved. Chromoviruses in the key Amniota lineage, the reptiles, have been analyzed by PCR to explain their evolutionary dynamics in amniotes. Phylogenetic analyses provide evidence for a novel centromere-specific chromoviral clade that is widespread and highly conserved in all seed plants. Chromoviral diversity in plants, fungi, and vertebrates, as shown by phylogenetic analyses, was found to be much greater than previously expected. The age of plant chromoviruses has been significantly extended by finding their representatives in the most basal plant lineages, the green and the red algae. The evolutionary origin of chromoviruses has been found to be no earlier than in Cercozoa. The evolutionary history and dynamics of chromoviruses can be explained simply by strict vertical transmission in plants, followed by more complex evolution in fungi and in Metazoa. The currently available data clearly show that chromoviruses indeed represent the oldest and the most widespread clade of Metaviridae.

Hellborg, L. and H. Ellegren (2004). "Low Levels of Nucleotide Diversity in Mammalian Y Chromosomes." Mol. Biol. Evol. **21**(1): 158-163.

<http://mbe.oupjournals.org/cgi/content/abstract/21/1/158>

Sex chromosomes provide a useful context for the study of the relative importance of evolutionary forces affecting genetic diversity. The human Y chromosome shows levels of nucleotide diversity 20% that of autosomes, which is significantly less than expected when differences in effective population size and sex-specific mutation rates are taken into account. To study the generality of low levels of Y chromosome variability in mammalian genomes, we investigated nucleotide diversity in intron sequences of X (1.1-3.0 kb) and Y (0.7-3.5 kb) chromosome genes of five mammals: lynx, wolf, reindeer, cattle, and field vole. For all species, nucleotide diversity was found to be lower on Y than on X, with no segregating site observed in Y-linked sequences of lynx, reindeer, and cattle. For X chromosome sequences, nucleotide diversity was in the range of 1.6×10^{-4} (lynx) to 8.0×10^{-4} (field vole). When differences in effective population size and the extent of the male mutation bias were taken into account, all five species showed evidence of reduced levels of Y chromosome variability. Reduced levels of Y chromosome variability have also been observed in *Drosophila* and in plants, as well as in the female-specific W chromosome of birds. Among the different factors proposed to explain low levels of genetic variability in the sex-limited chromosome (Y/W), we note that selection is the only factor that is broadly applicable irrespective of mode of reproduction and whether there is male or female heterogamety.

Hofreiter, M., C. Capelli, et al. (2002). "Ancient DNA Analyses Reveal High Mitochondrial DNA Sequence Diversity and Parallel Morphological Evolution of Late Pleistocene Cave Bears." Mol. Biol. Evol. **19**(8): 1244-1250.

<http://mbe.oupjournals.org/cgi/content/abstract/19/8/1244>

Cave bears (*Ursus spelaeus*) existed in Europe and western Asia until the end of the last glaciation some 10,000 years ago. To investigate the genetic diversity, population history, and relationship among different cave bear populations, we have determined mitochondrial DNA sequences from 12 cave bears that range in age from about 26,500 to at least 49,000 years and originate from nine caves. The samples include one individual from the type specimen population, as well as two small-sized high-Alpine bears. The results show that about 49,000 years ago, the mtDNA diversity among cave bears was about 1.8-fold lower than the current species-wide diversity of brown bears (*Ursus arctos*). However, the current brown bear mtDNA gene pool consists of three clades, and cave bear mtDNA diversity is similar to the diversity observed within each of these clades. The results also show that geographically separated populations of the high-Alpine cave bear form were polyphyletic with respect to their mtDNA. This suggests that small size may have been an ancestral trait in cave bears and that large size evolved at least twice independently.

Joshi, M. B., P. K. Rout, et al. (2004). "Phylogeography and Origin of Indian Domestic Goats." Mol. Biol. Evol. **21**(3): 454-462.

<http://mbe.oupjournals.org/cgi/content/abstract/21/3/454>

The Indian subcontinent contains 20 well-characterized goat breeds, which vary in their genetic potential for the production of milk, meat, and fibre; disease resistance; heat tolerance; and fecundity. Indian goats make up 20% of the world's goat population, but there has been no extensive study of these economically important animals. Therefore, we have undertaken the present investigation of 363 goats belonging to 10 different breeds from different geographic regions of India using mtDNA sequence data from the HVRI region. We find evidence for population structure and novel lineages in Indian goats and cannot reconcile the genetic diversity found within the major lineage with domestication starting 10,000 years ago from a single mtDNA ancestor. Thus, we propose a more complex origin for domestic goats.

Kitano, T., Y.-H. Liu, et al. (2004). "Human-Specific Amino Acid Changes Found in 103 Protein-Coding Genes." Mol. Biol. Evol. **21**(5): 936-944.

<http://mbe.oupjournals.org/cgi/content/abstract/21/5/936>

We humans have many characteristics that are different from those of the great apes. These human-specific characters must have arisen through mutations accumulated in the genome of our direct ancestor after the divergence of the last common ancestor with chimpanzee. Gene trees of human and great apes are necessary for extracting these human-specific genetic changes. We conducted a systematic analysis of 103 protein-coding genes for human, chimpanzee, gorilla, and orangutan. Nucleotide sequences for 18 genes were newly determined for this study, and those for the remaining genes were retrieved from the DDBJ/EMBL/GenBank database. The total number of amino acid changes in the human lineage was 147 for 26,199 codons (0.56%). The total number of amino acid changes in the human genome was, thus, estimated to be about 80,000. We applied the acceleration index test and Fisher's synonymous/nonsynonymous exact test for each gene tree to detect any human-specific

enhancement of amino acid changes compared with ape branches. Six and two genes were shown to have significantly higher nonsynonymous changes at the human lineage from the acceleration index and exact tests, respectively. We also compared the distribution of the differences of the nonsynonymous substitutions on the human lineage and those on the great ape lineage. Two genes were more conserved in the ape lineage, whereas one gene was more conserved in the human lineage. These results suggest that a small proportion of protein-coding genes started to evolve differently in the human lineage after it diverged from the ape lineage.

Kitano, T., C. Schwarz, et al. (2003). "Gene Diversity Patterns at 10 X-Chromosomal Loci in Humans and Chimpanzees." Mol. Biol. Evol. **20**(8): 1281-1289.

<http://mbe.oupjournals.org/cgi/content/abstract/20/8/1281>

We have investigated the pattern and extent of nucleotide diversity in 10 X-chromosomal genes where mutations are known to cause mental retardation in humans. For each gene, we sequenced the entire coding region from cDNA in humans, chimpanzees, and orangutans, as well as about 3 kb of genomic DNA in 20 humans sampled worldwide and in 10 chimpanzees representing two "subspecies." Overall nucleotide diversity in these genes is about twofold lower in humans than in chimpanzees, and nucleotide diversity within and between species is low, suggesting that a high level of functional constraint acts on these genes. Strikingly, we find that a summary of the allele frequency spectrum is significantly correlated in humans and chimpanzees, perhaps reflecting very similar levels of constraint at these genes in the two species. A possible exception is FMR2, which shows a higher number of nonsynonymous than synonymous substitutions on the human lineage, suggesting the action of positive selection.

McCartney, M. A. and H. A. Lessios (2004). "Adaptive Evolution of Sperm Bindin Tracks Egg Incompatibility in Neotropical Sea Urchins of the Genus *Echinometra*." Mol. Biol. Evol. **21**(4): 732-745.

<http://mbe.oupjournals.org/cgi/content/abstract/21/4/732>

Bindin is a gamete recognition protein known to control species-specific sperm-egg adhesion and membrane fusion in sea urchins. Previous analyses have shown that diversifying selection on bindin amino acid sequence is found when gametically incompatible species are compared, but not when species are compatible. The present study analyzes bindin polymorphism and divergence in the three closely related species of *Echinometra* in Central America: *E. lucunter* and *E. viridis* from the Caribbean, and *E. vanbrunti* from the eastern Pacific. The eggs of *E. lucunter* have evolved a strong block to fertilization by sperm of its neotropical congeners, whereas those of the other two species have not. As in the Indo-West Pacific (IWP) *Echinometra*, the neotropical species show high intraspecific bindin polymorphism in the same gene regions as in the IWP species. Maximum likelihood analysis shows that many of the polymorphic codon sites are under mild positive selection. Of the fixed amino acid replacements, most have accumulated along the bindin lineage of *E. lucunter*. We analyzed the data with maximum likelihood models of variation in positive selection across lineages and codon sites, and with models that consider sites and lineages simultaneously. Our results show that positive selection is concentrated along the *E. lucunter* bindin lineage, and that codon sites with amino acid replacements fixed in this species show by far the highest signal of positive selection. Lineage-specific positive selection paralleling egg incompatibility provides support that adaptive evolution of sperm proteins acts to maintain recognition of bindin by changing egg receptors. Because both egg incompatibility and bindin divergence are greater between allopatric species than between sympatric species, the hypothesis of selection against hybridization (reinforcement) cannot explain why adaptive evolution has been confined to a single lineage in the American *Echinometra*. Instead, processes

acting to varying degrees within species (e.g., sperm competition, sexual selection, and sexual conflict) are more promising explanations for lineage-specific positive selection on *bindin*.

Moum, T., U. Arnason, et al. (2002). "Mitochondrial DNA Sequence Evolution and Phylogeny of the Atlantic Alcidae, Including the Extinct Great Auk (*Pinguinus impennis*)."
Mol. Biol. Evol. **19**(9): 1434-1439.

<http://mbe.oupjournals.org/cgi/content/abstract/19/9/1434>

The Atlantic auk assemblage includes four extant species, razorbill (*Alca torda*), dovekie (*Alle alle*), common murre (*Uria aalge*), and thick-billed murre (*U. lomvia*), and one recently extinct species, the flightless great auk (*Pinguinus impennis*). To determine the phylogenetic relationships among the species, a contiguous 4.2-kb region of the mitochondrial genome from the extant species was amplified using PCR. This region included one ribosomal RNA gene, four transfer RNA genes, two protein-coding genes, the control region, and intergenic spacers. Sets of PCR primers for amplifying the same region from great auk were designed from sequences of the extant species. The authenticity of the great auk sequence was ascertained by alternative amplifications, cloning, and separate analyses in an independent laboratory. Phylogenetic analyses of the entire assemblage, made possible by the great auk sequence, fully resolved the phylogenetic relationships and split it into two primary lineages, *Uria* versus *Alle*, *Alca*, and *Pinguinus*. A sister group relationship was identified between *Alca* and *Pinguinus* to the exclusion of *Alle*. Phylogenetically, the flightless great auk originated late relative to other divergences within the assemblage. This suggests that three highly divergent species in terms of adaptive specializations, *Alca*, *Alle*, and *Pinguinus*, evolved from a single lineage in the Atlantic Ocean, in a process similar to the initial adaptive radiation of alcids in the Pacific Ocean.

Reiss, D., H. Quesneville, et al. (2003). "Hoppel, a P-like Element Without Introns: a P-Element Ancestral Structure or a Retrotranscription Derivative?"
Mol. Biol. Evol. **20**(6): 869-879.

<http://mbe.oupjournals.org/cgi/content/abstract/20/6/869>

An *in silico* search for P-transposable-element-related sequences in the *Drosophila melanogaster* genome allowed us to detect sequences that are similar to P-element transposases. These sequences are located in the central region of 3.4-kb Hoppel elements, a class II transposon. Polymerase chain reaction (PCR) analysis of the insertional polymorphism revealed that these elements are mobile. The 3.4-kb elements are the longest copies of this family ever found. They contain an open reading frame that is long enough to encode a transposase, suggesting that the 3.4-kb elements are the full-length copies of the Hoppel family. Multiple alignments of several P-element transposases from different species and the Hoppel-element-encoded peptide showed that all of the P-element introns and the 5' region of the transposase are absent from the Hoppel sequence. Sequence analysis combined with reverse transcriptase PCR analysis showed that the 3.4-kb Hoppel elements are intronless. P and Hoppel not only share similar amino acid sequences but also have terminal inverted repeats of the same length (31 bp), and their excision footprints present a similar structure, which suggests that their transposases are functionally very similar. Thus, we propose that the Hoppel element family be included in the P-element superfamily. Two evolutionary scenarios are discussed considering the presence /absence of introns within the P-element superfamily.

Staats, M., P. van Baarlen, et al. (2005). "Molecular Phylogeny of the Plant Pathogenic Genus *Botrytis*

and the Evolution of Host Specificity." *Mol. Biol. Evol.* **22**(2): 333-346.

<http://mbe.oupjournals.org/cgi/content/abstract/22/2/333>

The cosmopolitan genus *Botrytis* contains 22 recognized species and one hybrid. The current classification is largely based on morphological characters and, to a minor extent, on physiology and host range. In this study, a classification of the genus was constructed based on DNA sequence data of three nuclear protein-coding genes (RPB2, G3PDH, and HSP60) and compared with the traditional classification. Sexual reproduction and the host range, important fitness traits, were traced in the tree and used for the identification of major evolutionary events during speciation. The phylogenetic analysis corroborated the classical species delineation. In addition, the hybrid status of *B. allii* (*B. byssoidea* x *B. aclada*) was confirmed. Both individual gene trees and combined trees show that the genus *Botrytis* can be divided into two clades, radiating after the separation of *Botrytis* from other Sclerotiniaceae genera. Clade 1 contains four species that all colonize exclusively eudicot hosts, whereas clade 2 contains 18 species that are pathogenic on either eudicot (3) or monocot (15) hosts. A comparison of *Botrytis* and angiosperm phylogenies shows that cospeciation of pathogens and their hosts have not occurred during their respective evolution. Rather, we propose that host shifts have occurred during *Botrytis* speciation, possibly by the acquisition of novel pathogenicity factors. Loss of sexual reproduction has occurred at least three times and is supposed to be a consequence of negative selection.

Taylor, S. D., K. D. de la Cruz, et al. (2005). "Characterization of the Long-Wavelength Opsin from Mecoptera and Siphonaptera: Does a Flea See?" *Mol. Biol. Evol.* **22**(5): 1165-1174.

<http://mbe.oupjournals.org/cgi/content/abstract/22/5/1165>

Mecoptera and Siphonaptera represent two insect orders that have largely been overlooked in the study of insect vision. Recent phylogenetic evidence demonstrates that Mecoptera (scorpionflies) is paraphyletic, with the order Siphonaptera (fleas) nesting as sister to the family Boreidae (snow fleas), showing an evolutionary trend towards reduction in gross eye morphology within fleas. We provide the first molecular characterization of long-wavelength opsins from these three lineages (opsin gene from fleas [FL-Opsin], the Boreidae [B-Opsin], and a mecopteran family [M-Opsin]) and assess the effects of loss of visual acuity on the structure and function of the opsin gene. Phylogenetic analysis implies a physiological sensitivity in the red-green spectrum for these opsins. Analysis of intron splice sites reveals a high degree of similarity between FL-Opsin and B-Opsin as well as conserved splice sites across insect blue-green and long-wavelength opsins. Calculated rates of evolution and tests for destabilizing selection indicate that FL-Opsin, B-Opsin, and M-Opsin are evolving at similar rates with no radical selective pressures, implying conservative evolution and functional constraint across all three lineages.

Zhou, G., Y. Zhai, et al. (2004). "Haplotype Structure and Evidence for Positive Selection at the Human IL13 Locus." *Mol. Biol. Evol.* **21**(1): 29-36.

<http://mbe.oupjournals.org/cgi/content/abstract/21/1/29>

Interleukin-13 (IL13) is believed to play an important role in the pathogenesis of atopy and allergic asthma. To better understand genetic variation at the IL13 locus, we resequenced a 5.1-kb genomic region spanning the entire locus and identified 26 single-nucleotide polymorphisms (SNPs) in 74 individuals from three major populations--Chinese, Caucasian, and African. Our survey suggests exceptionally high and significant geographic structure at the IL13 locus between African and outside Africa populations. This unusual pattern suggests that positive selection that

acts in some local populations may have played a role on the IL13 locus. In support of this suggestion, we found a significant excess of high frequency-derived SNPs in the Chinese population and Caucasian population, respectively, as expected after a recent episode of positive selection. Further, the unusual haplotype structure indicates that different scenarios of the action of positive selection on the IL13 locus in different populations may exist. In the Caucasian population, the skewed haplotype distribution dominated by one common haplotype supports the hypothesis of simple directional selection. Whereas, in the Chinese population, the two-round hitchhiking hypothesis may explain the skewed haplotype structure with three dominant ones. These findings may provide insight into the likely relative roles of selection and population history in establishing present-day variation at the IL13 locus, and, motivate further studies of this locus as an important candidate in common diseases association studies.

Mol. Cancer Res. (2)

Jia, L., J. Kim, et al. (2003). "Androgen Receptor Activity at the Prostate Specific Antigen Locus: Steroidal and Non-Steroidal Mechanisms." Mol. Cancer Res. **1**(5): 385-392.

<http://mcr.aacrjournals.org/cgi/content/abstract/1/5/385>

Ligand-activated androgen receptors (ARs) occupy target genes and recruit histone modifiers that influence transcriptional competency. In LNCaP prostate cancer cells, the natural ligand 5 α -dihydrotestosterone (DHT) activates transiently transfected AR-responsive promoter constructs; concurrent treatment with the protein kinase A activator forskolin enhanced AR stimulation induced by DHT. Additional treatment with the cytokine IL-6, purportedly an AR activator, markedly inhibited receptor activity. To assess AR activity on natural chromatin-integrated promoters/enhancers, we determined AR occupancy of the endogenous prostate specific antigen (PSA) promoter/enhancer as well as PSA expression in LNCaP cells treated with DHT; AR occupancy of the PSA enhancer was rapid (within 1 h of stimulation), robust (10-fold over background), and sustained (8-16 h). In contrast, AR occupancy of the PSA promoter was only increased by 2-fold. Histone H3 acetylation at both the enhancer and promoter was evident 1-2 h after DHT treatment. Detectable pre- and mature PSA mRNA levels appeared after 1 and 6 h treatment, respectively. Substantial qualitative and quantitative differences in PSA expression and AR occupancy of the PSA enhancer were observed when DHT-induced and ligand-independent activations of the AR were compared; forskolin stimulated PSA mRNA and protein expression, whereas IL-6 inhibited both DHT- and forskolin-stimulated expression. IL-6 did not diminish DHT-dependent AR occupancy of the PSA enhancer but inhibited CBP/p300 recruitment, histone H3 acetylation, and cell proliferation. These findings provide a contextual framework for interpreting the contribution of non-steroidal activation of the AR to signaling in vivo, and have implications for prostate cancer cell growth.

Peng, S.-B., V. Peek, et al. (2005). "Akt Activation, but not Extracellular Signal-Regulated Kinase Activation, Is Required for SDF-1 α /CXCR4-Mediated Migration of Epitheloid Carcinoma Cells." Mol. Cancer Res. **3**(4): 227-236.

<http://mcr.aacrjournals.org/cgi/content/abstract/3/4/227>

Emerging evidence shows that the stromal cell-derived factor 1 (SDF-1)/CXCR4 interaction

regulates multiple cell signaling pathways and a variety of cellular functions such as cell migration, proliferation, and survival. There is little information linking the cellular functions and individual signaling pathways mediated by SDF-1 and CXCR4 in human cancer cells. In this study, we have shown that human epitheloid carcinoma HeLa cells express functional CXCR4 by reverse transcription-PCR, immunofluorescent staining, and ^{125}I -SDF-1 $\{\alpha\}$ ligand binding analyses. The treatment of HeLa cells with recombinant SDF-1 $\{\alpha\}$ results in time-dependent Akt and extracellular signal-regulated kinase 1/2 (ERK1/2) activations. The SDF-1 $\{\alpha\}$ -induced Akt and ERK1/2 activations are CXCR4 dependent as confirmed by their total inhibition by T134, a CXCR4-specific peptide antagonist. Cell signaling analysis with pathway-specific inhibitors reveals that SDF-1 $\{\alpha\}$ -induced Akt activation is not required for ERK1/2 activation and vice versa, indicating that activations of Akt and ERK1/2 occur independently. Functional analysis shows that SDF-1 $\{\alpha\}$ induces a CXCR4-dependent migration of HeLa cells. The migration can be totally blocked by phosphoinositide 3-kinase inhibitors, wortmannin or LY294002, whereas mitogen-activated protein/ERK kinase inhibitors, PD98059 and U0126, have no significant effect on SDF-1 $\{\alpha\}$ -induced migration, suggesting that Akt activation, but not ERK1/2 activation, is required for SDF-1 $\{\alpha\}$ -induced migration of epitheloid carcinoma cells.

Mol. Cancer Ther. (5)

Di Nicolantonio, F., L. A. Knight, et al. (2004). "The ex vivo characterization of XR5944 (MLN944) against a panel of human clinical tumor samples." *Mol. Cancer Ther.* **3**(12): 1631-1637.

<http://mct.aacrjournals.org/cgi/content/abstract/3/12/1631>

XR5944 (MLN944) is a novel DNA targeting agent with potent antitumor activity, both in vitro and in vivo, against several murine and human tumor models. We have used an ATP-tumor chemosensitivity assay to assess the ex vivo sensitivity of a variety of solid tumors ($n = 90$) and a CCRF-CEM leukemia cell line selected with XR5944. Differences in gene expression between the parental CCRF-CEM and the resistant subline were investigated by quantitative reverse transcription-PCR. Immunohistochemistry for topoisomerases I and II $\{\alpha\}$ and multidrug resistance (MDR1) protein was done on those tumors for which tissue was available ($n = 32$). The CCRF-CEM XR5944 line showed increased mRNA levels of MDR1, major vault protein, and MDR-associated protein 1 compared with the parental line, whereas the expression of topoisomerases I, II $\{\alpha\}$, and II $\{\beta\}$ was essentially unchanged, suggesting that XR5944 is susceptible to MDR mechanisms. The median IC₉₀ and IC₅₀ values for XR5944 in tumor-derived cells were 68 and 26 nmol/L, respectively, 6-fold greater than in resistant cell lines. XR5944 was 40- to 300-fold more potent than the other cytotoxics tested, such as doxorubicin, topotecan, and paclitaxel. Breast and gynecologic malignancies were most sensitive to XR5944, whereas gastrointestinal tumors showed greater resistance. A positive correlation ($r = 0.68$; $P < 0.0001$) was found between the IC₅₀ values of XR5944 and P-glycoprotein/MDR1 staining but not with either topoisomerase I or II $\{\alpha\}$ immunohistochemistry index. These data support the rapid introduction of XR5944 to clinical trials and suggest that it may be effective against a broad spectrum of tumor types, especially ovarian and breast cancer.

Ikeda, N., H. Uemura, et al. (2003). "Combination Treatment with 1 $\{\alpha\}$,25-Dihydroxyvitamin D₃ and 9-cis-Retinoic Acid Directly Inhibits Human Telomerase Reverse Transcriptase Transcription in Prostate Cancer Cells." *Mol. Cancer Ther.* **2**(8): 739-746.

<http://mct.aacrjournals.org/cgi/content/abstract/2/8/739>

The vitamin D3 receptor, which is the nuclear receptor for 1 α ,25-dihydroxyvitamin D3 (VD3), forms a heterodimer with the retinoid X receptor (RXR), which is the nuclear receptor for 9-cis-retinoic acid (9-cis-RA). The heterodimer binds to a specific response element consisting of two directly repeated pairs of motifs, AGGTGA, spaced by three nucleotides [direct repeat (DR) 3] and modulates the expression of VD3-responsive genes. Telomerase activity, which is seen in most immortal cells and germ cells, is a complex of enzymes that maintain the length of telomeres. One of the major components of human telomerase, human telomerase reverse transcriptase (hTERT), is the catalytic subunit, and the expression of hTERT might correlate most strongly with telomerase activity. We found that the sequence of 5'-AGTTCATGGAGTTCA-3' (DR3') is similar to that of DR3 in the promoter region of hTERT. Our results showed that the combination of VD3 and 9-cis-RA inhibited telomerase activity through direct interaction of the heterodimer of vitamin D3 receptor and RXR with the DR3' sequence in the hTERT promoter as well as the combination of VD3 and selective RXR ligand did. Also, in vivo data showed that the growth of xenografts in nude mice was inhibited by VD3 and 9-cis-RA. The results of the present study provide evidence on the molecular mechanism of the inhibition of cell growth by these agents, and they could be novel therapeutic agents for prostate cancer.

Johnson, P. H., R. P. Walker, et al. (2002). "Multiplex Gene Expression Analysis for High-Throughput Drug Discovery: Screening and Analysis of Compounds Affecting Genes Overexpressed in Cancer Cells." Mol. Cancer Ther. **1**(14): 1293-1304.

<http://mct.aacrjournals.org/cgi/content/abstract/1/14/1293>

Drug discovery strategies are needed that can rapidly exploit multiple therapeutic targets associated with the complex gene expression changes that characterize a polygenic disease such as cancer. We report a new cell-based high-throughput technology for screening chemical libraries against several potential cancer target genes in parallel. Multiplex gene expression (MGE) analysis provides direct and quantitative measurement of multiple endogenous mRNAs using a multiplexed detection system coupled to reverse transcription-PCR. A multiplex assay for six genes overexpressed in cancer cells was used to screen 9000 chemicals and known drugs in the human prostate cancer cell line PC-3. Active compounds that modulated gene expression levels were identified, and IC50 values were determined for compounds that bind DNA, cell surface receptors, and components of intracellular signaling pathways. A class of steroids related to the cardiac glycosides was identified that potently inhibited the plasma membrane Na⁺K⁺-ATPase resulting in the inhibition of four of the prostate target genes including transcription factors Hoxb-13, hPSE/PDEF, hepatocyte nuclear factor-3 α , and the inhibitor of apoptosis, survivin. Representative compounds selectively induced apoptosis in PC-3 cells compared with the nonmetastatic cell line BPH-1. The multiplex assay distinguished potencies among structural variants, enabling structure-activity analysis suitable for chemical optimization studies. A second multiplex assay for five toxicological markers, Hsp70, Gadd153, Gadd45, O6-methylguanine-DNA methyltransferase, and cyclophilin, detected compounds that caused DNA damage and cellular stress and was a more sensitive and specific indicator of potential toxicity than measurement of cell viability. MGE analysis facilitates rapid drug screening and compound optimization, the simultaneous measurement of toxicological end points, and gene function analysis.

Sale, S., R. Sung, et al. (2002). "Conservation of the Class I β -Tubulin Gene in Human Populations and Lack of Mutations in Lung Cancers and Paclitaxel-resistant Ovarian Cancers." Mol. Cancer Ther. **1**(3): 215-225.

<http://mct.aacrjournals.org/cgi/content/abstract/1/3/215>

The goal of this study was to determine the prevalence of sequence variants in the class I β -tubulin (clone m40) gene and their occurrence in human tumors and cancer cell lines. DNA was isolated from 93 control individuals representing a wide variety of ethnicities, 49 paclitaxel-naïve specimens (16 ovarian cancers, 17 non-small cell lung cancers, and 16 ovarian cancer cell lines), and 30 paclitaxel-resistant specimens (9 ovarian cancers, 9 ovarian cancer cell lines, and 12 ovarian cancer xenografts in nude mice). Denaturing high-performance liquid chromatography and direct sequence analysis detected two silent polymorphisms in exon 4, Leu217Leu (CTG/CTA) and Gly400Gly (GGC/GGT), with minor allele frequencies of 17 and 0.5%, respectively. Five nucleotide substitutions and one single-base deletion were detected in introns 1, 2, and 3 and in the 3' untranslated region. Analysis of 49 paclitaxel-naïve and 30 paclitaxel-resistant specimens revealed no additional polymorphisms in the coding region. In addition, no amino acid replacements were found in chimpanzee, gorilla, and orangutan in comparison to human. Our data demonstrate a very high degree of sequence conservation in class I β -tubulin, suggesting that all residues are important in tubulin structure and function. Individual variation in response to treatment with paclitaxel is not likely to be caused by genetic variations in the β -tubulin drug target. Moreover, acquired mutations in class I β -tubulin are unlikely to be a clinically relevant cause of drug resistance.

Sowers, R., J. Toguchida, et al. (2003). "mRNA Expression Levels of E2F Transcription Factors Correlate with Dihydrofolate Reductase, Reduced Folate Carrier, and Thymidylate Synthase mRNA Expression in Osteosarcoma." *Mol. Cancer Ther.* **2**(6): 535-541.

<http://mct.aacrjournals.org/cgi/content/abstract/2/6/535>

Previous studies have shown that decreased expression of the reduced folate carrier (RFC) and increased expression of dihydrofolate reductase (DHFR) are associated with intrinsic and acquired methotrexate resistance, respectively, in osteosarcoma (OS). It has also been shown in colorectal cancer that E2F-1 expression correlates with thymidylate synthase (TS) and, to a lesser extent, DHFR expression. To begin to investigate the regulation of DHFR and RFC expression in OS samples, mRNA expression of E2F-1 and E2F-4 were measured in OS tumor samples and related to DHFR, RFC, and TS mRNA expression. Using fluorescent quantitative real-time PCR, 112 human OS patient samples were investigated for potential E2F-1/E2F-4:DHFR, E2F-1/E2F-4:RFC, and E2F-1/E2F-4:TS correlations. The expression ranges for each gene are as follows: DHFR, 0.02-33.13 (median = 0.20); RFC, 0.02-229.13 (median = 1.91); TS, 0.01-9.99 (median = 0.15); E2F-1, 0.05-69.07 (median = 0.52); and E2F-4, 0.24-52.35 (median = 1.45). Spearman correlation coefficients (rs) for E2F-1:DHFR, E2F-1:RFC, E2F-1:TS, E2F-4:DHFR, E2F-4:RFC, and E2F-4:TS were calculated to be 0.53, 0.63, 0.60, 0.41, 0.58, and 0.33, respectively (P < 0.001). On the basis of this data, moderate correlations exist between E2F-1/E2F-4 and DHFR, RFC, and TS. These results suggest E2F-1/E2F-4 may play a role in the regulation of RFC expression, which has not been reported previously. The E2F transcription factors are also related to DHFR and TS expression in OS samples, suggesting a possible involvement in methotrexate resistance. Although E2F mRNA levels correlate with DHFR, RFC, and TS mRNA expression, additional experiments are necessary to determine the direct effects of these transcription factors and identify other proteins that may influence this relationship.

Baldwin, M. E., M. M. Halford, et al. (2005). "Vascular Endothelial Growth Factor D Is Dispensable for Development of the Lymphatic System." Mol. Cell. Biol. **25**(6): 2441-2449.

<http://mcb.asm.org/cgi/content/abstract/25/6/2441>

Vascular endothelial growth factor receptor 3 (Vegfr-3) is a tyrosine kinase that is expressed on the lymphatic endothelium and that signals for the growth of the lymphatic vessels (lymphangiogenesis). Vegf-d, a secreted glycoprotein, is one of two known activating ligands for Vegfr-3, the other being Vegf-c. Vegf-d stimulates lymphangiogenesis in tissues and tumors; however, its role in embryonic development was previously unknown. Here we report the generation and analysis of mutant mice deficient for Vegf-d. Vegf-d-deficient mice were healthy and fertile, had normal body mass, and displayed no pathologic changes consistent with a defect in lymphatic function. The lungs, sites of strong Vegf-d gene expression during embryogenesis in wild-type mice, were normal in Vegf-d-deficient mice with respect to tissue mass and morphology, except that the abundance of the lymphatics adjacent to bronchioles was slightly reduced. Dye uptake experiments indicated that large lymphatics under the skin were present in normal locations and were functional. Smaller dermal lymphatics were similar in number, location, and function to those in wild-type controls. The lack of a profound lymphatic phenotype in Vegf-d-deficient mice suggests that Vegf-d does not play a major role in lymphatic development or that Vegf-c or another, as-yet-unknown activating Vegfr-3 ligand can compensate for Vegf-d during development.

Bhattacharyya, S. N., S. Chatterjee, et al. (2002). "Mitochondrial RNA Import in *Leishmania tropica*: Aptamers Homologous to Multiple tRNA Domains That Interact Cooperatively or Antagonistically at the Inner Membrane." Mol. Cell. Biol. **22**(12): 4372-4382.

<http://mcb.asm.org/cgi/content/abstract/22/12/4372>

A large number of cytoplasmic tRNAs are imported into the kinetoplast-mitochondrion of *Leishmania* by a receptor-mediated process. To identify the sequences recognized by import receptors, mitochondria were incubated with a combinatorial RNA library. Repeated cycles of amplification of the imported sequences (SELEX) resulted in rapid selection of several import aptamers containing sequence motifs present in the anticodon arm, the D arm, the V-T region, and acceptor stem of known tRNAs, confirming or suggesting the presence of import signals in these domains. As predicted, truncated derivatives of tRNA^{Ala}(UAU) containing the D arm or the V-T region were imported in vitro. Four aptamers were studied in detail. All were imported in vitro as well as in transiently transfected cells, using the same pathway as tRNA, but their individual import efficiencies were different. Two types of aptamers were discernible: the A arm and D arm homologues (type I), which were efficiently transferred across the inner mitochondrial membrane, and the V-T homologues (type II), which were not. Remarkably, subnanomolar concentrations of type I RNAs stimulated the entry of type II RNAs into the matrix, whereas type II RNAs inhibited inner membrane transfer of type I RNAs. Moreover, tRNA^{Tyr}(GUA) and tRNA^{Ala}(UAU) interacted with one another as type I and type II, respectively. Such cooperative and antagonistic interactions may allow the use of a limited number of receptors to recognize a large number of tRNAs of variable affinity and enable the maintenance of a properly balanced tRNA pool for mitochondrial translation.

Cao, S., H. Bendall, et al. (2003). "The High-Mobility-Group Box Protein SSRP1/T160 Is Essential for Cell Viability in Day 3.5 Mouse Embryos." Mol. Cell. Biol. **23**(15): 5301-5307.

<http://mcb.asm.org/cgi/content/abstract/23/15/5301>

The high-mobility-group (HMG) SSRP1 protein is a member of a conserved chromatin-remodeling complex (FACT/DUF/CP) implicated in DNA replication, basal and regulated transcription, and DNA repair. To assist in the functional analysis of SSRP1, the *Ssrp1* gene was targeted in murine embryonic stem cells, and the mutation was introduced into the germ line. Embryos homozygous for the targeted allele die soon after implantation, and preimplantation blastocysts are defective for cell outgrowth and/or survival in vitro. The *Ssrp1* mutation was also crossed into a p53 null background without affecting growth and/or survival defects caused by loss of *Ssrp1* function. Thus, *Ssrp1* appears to encode nonredundant and p53-independent functions that are essential for cell viability.

Catalanotto, C., M. Pallotta, et al. (2004). "Redundancy of the Two Dicer Genes in Transgene-Induced Posttranscriptional Gene Silencing in *Neurospora crassa*." *Mol. Cell. Biol.* **24**(6): 2536-2545.

<http://mcb.asm.org/cgi/content/abstract/24/6/2536>

RNA interference (RNAi) in animals, cosuppression in plants, and quelling in fungi are homology-dependent gene silencing mechanisms in which the introduction of either double-stranded RNA (dsRNA) or transgenes induces sequence-specific mRNA degradation. These phenomena share a common genetic and mechanistic basis. The accumulation of short interfering RNA (siRNA) molecules that guide sequence-specific mRNA degradation is a common feature in both silencing mechanisms, as is the component of the RNase complex involved in mRNA cleavage. During RNAi in animal cells, dsRNA is processed into siRNA by an RNase III enzyme called Dicer. Here we show that elimination of the activity of two Dicer-like genes by mutation in the fungus *Neurospora crassa* eliminates transgene-induced gene silencing (quelling) and the processing of dsRNA to an siRNA form. The two Dicer-like genes appear redundant because single mutants are quelling proficient. This first demonstration of the involvement of Dicer in gene silencing induced by transgenes supports a model by which a dsRNA produced by the activity of cellular RNA-dependent RNA polymerases on transgenic transcripts is an essential intermediate of silencing.

Cazalla, D., K. Newton, et al. (2005). "A Novel SR-Related Protein Is Required for the Second Step of Pre-mRNA Splicing." *Mol. Cell. Biol.* **25**(8): 2969-2980.

<http://mcb.asm.org/cgi/content/abstract/25/8/2969>

The SR family proteins and SR-related polypeptides are important regulators of pre-mRNA splicing. A novel SR-related protein of an apparent molecular mass of 53 kDa was isolated in a gene trap screen that identifies proteins which localize to the nuclear speckles. This novel protein possesses an arginine- and serine-rich domain and was termed SRp53 (for SR-related protein of 53 kDa). In support for a role of this novel RS-containing protein in pre-mRNA splicing, we identified the mouse ortholog of the *Saccharomyces cerevisiae* U1 snRNP-specific protein Luc7p and the U2AF65-related factor HCC1 as interacting proteins. In addition, SRp53 is able to interact with some members of the SR family of proteins and with U2AF35 in a yeast two-hybrid system and in cell extracts. We show that in HeLa nuclear extracts immunodepleted of SRp53, the second step of pre-mRNA splicing is blocked, and recombinant SRp53 is able to restore splicing activity. SRp53 also regulates alternative splicing in a concentration-dependent manner. Taken together, these results suggest that SRp53 is a novel SR-related protein that has a role both in constitutive and in alternative splicing.

Chiang, Y. J., M. T. Hemann, et al. (2004). "Expression of Telomerase RNA Template, but Not Telomerase Reverse Transcriptase, Is Limiting for Telomere Length Maintenance In Vivo." Mol. Cell. Biol. **24**(16): 7024-7031.

<http://mcb.asm.org/cgi/content/abstract/24/16/7024>

Telomerase consists of two essential components, the telomerase RNA template (TR) and telomerase reverse transcriptase (TERT). The haplo-insufficiency of TR was recently shown to cause one form of human dyskeratosis congenita, an inherited disease marked by abnormal telomere shortening. Consistent with this finding, we recently reported that mice heterozygous for inactivation of mouse TR exhibit a similar haplo-insufficiency and are deficient in the ability to elongate telomeres in vivo. To further assess the genetic regulation of telomerase activity, we have compared the abilities of TR-deficient and TERT-deficient mice to maintain or elongate telomeres in interspecies crosses. Homozygous TERT knockout mice had no telomerase activity and failed to maintain telomere length. In contrast, TERT+/- heterozygotes had no detectable defect in telomere elongation compared to wild-type controls, whereas TR+/- heterozygotes were deficient in telomere elongation. Levels of TERT mRNA in heterozygous mice were one-third to one-half the levels expressed in wild-type mice, similar to the reductions in telomerase RNA observed in TR heterozygotes. These findings indicate that both TR and TERT are essential for telomere maintenance and elongation but that gene copy number and transcriptional regulation of TR, but not TERT, are limiting for telomerase activity under the in vivo conditions analyzed.

Daley, J. M. and T. E. Wilson (2005). "Rejoining of DNA Double-Strand Breaks as a Function of Overhang Length." Mol. Cell. Biol. **25**(3): 896-906.

<http://mcb.asm.org/cgi/content/abstract/25/3/896>

The ends of spontaneously occurring double-strand breaks (DSBs) may contain various lengths of single-stranded DNA, blocking lesions, and gaps and flaps generated by end annealing. To investigate the processing of such structures, we developed an assay in which annealed oligonucleotides are ligated onto the ends of a linearized plasmid which is then transformed into *Saccharomyces cerevisiae*. Reconstitution of a marker occurs only when the oligonucleotides are incorporated and repair is in frame, permitting rapid analysis of complex DSB ends. Here, we created DSBs with compatible overhangs of various lengths and asked which pathways are required for their precise repair. Three mechanisms of rejoining were observed, regardless of overhang polarity: nonhomologous end joining (NHEJ), a Rad52-dependent single-strand annealing-like pathway, and a third mechanism independent of the first two mechanisms. DSBs with overhangs of less than 4 bases were mainly repaired by NHEJ. Repair became less dependent on NHEJ when the overhangs were longer or had a higher GC content. Repair of overhangs greater than 8 nucleotides was as much as 150-fold more efficient, impaired 10-fold by rad52 mutation, and highly accurate. Reducing the microhomology extent between long overhangs reduced their repair dramatically, to less than NHEJ of comparable short overhangs. These data support a model in which annealing energy is a primary determinant of the rejoining efficiency and mechanism.

de la Serna, I. L., Y. Ohkawa, et al. (2005). "MyoD Targets Chromatin Remodeling Complexes to the Myogenin Locus Prior to Forming a Stable DNA-Bound Complex." Mol. Cell. Biol. **25**(10): 3997-4009.

<http://mcb.asm.org/cgi/content/abstract/25/10/3997>

The activation of muscle-specific gene expression requires the coordinated action of muscle regulatory proteins and chromatin-remodeling enzymes. Microarray analysis performed in the presence or absence of a dominant-negative BRG1 ATPase demonstrated that approximately one-third of MyoD-induced genes were highly dependent on SWI/SNF enzymes. To understand the mechanism of activation, we performed chromatin immunoprecipitations analyzing the myogenin promoter. We found that H4 hyperacetylation preceded Brg1 binding in a MyoD-dependent manner but that MyoD binding occurred subsequent to H4 modification and Brg1 interaction. In the absence of functional SWI/SNF enzymes, muscle regulatory proteins did not bind to the myogenin promoter, thereby providing evidence for SWI/SNF-dependent activator binding. We observed that the homeodomain factor Pbx1, which cooperates with MyoD to stimulate myogenin expression, is constitutively bound to the myogenin promoter in a SWI/SNF-independent manner, suggesting a two-step mechanism in which MyoD initially interacts indirectly with the myogenin promoter and attracts chromatin-remodeling enzymes, which then facilitate direct binding by MyoD and other regulatory proteins.

Delaloy, C., J. Lu, et al. (2003). "Multiple Promoters in the WNK1 Gene: One Controls Expression of a Kidney-Specific Kinase-Defective Isoform." Mol. Cell. Biol. **23**(24): 9208-9221.

<http://mcb.asm.org/cgi/content/abstract/23/24/9208>

WNK1 is a serine-threonine kinase, the expression of which is affected in pseudohypoaldosteronism type II, a Mendelian form of arterial hypertension. We characterized human WNK1 transcripts to determine the molecular mechanisms governing WNK1 expression. We report the presence of two promoters generating two WNK1 isoforms with a complete kinase domain. Further variations are achieved by the use of two polyadenylation sites and tissue-specific splicing. We also determined the structure of a kidney-specific isoform regulated by a third promoter and starting at a novel exon. This transcript is kinase defective and has a predominant expression in the kidney compared to the other WNK1 isoforms, with, furthermore, a highly restricted expression profile in the distal convoluted tubule. We confirmed that the ubiquitous and kidney-specific promoters are functional in several cells lines and identified core promoters and regulatory elements. In particular, a strong enhancer element upstream from the kidney-specific exon seems specific to renal epithelial cells. Thus, control of human WNK1 gene expression of kinase-active or -deficient isoforms is mediated predominantly through the use of multiple transcription initiation sites and tissue-specific regulatory elements.

Dunty, J. M., V. Gabarra-Niecko, et al. (2004). "FERM Domain Interaction Promotes FAK Signaling." Mol. Cell. Biol. **24**(12): 5353-5368.

<http://mcb.asm.org/cgi/content/abstract/24/12/5353>

From the results of deletion analyses, the FERM domain of FAK has been proposed to inhibit enzymatic activity and repress FAK signaling. We have identified a sequence in the FERM domain that is important for FAK signaling in vivo. Point mutations in this sequence had little effect upon catalytic activity in vitro. However, the mutant exhibits reduced tyrosine phosphorylation and dramatically reduced Src family kinase binding. Further, the abilities of the mutant to transduce biochemical signals and to promote cell migration were severely impaired. The results implicate a FERM domain interaction in cell adhesion-dependent activation of FAK and downstream signaling. We also show that the purified FERM domain of FAK interacts with full-length FAK in vitro, and mutation of this sequence disrupts the interaction. These findings are discussed in the context of models of FAK regulation by its FERM domain.

Edelstein, L. C., L. Lagos, et al. (2003). "NF- κ B-Dependent Assembly of an Enhanceosome-Like Complex on the Promoter Region of Apoptosis Inhibitor Bfl-1/A1." Mol. Cell. Biol. **23**(8): 2749-2761.

<http://mcb.asm.org/cgi/content/abstract/23/8/2749>

Expression of the prosurvival Bcl-2 homologue Bfl-1/A1 is induced by NF- κ B-activating stimuli, while B and T cells from c-rel knockout mice show an absolute defect in bfl-1/a1 gene activation. Here, we demonstrate NF- κ B-dependent assembly of an enhanceosome-like complex on the promoter region of bfl-1. Binding of NF- κ B subunit c-Rel to DNA nucleated the concerted binding of transcription factors AP-1 and C/EBP β to the 5'-regulatory region of bfl-1. Optimal stability of the complex was dependent on proper orientation and phasing of the NF- κ B site. Chromatin immunoprecipitation analyses demonstrated that T-cell activation triggers in vivo binding of endogenous c-Rel, c-Jun, C/EBP β , and HMG-IC to the bfl-1 regulatory region, coincident with selective recruitment of coactivators TAFII250 and p300, SWI/SNF chromatin remodeling factor component BRG-1, and basal transcription factors TATA-binding protein (TBP) and TFIIB, as well as hyperacetylation of histones H3 and H4. These results highlight a critical role for NF- κ B in bfl-1 transcription and point to the need for a complex and precise regulatory network to control bfl-1 expression. To our knowledge, this is the first demonstration of enhanceosome-mediated regulation of a cell death inhibitor.

Gonzalez-Suarez, E., J. M. Flores, et al. (2002). "Cooperation between p53 Mutation and High Telomerase Transgenic Expression in Spontaneous Cancer Development." Mol. Cell. Biol. **22**(20): 7291-7301.

<http://mcb.asm.org/cgi/content/abstract/22/20/7291>

Telomerase reintroduction in adult somatic tissues is envisioned as a way to extend their proliferative capacity. It is still a question, however, whether constitutive telomerase expression in adult tissues impacts the normal aging and spontaneous cancer incidence of an organism. Here, we studied the aging and spontaneous cancer incidence of mice with transgenic telomerase expression in a wide range of adult tissues, K5-Tert mice. For this, we maintained large colonies of K5-Tert mice for more than 2 years. K5-Tert mice showed a decreased life span compared to wild-type cohorts associated with a higher incidence of preneoplastic and neoplastic lesions in various tissue types. Neoplasias in K5-Tert mice were coincident with transgene expression in the affected tissues. These observations suggest that high telomerase activity may cooperate with genetic alterations that occur with age to promote tumorigenesis. Indeed, we demonstrate here that increased cancer incidence and the reduced viability of K5-Tert mice are aggravated in a p53 $\pm$ genetic background, indicating that telomerase cooperates with loss of p53 function in inducing tumorigenesis. Altogether, these results demonstrate that constitutive high levels of telomerase activity result in a decreased life span associated with an increased incidence of neoplasias as the organism ages.

Hermoso, M. A., T. Matsuguchi, et al. (2004). "Glucocorticoids and Tumor Necrosis Factor Alpha Cooperatively Regulate Toll-Like Receptor 2 Gene Expression." Mol. Cell. Biol. **24**(11): 4743-4756.

<http://mcb.asm.org/cgi/content/abstract/24/11/4743>

Tumor necrosis factor alpha (TNF- α) and glucocorticoids are widely recognized as mutually antagonistic regulators of adaptive immunity and inflammation. Surprisingly, we show here that they cooperatively regulate components of innate immunity. The Toll-like receptor 2 (TLR2) gene encodes a transmembrane receptor critical for triggering innate immunity. Although TLR2 mRNA and protein are induced by inflammatory molecules such as TNF- α , we show that TLR2 is also induced by the anti-inflammatory glucocorticoids in cells where they also regulate MKP-1 mRNA and protein levels. TNF- α and glucocorticoids cooperate to regulate the TLR2 promoter, through the involvement of a 3' NF- κ B site, a STAT-binding element, and a 3' glucocorticoid response element (GRE). Molecular studies show that the κ B α superrepressor or a STAT dominant negative element prevented TNF- α and dexamethasone stimulation of TLR2 promoter. Similarly, an AF-1 deletion mutant of glucocorticoid receptor or ablation of a putative GRE notably reduced the cooperative regulation of TLR2. Using chromatin immunoprecipitation assays, we demonstrate that all three transcription factors interact with both endogenous and transfected TLR2 promoters after stimulation by TNF- α and dexamethasone. Together, these studies define novel signaling mechanism for these three transcription factors, with a profound impact on discrimination of innate and adaptive immune responses.

Irvine, R. A., I. G. Lin, et al. (2002). "DNA Methylation Has a Local Effect on Transcription and Histone Acetylation." *Mol. Cell. Biol.* **22**(19): 6689-6696.

<http://mcb.asm.org/cgi/content/abstract/22/19/6689>

DNA methylation is commonly associated with gene silencing, and a link between histone deacetylation and DNA methylation has been established. However, the transcriptional impact of the position and length of methylated zones relative to the promoter and the coding region of a gene remains quite unclear. This study investigates the impact of regional methylation on transcription and the relationship between DNA methylation and histone acetylation. Using patch-methylated stable episomes in human cells, we establish the pivotal importance of the location of DNA methylation in the regulation of transcription. We further demonstrate that the size of the methylated patch is not a key determinant for transcriptional suppression. The impact of DNA methylation on transcription is greater when it is in the transcription unit, and it is primarily a local effect. However, methylation outside of the transcription unit may potentiate the effect of methylation within the transcription unit. Acetylated histones are associated with unmethylated DNA and are nearly absent from methylated DNA regions. This association appears to be local and does not propagate along the DNA.

Kang, S.-H. L., C. Mione Kiefer, et al. (2003). "Role of the Promoter in Maintaining Transcriptionally Active Chromatin Structure and DNA Methylation Patterns In Vivo." *Mol. Cell. Biol.* **23**(12): 4150-4161.

<http://mcb.asm.org/cgi/content/abstract/23/12/4150>

Establishment and maintenance of differential chromatin structure between transcriptionally competent and repressed genes are critical aspects of transcriptional regulation. The elements and mechanisms that mediate formation and maintenance of these chromatin states in vivo are not well understood. To examine the role of the promoter in maintaining chromatin structure and DNA methylation patterns of the transcriptionally active X-linked HPRT locus, 323 bp of the endogenous human HPRT promoter (from position -222 to +102 relative to the translation start site) was replaced by plasmid sequences by homologous recombination in cultured HT-1080 male fibrosarcoma cells. The targeted cells, which showed no detectable HPRT transcription, were then assayed for effects on DNase I hypersensitivity, general DNase I sensitivity, and DNA methylation patterns across the HPRT locus. In cells carrying the deletion, significantly

diminished DNase I hypersensitivity in the 5' flanking region was observed compared to that in parental HT-1080 cells. However, general DNase I sensitivity and DNA methylation patterns were found to be very similar in the mutated cells and in the parental cells. These findings suggest that the promoter and active transcription play a relatively limited role in maintaining transcriptionally potentiated epigenetic states.

Karlseder, J., L. Kachatrian, et al. (2003). "Targeted Deletion Reveals an Essential Function for the Telomere Length Regulator Trf1." Mol. Cell. Biol. **23**(18): 6533-6541.

<http://mcb.asm.org/cgi/content/abstract/23/18/6533>

The human telomeric DNA binding factor TRF1 (hTRF1) and its interacting proteins TIN2, tankyrase 1 and 2, and PINX1 have been implicated in the regulation of telomerase-dependent telomere length maintenance. Here we show that targeted deletion of exon 1 of the mouse gene encoding Trf1 causes early (day 5 to 6 postcoitus) embryonic lethality. The absence of telomerase did not alter the Terf1ex1{Delta}/ex1{Delta} lethality, indicating that the phenotype was not due to inappropriate telomere elongation by telomerase. Terf1ex1{Delta}/ex1{Delta} blastocysts had a severe growth defect of the inner cell mass that was accompanied by apoptosis. However, no evidence was found for telomere uncapping causing this cell death; chromosome spreads of Terf1ex1{Delta}/ex1{Delta} blastocysts did not reveal chromosome end-to-end fusions, and p53 deficiency only briefly delayed Terf1ex1{Delta}/ex1{Delta} lethality. These data suggest that murine Trf1 has an essential function that is independent of telomere length regulation.

Kirito, K., N. Fox, et al. (2004). "Thrombopoietin Induces HOXA9 Nuclear Transport in Immature Hematopoietic Cells: Potential Mechanism by Which the Hormone Favorably Affects Hematopoietic Stem Cells." Mol. Cell. Biol. **24**(15): 6751-6762.

<http://mcb.asm.org/cgi/content/abstract/24/15/6751>

Members of the homeobox family of transcription factors are major regulators of hematopoiesis. Overexpression of either HOXB4 or HOXA9 in primitive marrow cells enhances the expansion of hematopoietic stem cells (HSCs). However, little is known of how expression or function of these proteins is regulated during hematopoiesis under physiological conditions. In our previous studies we demonstrated that thrombopoietin (TPO) enhances levels of HOXB4 mRNA in primitive hematopoietic cells (K. Kirito, N. Fox, and K. Kaushansky, Blood 102:3172-3178, 2003). To extend our studies, we investigated the effects of TPO on HOXA9 in this same cell population. Although overall levels of the transcription factor were not affected, we found that TPO induced the nuclear import of HOXA9 both in UT-7/TPO cells and in primitive Sca-1+/c-kit+/Gr-1- hematopoietic cells in a mitogen-activated protein kinase-dependent fashion. TPO also controlled MEIS1 expression at mRNA levels, at least in part due to phosphatidylinositol 3-kinase activation. Collectively, TPO modulates the function of HOXA9 by leading to its nuclear translocation, likely mediated by effects on its partner protein MEIS1, and potentially due to two newly identified nuclear localization signals. Our data suggest that TPO controls HSC development through the regulation of multiple members of the Hox family of transcription factors through multiple mechanisms.

Kurisaki, K., A. Kurisaki, et al. (2003). "Nuclear Factor YY1 Inhibits Transforming Growth Factor {beta}- and Bone Morphogenetic Protein-Induced Cell Differentiation." Mol. Cell. Biol. **23**(13): 4494-4510.

<http://mcb.asm.org/cgi/content/abstract/23/13/4494>

Smad proteins transduce transforming growth factor β (TGF- β) and bone morphogenetic protein (BMP) signals that regulate cell growth and differentiation. We have identified YY1, a transcription factor that positively or negatively regulates transcription of many genes, as a novel Smad-interacting protein. YY1 represses the induction of immediate-early genes to TGF- β and BMP, such as the plasminogen activator inhibitor 1 gene (PAI-1) and the inhibitor of differentiation/inhibitor of DNA binding 1 gene (Id-1). YY1 inhibits binding of Smads to their cognate DNA elements in vitro and blocks Smad recruitment to the Smad-binding element-rich region of the PAI-1 promoter in vivo. YY1 interacts with the conserved N-terminal Mad homology 1 domain of Smad4 and to a lesser extent with Smad1, Smad2, and Smad3. The YY1 zinc finger domain mediates the association with Smads and is necessary for the repressive effect of YY1 on Smad transcriptional activity. Moreover, downregulation of endogenous YY1 by antisense and small interfering RNA strategies results in enhanced transcriptional responses to TGF- β or BMP. Ectopic expression of YY1 inhibits, while knockdown of endogenous YY1 enhances, TGF- β - and BMP-induced cell differentiation. In contrast, overexpression or knockdown of YY1 does not affect growth inhibition induced by TGF- β or BMP. Accordingly, YY1 does not interfere with the regulation of immediate-early genes involved in the TGF- β growth-inhibitory response, the cell cycle inhibitors p15 and p21, and the proto-oncogene c-myc. In conclusion, YY1 represses Smad transcriptional activities in a gene-specific manner and thus regulates cell differentiation induced by TGF- β superfamily pathways.

Lamia, K. A., O. D. Peroni, et al. (2004). "Increased Insulin Sensitivity and Reduced Adiposity in Phosphatidylinositol 5-Phosphate 4-Kinase β -/- Mice." *Mol. Cell. Biol.* **24**(11): 5080-5087.

<http://mcb.asm.org/cgi/content/abstract/24/11/5080>

Phosphorylated derivatives of the lipid phosphatidylinositol are known to play critical roles in insulin response. Phosphatidylinositol 5-phosphate 4-kinases convert phosphatidylinositol 5-phosphate to phosphatidylinositol 4,5-bis-phosphate. To understand the physiological role of these kinases, we generated mice that do not express phosphatidylinositol 5-phosphate 4-kinase β . These mice are hypersensitive to insulin and have reduced body weights compared to wild-type littermates. While adult male mice lacking phosphatidylinositol 5-phosphate 4-kinase β have significantly less body fat than wild-type littermates, female mice lacking phosphatidylinositol 5-phosphate 4-kinase β have increased insulin sensitivity in the presence of normal adiposity. Furthermore, in vivo insulin-induced activation of the protein kinase Akt is enhanced in skeletal muscle and liver from mice lacking phosphatidylinositol 5-phosphate 4-kinase β . These results indicate that phosphatidylinositol 5-phosphate 4-kinase β plays a role in determining insulin sensitivity and adiposity in vivo and suggest that inhibitors of this enzyme may be useful in the treatment of type 2 diabetes.

Lanz, R. B., S. S. Chua, et al. (2003). "Steroid Receptor RNA Activator Stimulates Proliferation as Well as Apoptosis In Vivo." *Mol. Cell. Biol.* **23**(20): 7163-7176.

<http://mcb.asm.org/cgi/content/abstract/23/20/7163>

Steroid receptor RNA activator (SRA) is an RNA that coactivates steroid hormone receptor-mediated transcription in vitro. Its expression is strongly up-regulated in many human tumors of the breast, uterus, and ovary, suggesting a potential role in pathogenesis. To assess SRA function in vivo, a transgenic-mouse model was generated to enable robust human SRA expression by using the transcriptional activity of the mouse mammary tumor virus long terminal

repeat. Transgenic SRA was expressed in the nuclei of luminal epithelial cells of the mammary gland and tissues of the male accessory sex glands. Distinctive evidence for SRA function in vivo was obtained from the elevated levels of estrogen-controlled expression of progesterone receptor in transgenic mammary glands. Although overexpression of SRA showed strong promoting activities on cellular proliferation and differentiation, no alterations progressed to malignancy. Epithelial hyperplasia was accompanied by increased apoptosis, and preneoplastic lesions were cleared by focal degenerative transformations. In bitransgenic mice, SRA also antagonized ras-induced tumor formation. This work indicates that although coactivation of steroid-dependent transcription by SRA is accompanied by a proliferative response, overexpression is not in itself sufficient to induce tumorigenesis. Our results underline an intricate relationship between the different physiological roles of steroid receptors in conjunction with the RNA activator in the regulation of development, tissue homeostasis, and reproduction.

Le Cam, L., M. Lacroix, et al. (2004). "The E4F Protein Is Required for Mitotic Progression during Embryonic Cell Cycles." Mol. Cell. Biol. **24**(14): 6467-6475.

<http://mcb.asm.org/cgi/content/abstract/24/14/6467>

The ubiquitously expressed E4F protein was originally identified as an E1A-regulated cellular transcription factor required for adenovirus replication. The function of this protein in normal cell physiology remains largely unknown. To address this issue, we generated E4F knockout mice by gene targeting. Embryos lacking E4F die at the peri-implantation stage, while in vitro-cultured E4F^{-/-} blastocysts exhibit defects in mitotic progression, chromosomal missegregation, and increased apoptosis. Consistent with these observations, we found that E4F localizes to the mitotic spindle during the M phase of early embryos. Our results establish a crucial role for E4F during early embryonic cell cycles and reveal an unexpected function for E4F in mitosis.

Lee, S. J., G. Gonzalez-Aseguinolaza, et al. (2002). "Disseminated Candidiasis and Hepatic Malarial Infection in Mannose-Binding-Lectin-A-Deficient Mice." Mol. Cell. Biol. **22**(23): 8199-8203.

<http://mcb.asm.org/cgi/content/abstract/22/23/8199>

To examine the physiological functions of mannose-binding lectin A (MBL-A), we generated mice that were deficient in MBL-A and examined their susceptibilities to the microbial pathogens *Candida albicans* and *Plasmodium yoelii*, an accepted experimental malaria model in mouse. We found no differences in the survival rates and fungal burdens of wild-type and MBL-A^{-/-} mice with disseminated *C. albicans* infection. The two mouse strains were also similar in their abilities to resist hepatic accumulation of *P. yoelii* parasites. We conclude that MBL-A deficiency does not alter resistance to disseminated candidiasis or initial hepatic invasion by *P. yoelii*.

Maier, H., J. Colbert, et al. (2003). "Activation of the Early B-Cell-Specific mb-1 (Ig- α) Gene by Pax-5 Is Dependent on an Unmethylated Ets Binding Site." Mol. Cell. Biol. **23**(6): 1946-1960.

<http://mcb.asm.org/cgi/content/abstract/23/6/1946>

Methylation of cytosine in CpG dinucleotides promotes transcriptional repression in mammals by blocking transcription factor binding and recruiting methyl-binding proteins that initiate chromatin remodeling. Here, we use a novel cell-based system to show that retrovirally expressed Pax-5 protein activates endogenous early B-cell-specific mb-1 genes in plasmacytoma cells, but only

when the promoter is hypomethylated. CpG methylation does not directly affect binding of the promoter by Pax-5. Instead, methylation of an adjacent CpG interferes with assembly of ternary complexes comprising Pax-5 and Ets proteins. In electrophoretic mobility shift assays, recruitment of Ets-1 is blocked by methylation of the Ets site (5'CCGGAG) on the antisense strand. In transfection assays, selective methylation of a single CpG within the Pax-5-dependent Ets site greatly reduces mb-1 promoter activity. Prior demethylation of the endogenous mb-1 promoter is required for its activation by Pax-5 in transduced cells. Although B-lineage cells have only unmethylated mb-1 genes and do not modulate methylation of the mb-1 promoter during development, other tissues feature high percentages of methylated alleles. Together, these studies demonstrate a novel DNA methylation-dependent mechanism for regulating transcriptional activity through the inhibition of DNA-dependent protein-protein interactions.

Martens, J. H. A., M. Verlaan, et al. (2002). "Scaffold/Matrix Attachment Region Elements Interact with a p300-Scaffold Attachment Factor A Complex and Are Bound by Acetylated Nucleosomes." Mol. Cell. Biol. **22**(8): 2598-2606.

<http://mcb.asm.org/cgi/content/abstract/22/8/2598>

The transcriptional coactivator p300 regulates transcription by binding to proteins involved in transcription and by acetylating histones and other proteins. These transcriptional effects are mainly at promoter and enhancer elements. Regulation of transcription also occurs through scaffold/matrix attachment regions (S/MARs), the chromatin regions that bind the nuclear matrix. Here we show that p300 binds to the S/MAR binding protein scaffold attachment factor A (SAF-A), a major constituent of the nuclear matrix. Using chromatin immunoprecipitations, we established that both p300 and SAF-A bind to S/MAR elements in the transiently silent topoisomerase I gene prior to its activation at G1 during cell cycle. This binding is accompanied by local acetylation of nucleosomes, suggesting that p300-SAF-A interactions at S/MAR elements of nontranscribed genes might poise these genes for transcription.

Martens, J. H. A., M. Verlaan, et al. (2003). "Cascade of Distinct Histone Modifications during Collagenase Gene Activation." Mol. Cell. Biol. **23**(5): 1808-1816.

<http://mcb.asm.org/cgi/content/abstract/23/5/1808>

Gene activation in eukaryotes requires chromatin remodeling, in part via histone modifications. To study the events at the promoter of a mitogen-inducible gene, we examined the induction of expression of the collagenase gene. It has been established that the collagenase gene can be activated by c-Jun and c-Fos and that the transcriptional coactivator p300 is involved in the activation. As expected, we found histone acetyltransferase activity at the collagenase promoter during activation. Interestingly, we also found histone methyltransferase and kinase activity. Strikingly, the first modification observed is methylation of histone H3 lysine 4, which correlates with the binding of the SET9 methyltransferase and the assembly of a complex consisting of c-Jun, c-Fos, TATA binding protein, and RNA polymerase II. The assembly of the preinitiation complex also shows an ordered binding of the acetyltransferase p300, the RSK2 kinase, and the SWI/SNF component Brg-1. Our results suggest that collagenase gene activation involves a dynamic recruitment of different factors and that in addition to acetylation, histone H3 lysine 4 di- and trimethylation and histone H3 serine 10 phosphorylation are important steps in the activation of this gene.

Narisawa, S., N. B. Hecht, et al. (2002). "Testis-Specific Cytochrome c-Null Mice Produce Functional Sperm but Undergo Early Testicular Atrophy." Mol. Cell. Biol. **22**(15): 5554-5562.

<http://mcb.asm.org/cgi/content/abstract/22/15/5554>

Differentiating male germ cells express a testis-specific form of cytochrome c (Cyt cT) that is distinct from the cytochrome c expressed in somatic cells (Cyt cS). To examine the role of Cyt cT in germ cells, we generated mice null for Cyt cT. Homozygous Cyt cT^{-/-} pups were statistically underrepresented (21%) but developed normally and were fertile. However, spermatozoa isolated from the cauda epididymis of Cyt cT-null animals were less effective in fertilizing oocytes in vitro and contain reduced levels of ATP compared to wild-type sperm. Sperm from Cyt cT-null mice contained a greater number of immotile spermatozoa than did samples from control mice, i.e., 53.1% {+/-} 13.7% versus 33.2% {+/-} 10.3% ($P < 0.0001$) for vas deferens sperm and 40.1% {+/-} 9.6% versus 33.2% {+/-} 7.5% ($P = 0.0104$) for epididymal sperm. Cyt cT-null mice often exhibit early atrophy of the testes after 4 months of age, losing germ cells as a result of increased apoptosis. However, no difference in the activation of caspase-3, -8, or -9 was detected between the Cyt cT^{-/-} testes and controls. Our data indicate that the Cyt cT-null testes undergo early atrophy equivalent to that which occurs during aging as a consequence of a reduction in oxidative phosphorylation.

Park, E. K., N. Warner, et al. (2002). "Low-Molecular-Weight Protein Tyrosine Phosphatase Is a Positive Component of the Fibroblast Growth Factor Receptor Signaling Pathway." Mol. Cell. Biol. **22**(10): 3404-3414.

<http://mcb.asm.org/cgi/content/abstract/22/10/3404>

Low-molecular-weight protein tyrosine phosphatase (LMW-PTP) has been implicated in the regulation of cell growth and actin rearrangement mediated by several receptor tyrosine kinases, including platelet-derived growth factor and epidermal growth factor. Here we identify the *Xenopus laevis* homolog of LMW-PTP1 (XLPTP1) as an additional positive regulator in the fibroblast growth factor (FGF) signaling pathway during *Xenopus* development. XLPTP1 has an expression pattern that displays substantial overlap with FGF receptor 1 (FGFR1) during *Xenopus* development. Using morpholino antisense technology, we show that inhibition of endogenous XLPTP1 expression dramatically restricts anterior and posterior structure development and inhibits mesoderm formation. In ectodermal explants, loss of XLPTP1 expression dramatically blocks the induction of the early mesoderm gene, *Xbra* (*Xbra*), by FGF and partially blocks *Xbra* induction by Activin. Moreover, FGF-induced activation of mitogen-activated protein (MAP) kinase is also inhibited by XLPTP1 morpholino antisense oligonucleotides; however, introduction of RNA encoding XLPTP1 is able to rescue morphological and biochemical effects of antisense inhibition. Inhibition of FGF-induced MAP kinase activity due to loss of XLPTP1 is also rescued by an active Ras, implying that XLPTP1 may act upstream of or parallel to Ras. Finally, XLPTP1 physically associates only with an activated FGFR1, and this interaction requires the presence of SNT1/FRS-2 (FGFR substrate 2). Although LMW-PTP1 has been shown to participate in other receptor systems, the data presented here also reveal XLPTP1 as a new and important component of the FGF signaling pathway.

Prioleau, M.-N., M.-C. Gendron, et al. (2003). "Replication of the Chicken {beta}-Globin Locus: Early-Firing Origins at the 5' HS4 Insulator and the {rho}- and {beta}A-Globin Genes Show Opposite Epigenetic Modifications." Mol. Cell. Biol. **23**(10): 3536-3549.

<http://mcb.asm.org/cgi/content/abstract/23/10/3536>

Chromatin structure is believed to exert a strong effect on replication origin function. We have studied the replication of the chicken β -globin locus, whose chromatin structure has been extensively characterized. This locus is delimited by hypersensitive sites (HSs) that mark the position of insulator elements. A stretch of condensed chromatin and another HS separate the β -globin domain from an adjacent folate receptor (FR) gene. We demonstrate here that in erythroid cells that express the FR but not the globin genes, replication initiates at four sites within the β -globin domain, one at the 5' HS4 insulator and the other three near the ρ - and β A-globin genes. Three origins consist of G+C-rich sequences enriched in CpG dinucleotides. The fourth origin is A+T rich. Together with previous work, these data reveal that the insulator origin has unmethylated CpGs, hyperacetylated histones H3 and H4, and lysine 4-methylated histone H3. In contrast, opposite modifications are observed at the other G+C-rich origins. We also show that the whole region, including the stretch of condensed chromatin, replicates early in S phase in these cells. Therefore, different early-firing origins within the same locus may have opposite patterns of epigenetic modifications. The role of insulator elements in DNA replication is discussed.

Regnier, V., P. Vagnarelli, et al. (2005). "CENP-A Is Required for Accurate Chromosome Segregation and Sustained Kinetochore Association of BubR1." *Mol. Cell. Biol.* **25**(10): 3967-3981.

<http://mcb.asm.org/cgi/content/abstract/25/10/3967>

CENP-A is an evolutionarily conserved, centromere-specific variant of histone H3 that is thought to play a central role in directing kinetochore assembly and in centromere function. Here, we have analyzed the consequences of disrupting the CENP-A gene in the chicken DT40 cell line. In CENP-A-depleted cells, kinetochore protein assembly is impaired, as indicated by mislocalization of the inner kinetochore proteins CENP-I, CENP-H, and CENP-C as well as the outer components Nuf2/Hec1, Mad2, and CENP-E. However, BubR1 and the inner centromere protein INCENP are efficiently recruited to kinetochores. Following CENP-A depletion, chromosomes are deficient in proper congression on the mitotic spindle and there is a transient delay in prometaphase. CENP-A-depleted cells further proceed through anaphase and cytokinesis with unequal chromosome segregation, suggesting that some kinetochore function remains following substantial depletion of CENP-A. We furthermore demonstrate that CENP-A-depleted cells exhibit a specific defect in maintaining kinetochore localization of the checkpoint protein BubR1 under conditions of checkpoint activation. Our data thus point to a specific role for CENP-A in assembly of kinetochores competent in the maintenance of mitotic checkpoint signaling.

Scamborova, P., A. Wong, et al. (2004). "An Intronic Enhancer Regulates Splicing of the Twintron of *Drosophila melanogaster* prospero Pre-mRNA by Two Different Spliceosomes." *Mol. Cell. Biol.* **24**(5): 1855-1869.

<http://mcb.asm.org/cgi/content/abstract/24/5/1855>

We have examined the alternative splicing of the *Drosophila melanogaster* prospero twintron, which contains splice sites for both the U2- and U12-type spliceosome and generates two forms of mRNA, pros-L (U2-type product) and pros-S (U12-type product). We find that twintron splicing is developmentally regulated: pros-L is abundant in early embryogenesis while pros-S displays the opposite pattern. We have established a Kc cell in vitro splicing system that accurately splices a minimal pros substrate containing the twintron and have examined the sequence requirements for pros twintron splicing. Systematic deletion and mutation analysis of intron sequences established that twintron splicing requires a 46-nucleotide purine-rich element located 32 nucleotides downstream of the U2-type 5' splice site. While this element regulates both splicing pathways, its alteration showed the severest effects on the U2-type splicing pathway. Addition of

an RNA competitor containing the wild-type purine-rich element to the Kc extract abolished U2-type splicing and slightly repressed U12-type splicing, suggesting that a trans-acting factor(s) binds the enhancer element to stimulate twintron splicing. Thus, we have identified an intron region critical for prospero twintron splicing as a first step towards elucidating the molecular mechanism of splicing regulation involving competition between the two kinds of spliceosomes.

Schuendeln, M. M., R. P. Piekorz, et al. (2004). "The Centrosomal, Putative Tumor Suppressor Protein TACC2 Is Dispensable for Normal Development, and Deficiency Does Not Lead to Cancer." Mol. Cell. Biol. **24**(14): 6403-6409.

<http://mcb.asm.org/cgi/content/abstract/24/14/6403>

TACC2 is a member of the transforming acidic coiled-coil-containing protein family and is associated with the centrosome-spindle apparatus during cell cycling. In vivo, the TACC2 gene is expressed in various splice forms predominantly in postmitotic tissues, including heart, muscle, kidney, and brain. Studies of human breast cancer samples and cell lines suggest a putative role of TACC2 as a tumor suppressor protein. To analyze the physiological role of TACC2, we generated mice lacking TACC2. TACC2-deficient mice are viable, develop normally, are fertile, and lack phenotypic changes compared to wild-type mice. Furthermore, TACC2 deficiency does not lead to an increased incidence of tumor development. Finally, in TACC2-deficient embryonic fibroblasts, proliferation and cell cycle progression as well as centrosome numbers are comparable to those in wild-type cells. Therefore, TACC2 is not required, nonredundantly, for mouse development and normal cell proliferation and is not a tumor suppressor protein.

Song, S., J. Cooperman, et al. (2004). "Identification of Cyclin D3 as a Direct Target of E2A Using DamID." Mol. Cell. Biol. **24**(19): 8790-8802.

<http://mcb.asm.org/cgi/content/abstract/24/19/8790>

The transcription factor E2A can promote precursor B cell expansion, promote G1 cell cycle progression, and induce the expressions of multiple G1-phase cyclins. To better understand the mechanism by which E2A induces these cyclins, we characterized the relationship between E2A and the cyclin D3 gene promoter. E2A transactivated the 1-kb promoter of cyclin D3, which contains two E boxes. However, deletion of the E boxes did not disrupt the transactivation by E2A, raising the possibility of indirect activation via another transcription factor or binding of E2A to non-E-box DNA elements. To distinguish between these two possibilities, promoter occupancy was examined using the DamID approach. A fusion construct composed of E2A and the Escherichia coli DNA adenosine methyltransferase (E47Dam) was subcloned in lentivirus vectors and used to transduce precursor B-cell and myeloid progenitor cell lines. In both cell types, specific adenosine methylation was identified at the cyclin D3 promoter. Chromatin immunoprecipitation analysis confirmed the DamID findings and localized the binding to within 1 kb of the two E boxes. The methylation by E47Dam was not disrupted by mutations in the E2A portion that block DNA binding. We conclude that E2A can be recruited to the cyclin D3 promoter independently of E boxes or E2A DNA binding activity.

Sorci, G., F. Riuzzi, et al. (2003). "S100B Inhibits Myogenic Differentiation and Myotube Formation in a RAGE-Independent Manner." Mol. Cell. Biol. **23**(14): 4870-4881.

<http://mcb.asm.org/cgi/content/abstract/23/14/4870>

S100B is a Ca²⁺-modulated protein of the EF-hand type with both intracellular and extracellular roles. S100B, which is most abundant in the brain, has been shown to exert trophic and toxic effects on neurons depending on the concentration attained in the extracellular space. S100B is also found in normal serum, and its serum concentration increases in several nervous and nonnervous pathological conditions, suggesting that S100B-expressing cells outside the brain might release the protein and S100B might exert effects on nonnervous cells. We show here that at picomolar to nanomolar levels, S100B inhibits myogenic differentiation of rat L6 myoblasts via inactivation of p38 kinase with resulting decrease in the expression of the myogenic differentiation markers, myogenin, muscle creatine kinase, and myosin heavy chain, and reduction of myotube formation. Although myoblasts express the multiligand receptor RAGE, which has been shown to transduce S100B effects on neurons, S100B produces identical effects on myoblasts overexpressing either full-length RAGE or RAGE lacking the transducing domain. This suggests that S100B affects myoblasts by interacting with another receptor and that RAGE is not the only receptor for S100B. Our data suggest that S100B might participate in the regulation of muscle development and regeneration by inhibiting crucial steps of the myogenic program in a RAGE-independent manner.

Van Sloun, P. P. H., I. Varlet, et al. (2002). "Involvement of Mouse Rev3 in Tolerance of Endogenous and Exogenous DNA Damage." *Mol. Cell. Biol.* **22**(7): 2159-2169.

<http://mcb.asm.org/cgi/content/abstract/22/7/2159>

The Rev3 gene of *Saccharomyces cerevisiae* encodes the catalytic subunit of DNA polymerase {zeta} that is implicated in mutagenic translesion synthesis of damaged DNA. To investigate the function of its mouse homologue, we have generated mouse embryonic stem cells and mice carrying a targeted disruption of Rev3. Although some strain-dependent variation was observed, Rev3^{-/-} embryos died around midgestation, displaying retarded growth in the absence of consistent developmental abnormalities. Rev3^{-/-} cell lines could not be established, indicating a cell-autonomous requirement of Rev3 for long-term viability. Histochemical analysis of Rev3^{-/-} embryos did not reveal aberrant replication or cellular proliferation but demonstrated massive apoptosis in all embryonic lineages. Although increased levels of p53 are detected in Rev3^{-/-} embryos, the embryonic phenotype was not rescued by the absence of p53. A significant increase in double-stranded DNA breaks as well as chromatid and chromosome aberrations was observed in cells from Rev3^{-/-} embryos. The inner cell mass of cultured Rev3^{-/-} blastocysts dies of a delayed apoptotic response after exposure to a low dose of N-acetoxy-2-acetylaminofluorene. These combined data are compatible with a model in which, in the absence of polymerase {zeta}, double-stranded DNA breaks accumulate at sites of unreplicated DNA damage, eliciting a p53-independent apoptotic response. Together, these data are consistent with involvement of polymerase {zeta} in translesion synthesis of endogenously and exogenously induced DNA lesions.

Waggoner, S. A. and S. A. Liebhaber (2003). "Identification of mRNAs Associated with {alpha}CP2-Containing RNP Complexes." *Mol. Cell. Biol.* **23**(19): 7055-7067.

<http://mcb.asm.org/cgi/content/abstract/23/19/7055>

Posttranscriptional controls in higher eukaryotes are central to cell differentiation and developmental programs. These controls reflect sequence-specific interactions of mRNAs with one or more RNA binding proteins. The {alpha}-globin poly(C) binding proteins ({alpha}CPs) comprise a highly abundant subset of K homology (KH) domain RNA binding proteins and have a characteristic preference for binding single-stranded C-rich motifs. {alpha}CPs have been implicated in translation control and stabilization of multiple cellular and viral mRNAs. To explore

the full contribution of α CPs to cell function, we have identified a set of mRNAs that associate in vivo with the major α CP2 isoforms. One hundred sixty mRNA species were consistently identified in three independent analyses of α CP2-RNP complexes immunopurified from a human hematopoietic cell line (K562). These mRNAs could be grouped into subsets encoding cytoskeletal components, transcription factors, proto-oncogenes, and cell signaling factors. Two mRNAs were linked to ceroid lipofuscinosis, indicating a potential role for α CP2 in this infantile neurodegenerative disease. Surprisingly, α CP2 mRNA itself was represented in α CP2-RNP complexes, suggesting autoregulatory control of α CP2 expression. In vitro analyses of representative target mRNAs confirmed direct binding of α CP2 within their 3' untranslated regions. These data expand the list of mRNAs that associate with α CP2 in vivo and establish a foundation for modeling its role in coordinating pathways of posttranscriptional gene regulation.

Zhao, F., R. McCarrick-Walmsley, et al. (2003). "Inhibition of p300/CBP by Early B-Cell Factor." Mol. Cell. Biol. **23**(11): 3837-3846.

<http://mcb.asm.org/cgi/content/abstract/23/11/3837>

Early B-cell factor (EBF) is a DNA binding protein required for early B-cell development. It activates transcription of several B-cell-specific genes, including the λ 5 gene, which encodes a protein necessary for signaling by the pre-B-cell receptor. In an effort to understand the mechanism by which EBF activates transcription, we examined its interaction with the coactivator protein p300/CBP. We found that two domains of EBF each bind the histone acetyltransferase (HAT)/CH3 domain of p300/CBP both in vitro and in vivo. Surprisingly, transcriptional activation by EBF was not sensitive to E1A, a potent p300/CBP inhibitor. In fact, overexpressed EBF mimicked E1A by severely repressing the activity of several other transcription factors, including E47, a protein that acts cooperatively with EBF to promote transcription of the λ 5 gene. This broad inhibitory profile correlated with EBF's ability to repress the HAT activity of p300/CBP in vivo and in vitro. However, such a repressed complex is not likely to form at the λ 5 promoter in vivo since (i) EBF could not bind p300/CBP and DNA simultaneously and (ii) the cooperativity imparted by E47 was sensitive to E1A. Our data reveal an intriguing inhibitory property of EBF--a property shared only by E1A, Twist, Pu.1, and the Hox family of homeodomain proteins--and suggest that E47 and EBF play distinct roles during λ 5 promoter activation.

Zofall, M., J. Persinger, et al. (2004). "Functional Role of Extranucleosomal DNA and the Entry Site of the Nucleosome in Chromatin Remodeling by ISW2." Mol. Cell. Biol. **24**(22): 10047-10057.

<http://mcb.asm.org/cgi/content/abstract/24/22/10047>

A minimal amount of extranucleosomal DNA was required for nucleosome mobilization by ISW2 as shown by using a photochemical histone mapping approach to analyze nucleosome movement on a set of nucleosomes with varied lengths of extranucleosomal DNA. ISW2 was ineffective in repositioning or mobilizing nucleosomes with ≥ 20 bp of extranucleosomal DNA. In addition, ISW2 was able to slide nucleosomes to within only 10 to 13 bp of the edge of DNA fragments. The nucleosome mobilization was promoted by extranucleosomal single-stranded DNA with modest strand preference. Gaps (10 bp) just inside the nucleosome and in the extranucleosomal DNA showed that the transfer of torsional strain (twist) into the nucleosomal DNA region was not required for mobilizing nucleosomes. However, indications are that the extranucleosomal DNA immediately adjacent to the nucleosome has an important role in the initial stage of nucleosome movement by ISW2.

Ahmed, S. A., K. Snow-Bailey, et al. (2005). "Nine Novel Germline Gene Variants in the RET Proto-Oncogene Identified in Twelve Unrelated Cases." Mol. Diagn. 7(2): 283-288.

<http://jmd.amjpathol.org/cgi/content/abstract/7/2/283>

We report nine novel DNA alterations in the RET proto-oncogene in 12 unrelated cases identified by DNA sequencing of exons 10 and 11 of the gene. The novel variants K666E, IVS9-11G[-&gt]A, D631V in cis with H665Q, D631E (with C634Y), E623K (in trans with C618S), 616delGAG (in trans with C609Y), Y606C, C630R, and R635-T636insELCR;T636P were detected in patients with various clinical presentations ranging from thyroid goiter, medullary thyroid carcinoma, and pheochromocytoma to classic multiple endocrine neoplasia type 2A. When novel DNA alterations are found, extended family studies can be helpful in determining the clinical significance of such findings. Segregation within families suggests that K666E and T636insELCR;T636P are likely to be disease-causing mutations. However, the mechanism by which they affect the normal activity of the RET receptor is unclear. Absence of segregation with disease was observed for E623K and 616delGAG. For the remainder of the DNA alterations, family studies were not possible, and the clinical significance of these novel variants needs further assessment. Additional case reports, animal models, and/or functional studies are needed to determine the clinical significance of these newly identified variants.

Ahrens, K., R. Braylan, et al. (2002). "IgH PCR of Zinc Formalin-Fixed, Paraffin-Embedded Non-Lymphomatous Gastric Samples Produces Artifactual "Clonal" Bands Not Observed in Paired Tissues Unexposed to Zinc Formalin." Mol. Diagn. 4(3): 159-163.

<http://jmd.amjpathol.org/cgi/content/abstract/4/3/159>

Helicobacter pylori (HP) causes dense gastritis that can be difficult to distinguish morphologically from MALT-type lymphoma (ML). Immunoglobulin heavy chain (IgH) gene analysis by polymerase chain reaction (PCR) is often used to resolve diagnosis. However, monoclonal bands have been reported in nonmalignant cases of gastritis. Retrospectively, 16 gastric ML with both formalin-fixed, paraffin-embedded (FF-PE) and ethanol-fixed samples (EF), and 9 cases of FF-PE HP-gastritis were analyzed by IgH PCR to document the presence of non-reproducible bands in HP-gastritis, but not ML samples. In duplicate analyses, 12 of 16 ML yielded identical monoclonal bands in FF-PE and EF samples whereas 3 of 9 FF-PE gastritis cases yielded different-sized (ie, non-reproducible) "clonal" bands. Sequencing of two PCR products from a gastritis case confirmed IgH gene sequences. To investigate whether FF-PE had a direct effect on producing these non-reproducible bands, 7 gastrectomy samples were prospectively divided into EF and FF-PE halves for IgH PCR. All 7 samples demonstrated polyclonal smears in EF portions while 4 of 7 FF-PE portions yielded either multiple distinct bands or non-reproducible bands. In conclusion, IgH PCR of FF-PE tissue can create artifactual "clonal" bands, which are the appropriate product size, contain IgH sequences, and, if not performed in duplicate, may confuse interpretation of B-cell clonality.

Antonescu, C. R., S. J. Tschernyavsky, et al. (2002). "Molecular Diagnosis of Clear Cell Sarcoma: Detection of EWS-ATF1 and MITF-M Transcripts and Histopathological and Ultrastructural Analysis of 12 Cases." Mol. Diagn. **4**(1): 44-52.

<http://jmd.amjpathol.org/cgi/content/abstract/4/1/44>

Clear cell sarcoma (CCS), also known as melanoma of soft parts, is an uncommon deep soft tissue tumor presenting typically in the lower extremities of young adults. Previous cytogenetic studies have established the specificity of the recurrent t(12;22)(q13;q12), resulting in a EWS-ATF1 fusion, for CCS. The prevalence of the EWS-ATF1 fusion in CCS remains unclear, since most genetically confirmed CCS have been reported as isolated cytogenetic or molecular diagnostic case reports. We therefore studied histologically confirmed CCS from 12 patients for the presence of EWS-ATF1 by reverse-transcriptase polymerase chain reaction (RT-PCR), using RNA extracted from either frozen (four cases) or formalin-fixed paraffin-embedded (eight cases) material. All primary tumors were located in the deep soft tissues of the extremities. Histologically, 10 cases had a typical epithelioid nested appearance. Most or all cases showed immunostaining for HMB45 (12 of 12), S-100 protein (10 of 12), and MITF (12 of 12). Ultrastructural analysis showed melanosomes in six of seven cases. The presence of an EWS-ATF1 fusion transcript was identified by RT-PCR in 11 of 12 cases (91%), all of which showed the same fusion transcript structure, namely the previously described in-frame fusion of EWS exon 8 to ATF1 codon 65. RT-PCR analysis for the melanocyte-specific splice form of the MITF transcript was positive in all cases tested (4 of 4). These data confirm that EWS-ATF1 detection can be used as a highly sensitive diagnostic test for CCS and that CCS expresses the melanocyte-specific form of the MITF transcript, further supporting its genuine melanocytic differentiation.

Barrans, S. L., P. A. S. Evans, et al. (2003). "The Detection of t(14;18) in Archival Lymph Nodes: Development of a Fluorescence in Situ Hybridization (FISH)-Based Method and Evaluation by Comparison with Polymerase Chain Reaction." Mol. Diagn. **5**(3): 168-175.

<http://jmd.amjpathol.org/cgi/content/abstract/5/3/168>

Fluorescence in situ hybridization (FISH) has been used to demonstrate the t(14;18) in up to 100% of follicular lymphoma (FL) cases, however, there is little reproducible data using fixed tissue. The aim was therefore to develop a robust FISH method for the demonstration of translocations in archival tissue. The technique was evaluated by comparison with multiplex polymerase chain reaction (PCR), capable of detecting the majority of known breakpoints. Twenty-eight paired frozen and fixed cases of FL and 20 reactive controls were analyzed. The t(14;18) was detected in 23 of 28 cases using PCR on frozen material and 8 of 20 in paraffin. Using FISH, 24 of 26 frozen and 26 of 28 paraffin cases had a demonstrable translocation. All 20 reactive nodes were negative for the t(14;18) by PCR. Using FISH, one of the reactive cases had occasional cells with a translocation FISH pattern, demonstrable in frozen and paraffin samples. This is consistent with the presence of the t(14;18), which is well described in normal individuals. Both PCR and FISH are highly effective for t(14;18) analysis in unfixed tissue. When only paraffin blocks are available, FISH is the method of choice, and a result was achieved in 100% of cases. The method is applicable to the retrospective analysis of a range of translocations.

Baudhuin, L. M., M. Mai, et al. (2005). "Analysis of hMLH1 and hMSH2 Gene Dosage Alterations in Hereditary Nonpolyposis Colorectal Cancer Patients by Novel Methods." Mol. Diagn. **7**(2): 226-235.

<http://jmd.amjpathol.org/cgi/content/abstract/7/2/226>

A significant fraction of hereditary nonpolyposis colorectal cancer cases with defective mismatch repair (ie, Lynch syndrome) have large genomic deletions or duplications in the mismatch repair genes, hMLH1 and hMSH2, which can be challenging to detect by traditional methods. For this study, we developed and validated a novel Southern blot analysis method that allows for ascertainment of the extent of the dosage alterations on an exon-by-exon basis and compared this method to a second novel technique, multiplex ligation-dependent probe amplification (MLPA). From a total of 254 patients referred for Lynch syndrome testing, 20 of the 118 MLH1 cases and 42 of the 136 MSH2 cases had large genomic alterations, as detected by Southern blot. MLPA and Southern blot results were concordant with the exception of three major discrepancies: one because of a lack of MLPA probes for the region altered, another because of a point mutation near the MLPA probe ligation site, and another that was unexplained. Compared to Southern blot, MLPA has a shorter turn-around time, the analysis is less costly, less time-consuming, and less labor-intensive, and results are generally clear and unambiguous. However, concerns with MLPA include the presence of false-negatives and -positives because of positioning of probes and DNA variants near the probe ligation site. Overall, both Southern blot and MLPA provide important tools for the complete evaluation of patients with Lynch syndrome.

Carbone, A., A. Gloghini, et al. (2005). "Kaposi's Sarcoma-Associated Herpesvirus/Human Herpesvirus Type 8-Positive Solid Lymphomas: A Tissue-Based Variant of Primary Effusion Lymphoma." Mol. Diagn. 7(1): 17-27.

<http://jmd.amjpathol.org/cgi/content/abstract/7/1/17>

Kaposi's sarcoma-associated herpesvirus (KSHV), also termed human herpesvirus type 8, is consistently identified in Kaposi's sarcoma, primary effusion lymphoma (PEL), and multicentric Castleman's disease. Here we report four cases of KSHV-bearing solid lymphomas that occurred in AIDS patients (cases 1 to 3) and in a human immunodeficiency virus (HIV)-seronegative person (case 4). The patients presented extranodal masses in the abdomen (cases 1, 3, and 4) or skin (case 2), and nodal involvement, together with Kaposi's sarcoma (case 3). The gastrointestinal tract was involved in two patients (cases 1 and 3). The patients did not develop a lymphomatous effusion. KSHV was detected in the tumor cells of all cases by immunohistochemistry and by polymerase chain reaction. Epstein-Barr virus was detected in two of the HIV-related cases. All KSHV-positive solid lymphomas exhibited PEL-like cell morphology. To investigate the relationship of these disorders to PEL and to other AIDS-associated diffuse large cell lymphomas, KSHV-positive solid lymphomas were tested for the expression of a set of genes that were previously shown by gene profiling analysis to define PEL tumor cells. The results showed that expression of this set of genes in KSHV-positive lymphomas is similar to that of PEL but distinct from KSHV-negative AIDS-associated diffuse large cell lymphomas. Because pathobiological features of KSHV-positive solid lymphomas closely mimic those of PEL, our results suggest that KSHV-positive solid lymphomas should be considered as a tissue-based variant of classical PEL, irrespective of HIV status.

Dorschner, M. O., D. Barden, et al. (2002). "Diagnosis of Five Spinocerebellar Ataxia Disorders by Multiplex Amplification and Capillary Electrophoresis." Mol. Diagn. 4(2): 108-113.

<http://jmd.amjpathol.org/cgi/content/abstract/4/2/108>

The autosomal-dominant spinocerebellar ataxias (ADCA) are a heterogeneous group of neurodegenerative disorders with variable expression and phenotypic overlap. An accurate diagnosis relies on detection of a mutation in a specific causative gene, which is typically an abnormal number of CAG trinucleotide repeats. To streamline testing in a clinical setting, we converted our current panel of tests for the spinocerebellar ataxias (SCA) types SCA1, SCA2,

SCA3, SCA6, and SCA7 from five independent amplification reactions analyzed by polyacrylamide gel electrophoresis (PAGE) to a single multiplex amplification reaction analyzed by capillary electrophoresis (CE). Multiplex amplification was facilitated by the use of chimeric primers; different lengths and fluorochromes distinguished the amplicons. During CE with commercially available molecular weight standards, the SCA amplicons migrated faster than predicted, thereby underestimating their length compared to that determined previously by PAGE. This was observed to varying degrees for each of the five loci, with the greatest size differential occurring in amplicons with greater (CAG)_n. To determine accurate amplicon length, and therefore an accurate number of CAG repeats, a size correction formula was calculated for each locus. This multiplex semi-automated assay has been reliable during 1 year of use in a clinical setting during which 57 samples were tested and five positive samples were detected.

Hadd, A. G., W. Laosinchai-Wolf, et al. (2004). "Microsphere Bead Arrays and Sequence Validation of 5/7/9T Genotypes for Multiplex Screening of Cystic Fibrosis Polymorphisms." *Mol. Diagn.* **6**(4): 348-355.

<http://jmd.amjpathol.org/cgi/content/abstract/6/4/348>

The development of simple and rapid methods for the detection of the common genetic mutations associated with cystic fibrosis (CF) requires access to positive-control samples including the 5/7/9T variants of intron 8. We used PCR and a simple multiplex bead-array assay to identify 5/7/9T control samples from 29 commercially available DNA samples. Unpurified PCR products were directly hybridized to color-coded beads containing allele-specific capture probes for 5/7/9T detection. The performance of the assay was investigated using reverse-complement oligonucleotides, individual PCR products, and multiplex PCR products for 5/7/9T detection within a complex CFTR screening assay. Samples were genotyped by grouping the relative signal intensities from each capture probe. Of 29 commercially available DNA samples analyzed, 2 5T/7T, 2 5T/9T, 9 7T/9T, 11 7T/7T, and 5 9T/9T genotypes were identified. The genotype within each sample group was confirmed by DNA sequencing. The assay was compatible with the analysis of 10 to 1000 ng of genomic DNA isolated from whole blood and allowed for the separate identification of primary CFTR mutations from reflex variants. The correct identification of positive controls demonstrated the utility of a simple bead-array assay and provided accessible samples for assay optimization and for routine quality control in the clinical laboratory.

Hamajima, N., T. Saito, et al. (2002). "Competitive Amplification and Unspecific Amplification in Polymerase Chain Reaction with Confronting Two-Pair Primers." *Mol. Diagn.* **4**(2): 103-107.

<http://jmd.amjpathol.org/cgi/content/abstract/4/2/103>

Polymerase chain reaction with confronting two-pair primers (PCR-CTPP) is an inexpensive, time-saving genotyping method that is applicable for most single nucleotide polymorphisms. To date, we have applied PCR-CTPP successfully for the genotyping of more than 30 polymorphisms. This paper demonstrates the differences in DNA amplification among different annealing temperatures of PCR-CTPP with given melting temperatures for four primers. The NQO1 C609T (Pro187Ser) polymorphism was used as an example. Two sets of four primers were applied for PCR-CTPP; the first set with different melting temperatures (T_{ms}), and the second with similar T_{ms}. The comparisons with one-pair primer PCR (allele-specific PCR) revealed that PCR-CTPP amplified DNA more specifically than allele-specific PCR. The primers with different T_{ms} caused competitive DNA amplification for heterozygous genotype. Four primers with similar T_{ms} amplified both alleles unspecifically at a lower annealing temperature, while the same DNA samples were correctly genotyped under an optimal annealing temperature. These findings are unique for PCR-CTPP, and important characteristics when the primers and

annealing temperatures in PCR-CTPP are designed. The knowledge of these characteristics will extend the applicability of PCR-CTPP for polymorphism genotyping.

Jebbink, J., X. Bai, et al. (2003). "Development of Real-Time PCR Assays for the Quantitative Detection of Epstein-Barr Virus and Cytomegalovirus, Comparison of TaqMan Probes, and Molecular Beacons." Mol. Diagn. **5**(1): 15-20.

<http://jmd.amjpathol.org/cgi/content/abstract/5/1/15>

Human Epstein-Barr virus (EBV) and cytomegalovirus (CMV) can cause serious complications in immunocompromised patients. Rapid diagnosis of EBV and CMV infection is critical in the management of the disease so that anti-viral therapy can be started early. Here we describe the development of real-time PCR assays using TaqMan probes and molecular beacons and compare the performance of both assays with a well-established, validated, gel-based PCR method for the quantification of EBV and CMV in patients' samples. The TaqMan and molecular beacon assays were linear between 10 to 10⁷ viral genomes/reaction. Both assays generated calibration curves with strong correlation and low intra-assay and interassay variation. Results of EBV and CMV viral load determination inpatient samples obtained by the gel-based and real-time PCR were very similar. The real-time PCR assays showed increases in viral load before clinical measures of viral disease and decreases in viral load during anti-viral therapy in two of six pediatric patients. The data indicate that these TaqMan and molecular beacon approaches are accurate, rapid, and reliable assays for the diagnosis and monitoring of EBV and CMV infections in patients.

Koga, T., Y. Horio, et al. (2004). "Identification of MGB1 as a Marker in the Differential Diagnosis of Lung Tumors in Patients with a History of Breast Cancer by Analysis of Publicly Available SAGE Data." Mol. Diagn. **6**(2): 90-95.

<http://jmd.amjpathol.org/cgi/content/abstract/6/2/90>

The risk of developing second primary cancers is increased in patients with breast cancer. The lung is one of the major target organs, and therefore a differential diagnosis between primary and metastatic cancers is required for the treatment of lung tumors in patients with a history of breast cancer. However, biopsy specimens frequently result in small, fragmented tissues containing only a few, degenerated cancer cells. We attempted to find a useful marker for differential diagnosis, using the online SAGE database. We selected three molecules, small breast epithelial mucin (SBEM), prostate epithelium-specific Ets transcription factor (PDEF), and mammaglobin (MGB1), as potential markers for breast cancer. SBEM and PDEF proved of no use for practical differential diagnosis because they are expressed in the normal bronchus. In contrast, expression of MGB1 was detected in all 22 primary breast cancers, but not in 22 normal lung tissues. Furthermore, all 12 metastatic breast cancers examined demonstrated positive MGB1 transcripts, whereas one of 48 primary lung adenocarcinomas expressed MGB1. This suggests that MGB1 can serve as a differential molecular marker. In practice, prospective examination, using the nine cases with a history of breast cancer, confirmed the usefulness of MGB1 in differential diagnosis.

Mitas, M., L. Hoover, et al. (2003). "Lunx Is a Superior Molecular Marker for Detection of Non-Small Lung Cell Cancer in Peripheral Blood." Mol. Diagn. **5**(4): 237-242.

<http://jmd.amjpathol.org/cgi/content/abstract/5/4/237>

The clinical management of non-small cell lung cancer (NSCLC) would benefit greatly by a test that was able to detect small amounts of NSCLC in the peripheral blood. In this report, we used a novel strategy to enrich tumor cells from the peripheral blood of 24 stage I to IV NSCLC patients and determined expression levels for six cancer-associated genes (lunx, muc1, KS1/4, CEA, CK19, and PSE). Using thresholds established at three standard deviations above the mean observed in 15 normal controls, we observed that lunx (10 of 24, 42%), muc1 (5 of 24, 21%), and CK19 (5 of 24, 21%) were overexpressed in 14 of 24 (58%) peripheral blood samples obtained from NSCLC patients. Patients who overexpressed either KS1/4 (n = 2) or PSE (n = 1) also overexpressed either lunx or muc1. Of patients with presumed curable and resectable stage I to II disease (n = 7), at least one marker was overexpressed in three (43%) patients. In advanced stage III to IV patients (n = 17), at least one marker was overexpressed in 11 patients (65%). These results provide evidence that circulating tumor cells can be detected in NSCLC patients by a high throughput molecular technique. Further studies are needed to determine the clinical relevance of gene overexpression.

Murphy, K. M., T. Geiger, et al. (2003). "A Single Nucleotide Primer Extension Assay to Detect the APC I1307K Gene Variant." Mol. Diagn. **5**(4): 222-226.

<http://jmd.amjpathol.org/cgi/content/abstract/5/4/222>

Adenomatous polyposis coli (APC) is a tumor suppressor gene important in colorectal tumorigenesis. A genetic variant of APC, I1307K, results from a T-to-A transversion at nucleotide 3920 which converts the wild-type sequence to a homopolymer tract (A8). The I1307K alteration is not itself oncogenic, but creates a hypermutable region (A8) that is prone to frame-shift mutations. The APC I1307K variant occurs in approximately 6% of the Ashkenazi Jewish population and is reported to approximately double an individual's risk for colorectal cancer. Here we describe a single nucleotide primer extension assay for the detection of the APC I1307K mutation. Following PCR amplification, nucleotide 3920 of the APC gene is directly sequenced using single nucleotide primer extension technology. The assay is in a multiplex format allowing simultaneous forward and reverse sequencing of the I1307K variant, which provides an internal, independent confirmation of each testing result. The assay was validated against 60 samples previously characterized by an allele-specific oligonucleotide (ASO) hybridization assay, with 100% concordance of results. Compared to the ASO assay, this single nucleotide primer extension assay requires significantly less technical time to perform, and has a greatly increased throughput capacity. The single nucleotide extension assay provides a highly sensitive and specific assay to identify individuals with the APC I1307K gene variant who may benefit from increased colorectal screening.

Patnaik, M., J. S. Dlott, et al. (2004). "Detection of Genomic Polymorphisms Associated with Venous Thrombosis Using the Invader Biplex Assay." Mol. Diagn. **6**(2): 137-144.

<http://jmd.amjpathol.org/cgi/content/abstract/6/2/137>

A multi-site study to assess the accuracy and performance of the biplex Invader assay for genotyping five polymorphisms implicated in venous thrombosis was carried out in seven laboratories. Genotyping results obtained using the Invader biplex assay were compared to those obtained from a reference method, either allele-specific polymerase chain reaction (AS-PCR), restriction fragment length polymorphism (PCR-RFLP) or PCR-mass spectrometry. Results were compared for five loci associated with venous thrombosis: Factor V Leiden, Factor II (prothrombin) G20210A, methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C, and plasminogen activator inhibitor (PAI-1) 4G/5G. Of a total of 1448 genotypes tested in this study, there were 22 samples that gave different results between the Invader biplex assay and the PCR-

based methods. On further testing, 21 were determined to be correctly genotyped by the Invader Assay and only a single discrepancy was resolved in favor of the PCR-based assays. The compiled results demonstrate that the Invader biplex assay provides results more than 99.9% concordant with standard PCR-based techniques and is a rapid and highly accurate alternative to target amplification-based methods.

Plumbley, J. A., H. Fan, et al. (2002). "Lymphoid Tissues from Patients with Infectious Mononucleosis Lack Monoclonal B and T Cells." Mol. Diagn. 4(1): 37-43.

<http://jmd.amjpathol.org/cgi/content/abstract/4/1/37>

In typical cases of infectious mononucleosis (IM), lymphoid tissue is rarely submitted for pathological examination. When lymphoid tissues from IM cases are examined, the histological appearance of IM may be difficult to distinguish from malignant lymphoma. The purpose of this study was to address the utility of clinical molecular assays for T and B cell clonality in distinguishing IM from lymphoid malignancy. DNA was recovered from paraffin-embedded archival lymphoid tissues of 18 cases of IM and 13 control cases representing other reactive lymphoid hyperplasias. T cell receptor {gamma} (TCR-{gamma}) and immunoglobulin heavy chain (IgH) gene rearrangements were assayed using our standard clinical polymerase chain reaction procedures targeting each of the four functional variable (V) families and the three joining (J) families of the TCR-{gamma} gene, and framework III of the IgH gene, respectively. In 17 of 18 cases of IM, no monoclonal T or B cell populations were detectable. One case, the only spleen specimen in the study, had an oligoclonal pattern of TCR-{gamma} rearrangements. The control cases representing other reactive hyperplasias also lacked monoclonality. The assays used were sensitive to clonal populations as small as 5% of cells. In this case series, no monoclonal lymphoid populations were identified in any case of IM. This finding suggests that molecular studies are useful in distinguishing IM from lymphoid neoplasms.

Sanchez-Vega, B., F. Vega, et al. (2002). "Quantification of bcl-2/JH Fusion Sequences and a Control Gene by Multiplex Real-Time PCR Coupled with Automated Amplicon Sizing by Capillary Electrophoresis." Mol. Diagn. 4(4): 223-229.

<http://jmd.amjpathol.org/cgi/content/abstract/4/4/223>

Follicular lymphoma is characterized by the presence of the t(14;18)(q32;q21) chromosomal translocation which juxtaposes the bcl-2 gene at 18q21 with the immunoglobulin heavy chain locus at 14q32. Quantification of t(14;18) carrying cells in FL patients can be achieved by real-time PCR, a highly sensitive technique for evaluating treatment efficacy and minimal residual disease. Despite the many advantages of real-time technology for this purpose, one disadvantage is that current real-time t(14;18) PCR assays amplify a control gene as a normalizer in a separate reaction. Since each PCR reaction has its own kinetics, separate PCR assays for target and control sequences can potentially result in inaccurate quantification of t(14;18)-positive cells. In addition, the real-time t(14;18) PCR assays do not determine the size of the amplified fusion sequence, which is helpful for excluding contamination and is commonly used to demonstrate clonal identity between pre- and post-treatment specimens from a patient. To address these limitations, we designed a multiplex real-time PCR protocol that allows amplification of control and target genes in the same reaction and precise size determination of bcl-2/JH fusion sequences by capillary electrophoresis. This multiplex PCR assay is equally sensitive to previous assays, allows more accurate quantification of bcl-2/JH fusion sequences, and is more convenient.

Schrijver, I., S. Ramalingam, et al. (2005). "Diagnostic Testing by CFTR Gene Mutation Analysis in a Large Group of Hispanics: Novel Mutations and Assessment of a Population-Specific Mutation Spectrum." Mol. Diagn. 7(2): 289-299.

<http://jmd.amjpathol.org/cgi/content/abstract/7/2/289>

Characterization of CFTR mutations in the U.S. Hispanic population is vital to early diagnosis, genetic counseling, patient-specific treatment, and the understanding of cystic fibrosis (CF) pathogenesis. The mutation spectrum in Hispanics, however, remains poorly defined. A group of 257 self-identified Hispanics with clinical manifestations consistent with CF were studied by temporal temperature gradient electrophoresis and/or DNA sequencing. A total of 183 mutations were identified, including 14 different amino acid-changing novel variants. A significant proportion (78/85) of the different mutations identified would not have been detected by the ACMG/ACOG-recommended 25-mutation screening panel. Over one third of the mutations (27/85) occurred with a relative frequency >1%, which illustrates that the identified mutations are not all rare. This is supported by a comparison with other large CFTR studies. These results underscore the disparity in mutation identification between Caucasians and Hispanics and show utility for comprehensive diagnostic CFTR mutation analysis in this population.

Shadrach, B., M. Commane, et al. (2004). "A Rare Mutation in the Primer Binding Region of the Amelogenin Gene Can Interfere with Gender Identification." Mol. Diagn. 6(4): 401-405.

<http://jmd.amjpathol.org/cgi/content/abstract/6/4/401>

PCR amplification of part of the X-Y homologous amelogenin gene with a single primer pair has been used as a sex identification test because it generates different length products from the X and Y chromosomes. Using a commercially available kit that contains amelogenin primers, we report a single phenotypically normal Caucasian male out of 327 males tested to date that failed to show an X chromosome-specific PCR product. Using alternative amelogenin primers external to but encompassing the initial amplicon, an X chromosome-specific product was seen. Sequence analysis of this X-specific PCR product revealed a C to G mutation at the most 3' base of the initial reverse amelogenin PCR primer. An alternative reverse PCR primer with this most 3' base deleted showed X- and Y-specific products from the case study male. Rare mutations that result in a failure to amplify sex chromosome-specific products can result in incorrect gender identification.

Taylor, M. L., D. Sehgal, et al. (2004). "Demonstration That Mast Cells, T Cells, and B Cells Bearing the Activating Kit Mutation D816V Occur in Clusters within the Marrow of Patients with Mastocytosis." Mol. Diagn. 6(4): 335-342.

<http://jmd.amjpathol.org/cgi/content/abstract/6/4/335>

Mastocytosis is characterized by focal heterotypic clusters of mast cells and lymphocytes in the bone marrow and by a somatically acquired activating Kit mutation, D816V. The relationship of the occurrence of this mutation to the heterotypic clusters of mast cells and lymphocytes in bone marrow is unknown. We hypothesized that these two unique features of mastocytosis were related. To explore this hypothesis, laser capture microdissected mast cells, B cells, and T cells, from both lesional and non-lesional areas of bone marrow biopsy tissues from patients with mastocytosis, were examined for the D816V mutation in their DNA, using HinfI restriction

digestion of nested PCR products amplified from extracts of dissected cells. The D816V mutation was detected in mast cells, B cells, and T cells from lesional but not non-lesional areas of bone marrow tissues. B cells obtained from lesional areas of tissue were also assessed for clonality and were found to at least represent an oligoclonal population. Thus, mast cells and lymphocytes within focal aggregates in the bone marrow of those with mastocytosis are more frequently positive for the codon 816 activating mutation. Further, the B cell population is oligoclonal, suggesting that clonal proliferation is unlikely to be the basis of clustering.

Uchiyama, M., C. Maesawa, et al. (2005). "Consensus JH Gene Probes with Conjugated 3'-Minor Groove Binder for Monitoring Minimal Residual Disease in Acute Lymphoblastic Leukemia." Mol. Diagn. 7(1): 121-126.

<http://jmd.amjpathol.org/cgi/content/abstract/7/1/121>

Several approaches for the detection of minimal residual disease (MRD) in childhood acute lymphoblastic leukemia (ALL) have shown the importance of determining the level of MRD precisely. In the present study, we tested a new real-time quantitative polymerase chain reaction (RQ-PCR) strategy with minor groove binder (MGB) technology for immunoglobulin heavy chain gene rearrangements by positioning a MGB probe at the germline JH segments and one of the primers at the downstream introns in combination with an allele-specific oligonucleotide (ASO) primer complementary to the VH-DH or DH-JH junctional region. A MGB probe forms extremely stable duplexes with single-stranded DNA targets, allowing the use of shorter probes for hybridization-based assays. Therefore, it shows positional flexibility. We have designed two novel consensus MGB JH germline probes for analyzing all of the germline rearrangements registered in the V BASE database, and demonstrated that the MRD was detectable with the probes in 17 cases of childhood ALL. The actual copy number for the targets and dynamic changes before and after treatment were almost identical between the JH MGB probe and conventional non-MGB probes in each patient. MGB technology will undoubtedly contribute to MRD-PCR studies of childhood ALL.

Wang, Z.-Y., Q. Zhang, et al. (2003). "Detection of Protein Tyrosine-Kinase (PTK) Gene Expression Pattern in Normal and Malignant T Lymphocytes by Combined PTK-Specific Polymerase Chain Reaction and Parallel Denaturing Gradient Gel Electrophoresis." Mol. Diagn. 5(2): 113-120.

<http://jmd.amjpathol.org/cgi/content/abstract/5/2/113>

Protein tyrosine kinases (PTKs) control key functions of normal and malignant cells. Comparison of PTK gene expression among various cell populations may be achieved by amplification of the PTK cDNAs using degenerate primers which recognize two relatively invariable regions within their catalytic domain. This approach produces a mixture of PTK cDNA fragments with identical or very similar lengths which are difficult to separate by standard gel electrophoresis. These mixed products are then analyzed in a random fashion which leads to redundant cloning of some and potential omission of other PTKs. By using parallel denaturing gradient gel electrophoresis (DGGE) we have been able to separate the amplified PTK cDNAs derived from the same T-lymphocyte population and compare their expression between various types of normal and malignant T lymphocytes. One such PTK is the type I receptor for insulin-like growth factor, which we found to be preferentially expressed by neoplastic T cells on the both mRNA and protein levels. The combination of PCR which uses PTK-specific primers and parallel DGGE of the amplified PTK cDNAs may prove useful in studying mechanisms of cell activation and malignant transformation and in identifying targets for therapies based on selective inhibition of oncogenic PTKs.

Apostolakis, E. M., R. Lanz, et al. (2004). "Pituitary Adenylate Cyclase-Activating Peptide: A Pivotal Modulator of Steroid-Induced Reproductive Behavior in Female Rodents." Mol. Endocrinol. **18**(1): 173-183.

<http://mend.endojournals.org/cgi/content/abstract/18/1/173>

Pituitary adenylate cyclase-activating polypeptide (PACAP) regulates the secretion of GnRH into the hypothalamic hypophyseal portal system and sensitizes the pituitary for release of hormones that trigger ovulation. Because reproductive behavior is synchronized with GnRH release, the present study was undertaken to determine whether PACAP in the ventromedial nucleus (VMN) plays a role in receptivity. To this end, we used rat and mouse reproductive behavioral models to determine the biological relationship between PACAP and steroid receptor function in females. We provide evidence for the requirement of PACAP in the VMN for progesterone (P)-dependent sexual behavior in estrogen (E)-primed females. We clarify the biological and molecular mechanisms of PACAP activity by showing 1) that inhibition of endogenous PACAP suppresses P receptor (PR)-dependent sexual behavior facilitated by the steroid P or D1-like agonist SKF38393 and 2) that PR, steroid receptor coactivators-1 and -2, and new protein synthesis are essential for ligand independent PACAP-facilitated behavior. These findings are consistent with convergence of PACAP-mediated cellular signals on PR for genomic activation and subsequent behavioral changes. Further, we show that steroids regulate both endogenous PACAP mRNA in the VMN and immunoreactive PACAP in the medial basal hypothalamus and cerebral spinal fluid for ligand-dependent, steroid receptor-dependent receptivity. The present findings delineate a novel, steroid-dependent mechanism within the female hypothalamus by which the neuropeptide PACAP acts as a feed-forward, paracrine, and/or autocrine factor for synchronization of behavior coordinate with hypothalamic control of ovulation.

Eijken, M., M. Hewison, et al. (2005). "11{beta}-Hydroxysteroid Dehydrogenase Expression and Glucocorticoid Synthesis Are Directed by a Molecular Switch during Osteoblast Differentiation." Mol. Endocrinol. **19**(3): 621-631.

<http://mend.endojournals.org/cgi/content/abstract/19/3/621>

11{beta}-Hydroxysteroid dehydrogenase type 1 (11{beta}-HSD1) plays an important role in the prereceptor regulation of corticosteroids by locally converting cortisone into active cortisol. To investigate the impact of this mechanism on osteoblast development, we have characterized 11{beta}-HSD1 activity and regulation in a differentiating human osteoblast cell line (SV-HFO). Continuous treatment with the synthetic glucocorticoid dexamethasone induces differentiation of SV-HFO cells during 21 d of culture. Using this cell system, we showed an inverse relationship between 11{beta}-HSD1 activity and osteoblast differentiation. 11{beta}-HSD1 mRNA expression and activity were low and constant in differentiating osteoblasts. However, in the absence of differentiation (no dexamethasone), 11{beta}-HSD1 mRNA and activity increased strongly from d 12 of culture onward, with a peak around d 19. Promoter reporter studies provided evidence that specific regions of the 11{beta}-HSD1 gene are involved in this differentiation controlled regulation of the enzyme. Functional implication of these changes in 11{beta}-HSD1 is shown by the induction of osteoblast differentiation in the presence of cortisone. The current study

demonstrates the presence of an intrinsic differentiation-driven molecular switch that controls expression and activity of 11 β -HSD1 and thereby cortisol production by human osteoblasts. This efficient mechanism by which osteoblasts generate cortisol in an autocrine fashion to ensure proper differentiation will help to understand the complex effects of cortisol on bone metabolism.

Hewetson, A., E. C. Hendrix, et al. (2002). "Identification of the RUSH Consensus-Binding Site by Cyclic Amplification and Selection of Targets: Demonstration that RUSH Mediates the Ability of Prolactin to Augment Progesterone-Dependent Gene Expression." *Mol. Endocrinol.* **16**(9): 2101-2112.

<http://mend.endojournals.org/cgi/content/abstract/16/9/2101>

RUSH-1 α / β transcription factors were cloned by recognition site screening with an 85-bp region (-170/-85) of the rabbit uteroglobin gene. Deletion analysis showed this region was essential to prolactin (PRL) action, but conclusions were limited by the complexity of the large deletion. Cyclic amplification and selection of targets (CASTing) was used to identify the RUSH-binding site (-126/-121). Endometrial nuclear proteins were incubated with a pool of degenerate oligonucleotides and immunoprecipitated with RUSH-1 α / β antibodies. Bound DNA was amplified by PCR. The consensus motif (MCWTDK) was identified after five rounds of CASTing, authenticated by CASTing with RUSH-1 α -specific antibodies and recombinant protein, and refined with EMSA. Dissociation rate constants (K_d = 0.1-1.0 nM; r = 0.99) revealed high-affinity binding. Chromatin immunoprecipitation confirmed *in vivo* binding of RUSH to the transcriptionally active uteroglobin promoter. CASTing also revealed RUSH-GATA transcription factor interactions. Endometrial GATA-4 expression is progesterone dependent (Northern analysis) and preferentially localized in the epithelium (in situ hybridization). Although physically affiliated with RUSH, uterine forms of GATA-4 were not required for RUSH-DNA binding. Site-directed mutagenesis and transient transfection assays showed the RUSH motif mediates the ability of PRL to augment progesterone-dependent uteroglobin transcription. RUSH is central to the mechanism whereby PRL augments progesterone-dependent gene transcription.

Hewitt, S. C., B. J. Deroo, et al. (2003). "Estrogen Receptor-Dependent Genomic Responses in the Uterus Mirror the Biphasic Physiological Response to Estrogen." *Mol. Endocrinol.* **17**(10): 2070-2083.

<http://mend.endojournals.org/cgi/content/abstract/17/10/2070>

The physiological responses of the rodent uterus to acute estrogen (E) dosing can be divided into early and late events. Examples of early responses include increased RNA transcription, hyperemia, and water imbibition 2 and 6 h following E administration respectively, whereas later responses include cycles of DNA synthesis and mitosis of epithelial cells beginning 10 and 16 h after E. The development of estrogen receptor (ER) knockout (ERKO) mice, combined with microarray technology, has allowed us to design a genomic approach to study the acute response of the rodent reproductive tract to E. To determine whether early and late biological responses are correlated with altered regulation of a single set of genes or distinct sets of genes characteristic of early and late responses, uterine RNA was obtained from ovariectomized mice that were treated with vehicle or with estradiol for 2 h (early) or 24 h (late). Samples were also prepared from identically treated mice that lacked either ER α (α ERKO) or ER β (β ERKO) to address the relative contributions of the ERs in the uterine responses. Microarray analysis of the relative expression of 8700 mouse cDNAs indicated distinct clusters of genes that were regulated both positively and negatively by E in the early or late phases as well as clusters of genes regulated at both times. Both early and late responses by the β ERKO samples were indistinguishable from those of WT samples, whereas the α ERKO showed little change in gene expression in response to E, indicating the predominant role for ER α in

the genomic response. Further studies indicated that the genomic responses in samples from intermediate time points (6 h, 12 h) fall within the early or late clusters, rather than showing unique clusters regulated in the intermediary period. The use of this genomic approach has illustrated how physiological responses are reflected in genomic patterns. Furthermore, the identification of functional gene families that are regulated by E in the uterus combined with the utilization of genetically altered experimental animal models can help to uncover and define novel mechanisms of E action.

Hwang, E. S., D. W. Kim, et al. (2004). "Regulation of Signal Transducer and Activator of Transcription 1 (STAT1) and STAT1-Dependent Genes by RET/PTC (Rearranged in Transformation/Papillary Thyroid Carcinoma) Oncogenic Tyrosine Kinases." *Mol. Endocrinol.* **18**(11): 2672-2684.

<http://mend.endojournals.org/cgi/content/abstract/18/11/2672>

Chimeric RET/PTC (rearranged in transformation/papillary thyroid carcinoma) oncoproteins are constitutively active tyrosine kinases found in thyroid papillary carcinoma and nonneoplastic Hashimoto's thyroiditis. Although several proteins have been identified to be substrates of RET/PTC kinases, the pathogenic roles played by RET/PTC in malignant and benign thyroid diseases and the molecular mechanisms that are involved are not fully understood. We found that RET/PTC expression phosphorylates the Y701 residue of STAT1, a type II interferon (IFN)-responsive protein. RET/PTC-mediated signal transducer and activator of transcription 1 (STAT1) phosphorylation requires RET/PTC kinase activity to be intact but other tyrosine kinases, such as Janus kinases or c-Src, are not involved. RET/PTC-induced STAT1 transcriptional activation was not inhibited by suppressor of cytokine signaling-1 or -3, or protein inhibitors of activated STAT3 [(protein inhibitor of activated STAT (PIAS3)], but PIAS1 strongly repressed the RET/PTC-induced transcriptional activity of STAT1. RET/PTC-induced STAT1 activation caused IFN regulatory factor-1 expression. We found that STAT1 and IFN regulatory factor-1 cooperated to significantly increase transcription from type IV IFN- γ -responsive promoters of class II transactivator genes. Significantly, cells stably expressing RET/PTC expressed class II transactivator and showed enhanced de novo membrane expression of major histocompatibility complex (MHC) class II proteins. Furthermore, RET/PTC1-bearing papillary thyroid carcinoma cells strongly expressed MHC class II (human leukocyte-associated antigen-DR α) genes, whereas the surrounding normal tissues did not. Thus, RET/PTC is able to phosphorylate and activate STAT1. This may lead to enhanced MHC class II expression, which may explain why the tissues surrounding RET/PTC-positive cancers are infiltrated with lymphocytes. Such immune response-promoting activity of RET/PTC may also relate to the development of Hashimoto's thyroiditis.

Ishii, S., M. Yamada, et al. (2004). "Aberrant Dynamics of Histone Deacetylation at the Thyrotropin-Releasing Hormone Gene in Resistance to Thyroid Hormone." *Mol. Endocrinol.* **18**(7): 1708-1720.

<http://mend.endojournals.org/cgi/content/abstract/18/7/1708>

Histone acetylation status influences transcriptional activity, and the mechanism of negative gene regulation by thyroid hormone remains unclear, although its impairment by a mutant thyroid hormone receptor (TR) is critical for resistance to thyroid hormone (RTH). We found a novel RTH mutant, F455S, that exhibited impaired repression of the TRH gene and had a strong dominant-negative effect on the gene. F455S strongly interacted with nuclear receptor corepressor (NCoR) and was hard to dissociate from it. To analyze the dynamics of histone acetylation status in vivo, we established cell lines stably expressing the TRH promoter and wild-type or F455S TR. Treatment with a histone deacetylase (HDAC) inhibitor completely abolished the repression of the

gene by T3. The histones H3 and H4 at the TRH promoter were acetylated, and addition of T3 caused recruitment of HDACs 2 and 3 within 15 min, resulting in a transient deacetylation of the histone tails. TR and NCoR were located on the promoter, and T3 caused NCoR dissociation and steroid receptor coactivator-1 recruitment. In the presence of F455S, the histones were hyperacetylated, and HDAC recruitment and histone deacetylation were significantly impaired. This is the first report demonstrating the direct involvement of aberrant dynamics of chromatin modification in RTH.

Jansen, E., J. S. E. Laven, et al. (2004). "Abnormal Gene Expression Profiles in Human Ovaries from Polycystic Ovary Syndrome Patients." Mol. Endocrinol. **18**(12): 3050-3063.

<http://mend.endojournals.org/cgi/content/abstract/18/12/3050>

Polycystic ovary syndrome (PCOS) represents the most common cause of anovulatory infertility and affects 5-10% of women of reproductive age. The etiology of PCOS is still unknown. The current study is the first to describe consistent differences in gene expression profiles in human ovaries comparing PCOS patients vs. healthy normoovulatory individuals. The microarray analysis of PCOS vs. normal ovaries identifies dysregulated expression of genes encoding components of several biological pathways or systems such as Wnt signaling, extracellular matrix components, and immunological factors. Resulting data may provide novel clues for ovarian dysfunction in PCOS. Intriguingly, the gene expression profiles of ovaries from (long-term) androgen-treated female-to-male transsexuals (TSX) show considerable overlap with PCOS. This observation provides supportive evidence that androgens play a key role in the pathogenesis of PCOS. Presented data may contribute to a better understanding of dysregulated pathways in PCOS, which might ultimately reveal novel leads for therapeutic intervention.

Oksche, A., G. Leder, et al. (2002). "Variant Amino Acids in the Extracellular Loops of Murine and Human Vasopressin V2 Receptors Account for Differences in Cell Surface Expression and Ligand Affinity." Mol. Endocrinol. **16**(4): 799-813.

<http://mend.endojournals.org/cgi/content/abstract/16/4/799>

Cloning and sequencing of the murine chromosomal region XB harboring the murine vasopressin V2 receptor (mV2R) gene and comparison with the orthologous human Xq28 region harboring the human vasopressin V2 receptor (hV2R) revealed conservation of the genomic organization and a high degree of sequence identity in the V2R coding regions. Despite an identity of 87% of the amino acid sequences, both receptors show marked functional differences upon stable expression in Chinese hamster ovary cells: the mV2R displayed a 5-fold higher affinity for [3H]AVP than the human ortholog; similar differences were found for the AVP-mediated activation of adenylyl cyclase. Saturation binding experiments with transiently transfected intact COS.M6 cells showed that the mV2R was 3- to 5-fold less abundantly expressed at the cell surface than the hV2R. Laser scanning microscopy of fusion proteins consisting of the V2Rs and green fluorescent protein (GFP) (mV2R/GFP, hV2R/GFP) demonstrated that the hV2R/GFP was efficiently transported to the plasma membrane, whereas the mV2R/GFP was localized mainly within the endoplasmic reticulum. Chimeric hV2Rs, in which the first and/or second extracellular loop(s) were replaced by the corresponding loop(s) of the mV2R, revealed that the second extracellular loop accounts for the differences in ligand binding, but the first extracellular loop accounts for the reduced cell surface expression. The exchange of lysine 100 by aspartate in the first extracellular loop of hV2R was sufficient to reduce cell surface expression, which was accompanied by intracellular retention as observed in laser scanning microscopy analysis. Conversely, the exchange of aspartate 100 by lysine in the mV2R increased the cell surface expression and resulted in predominant plasma membrane localization. Thus, a single amino acid

difference in the first extracellular loop between mV2R and hV2R determines the efficiency of cell surface expression.

Ousova, O., V. Guyonnet-Duperat, et al. (2004). "Corticosteroid Binding Globulin: A New Target for Cortisol-Driven Obesity." Mol. Endocrinol. **18**(7): 1687-1696.

<http://mend.endojournals.org/cgi/content/abstract/18/7/1687>

We present data suggesting that corticosteroid-binding globulin (CBG) may be the causal gene of a previously identified quantitative trait locus (QTL) associated with cortisol levels, fat, and muscle content in a pig intercross. Because Cbg in human and mouse maps in the region orthologous to the pig region containing this QTL, we considered Cbg as an interesting positional candidate gene because CBG plays a major role in cortisol bioavailability. Firstly, we cloned pig Cbg from a bacterial artificial chromosome library and showed by fluorescent in situ hybridization and radiation hybrid mapping that it maps on 7q26 at the peak of the QTL interval. Secondly, we detected in a subset of the pig intercross progeny a highly significant genetic linkage between CBG plasma binding capacity values and the chromosome 7 markers flanking the cortisol-associated QTL. In this population, CBG capacity is correlated positively to fat and negatively to muscle content. Thirdly, CBG capacity was three times higher in Meishan compared with Large White parental breeds and a 7-fold difference was found in Cbg mRNA expression between the two breeds. Overall, the data accumulated in this study point to Cbg gene as a key regulator of cortisol levels and obesity susceptibility.

Pasquali, C., M.-L. Curchod, et al. (2003). "Identification of Protein Tyrosine Phosphatases with Specificity for the Ligand-Activated Growth Hormone Receptor." Mol. Endocrinol. **17**(11): 2228-2239.

<http://mend.endojournals.org/cgi/content/abstract/17/11/2228>

Protein tyrosine phosphatases (PTPs) play key roles in switching off tyrosine phosphorylation cascades, such as initiated by cytokine receptors. We have used substrate-trapping mutants of a large set of PTPs to identify members of the PTP family that have substrate specificity for the phosphorylated human GH receptor (GHR) intracellular domain. Among 31 PTPs tested, T cell (TC)-PTP, PTP- β , PTP1B, stomach cancer-associated PTP 1 (SAP-1), Pyst-2, Meg-2, and PTP-H1 showed specificity for phosphorylated GHR that had been produced by coexpression with a kinase in bacteria. We then used GH-induced, phosphorylated GH receptor, purified from overexpressing mammalian cells, in a Far Western-based approach to test whether these seven PTPs were also capable of recognizing ligand-induced, physiologically phosphorylated GHR. In this assay, only TC-PTP, PTP1B, PTP-H1, and SAP-1 interacted with the mature form of the phosphorylated GHR. In parallel, we show that these PTPs recognize very different subsets of the seven GHR tyrosines that are potentially phosphorylated. Finally, mRNA tissue distribution of these PTPs by RT-PCR analysis and coexpression of the wild-type PTPs to test their ability to dephosphorylate ligand-activated GHR suggest PTP-H1 and PTP1B as potential candidates involved in GHR signaling.

Pi, M., R. H. Oakley, et al. (2005). " β -Arrestin- and G Protein Receptor Kinase-Mediated Calcium-Sensing Receptor Desensitization." Mol. Endocrinol. **19**(4): 1078-1087.

<http://mend.endojournals.org/cgi/content/abstract/19/4/1078>

Extracellular calcium rapidly controls PTH secretion through binding to the G protein-coupled calcium-sensing receptor (CASR) expressed in parathyroid glands. Very little is known about the regulatory proteins involved in desensitization of CASR. G protein receptor kinases (GRK) and β -arrestins are important regulators of agonist-dependent desensitization of G protein-coupled receptors. In the present study, we investigated their role in mediating agonist-dependent desensitization of CASR. In heterologous cell culture models, we found that the transfection of GRK4 inhibits CASR signaling by enhancing receptor phosphorylation and β -arrestin translocation to the CASR. In contrast, we found that overexpression of GRK2 desensitizes CASR by classical mechanisms as well as through phosphorylation-independent mechanisms involving disruption of G α q signaling. In addition, we observed lower circulating PTH levels and an attenuated increase in serum PTH after hypocalcemic stimulation in β -arrestin2 null mice, suggesting a functional role of β -arrestin2-dependent desensitization pathways in regulating CASR function in vivo. We conclude that GRKs and β -arrestins play key roles in regulating CASR responsiveness in parathyroid glands.

Piekorz, R. P., S. Gingras, et al. (2005). "Regulation of Progesterone Levels during Pregnancy and Parturition by Signal Transducer and Activator of Transcription 5 and 20 α -Hydroxysteroid Dehydrogenase." *Mol. Endocrinol.* **19**(2): 431-440.

<http://mend.endojournals.org/cgi/content/abstract/19/2/431>

The two highly related signal transducers and activators of transcription (Stats), Stat5a and Stat5b, are major mediators of prolactin signaling in both the mammary gland and in the ovary. Deficiencies in Stat5b, or in both Stat5a and Stat5b, result in loss of pregnancy during midgestation and are correlated with an increase in ovarian 20 α -hydroxysteroid dehydrogenase (20 α -HSD) and a decrease in serum progesterone, which normally declines only immediately before parturition. To determine the relative contribution of 20 α -HSD to progesterone metabolism and Stat5 function during pregnancy and parturition, we created a 20 α -HSD-deficient strain of mice by gene disruption. Mice deficient for 20 α -HSD sustain high progesterone levels and display a delay in parturition of several days demonstrating that 20 α -HSD regulates parturition downstream of the prostaglandin F2 α receptor in an essential and nonredundant manner. Moreover, 20 α -HSD deficiency partially corrected the abortion of pregnancies associated with Stat5b deficiency, supporting the concept that prolactin activation of Stat5b is important in suppressing 20 α -HSD gene expression and thereby allowing the maintenance of progesterone levels that are required to sustain pregnancy.

Qin, J., D.-m. Gao, et al. (2004). "Prospero-Related Homeobox (Prox1) Is a Corepressor of Human Liver Receptor Homolog-1 and Suppresses the Transcription of the Cholesterol 7- α -Hydroxylase Gene." *Mol. Endocrinol.* **18**(10): 2424-2439.

<http://mend.endojournals.org/cgi/content/abstract/18/10/2424>

Cholesterol 7- α -hydroxylase (CYP7A1) catalyzes a rate-limiting step in bile acid synthesis in liver, and its gene transcription is under complex regulation by multiple nuclear receptors in response to bile acids, cholesterol derivatives, and hormones. The liver receptor homolog-1 (LRH-1), a member of the fushi tarazu factor 1 subfamily of nuclear receptors, has emerged as an essential regulator for the expression of cyp7a1. In this report, we demonstrate Prox1, a prospero-related homeobox transcription factor, identified through a yeast two-hybrid screening, can directly interact with human LRH-1 (hLRH-1) and suppresses hLRH-1-mediated transcriptional activation of human cyp7a1 gene. Biochemical analysis demonstrates that Prox1 interacts with both the ligand binding domain (LBD) and the DNA binding domain (DBD) of hLRH-1. An LRKLL motif in Prox1 is important for the interaction with the LBD but not the DBD of hLRH-1.

1. In hLRH-1 LBD, helices 2 and 10 are essential for Prox1 recruitment. The suppression by Prox1 on the transcriptional activity of hLRH-1 can be mediated through its interaction with the LBD or the DBD of hLRH-1. Gel shift assays reveal that Prox1 impairs the binding of hLRH-1 to the promoter of human cyp7a1 gene.

Sales, K. J., S. Maudsley, et al. (2004). "Elevated Prostaglandin EP2 Receptor in Endometrial Adenocarcinoma Cells Promotes Vascular Endothelial Growth Factor Expression via Cyclic 3',5'-Adenosine Monophosphate-Mediated Transactivation of the Epidermal Growth Factor Receptor and Extracellular Signal-Regulated Kinase 1/2 Signaling Pathways." *Mol. Endocrinol.* **18**(6): 1533-1545.

<http://mend.endojournals.org/cgi/content/abstract/18/6/1533>

Prostaglandin (PG) E2 E-series prostanoid-2 (EP2) receptor is elevated in numerous carcinomas including the endometrium and has been implicated in mediating the effects of PGE2 on vascular function. In this study, we investigated the intracellular signaling pathways that are activated by the EP2 receptor and their role in regulation of the expression of vascular endothelial growth factor in endometrial adenocarcinoma (Ishikawa) cells. Ishikawa cells were stably transfected with EP2 receptor cDNA in the sense or antisense directions. Treatment of Ishikawa cells with PGE2 rapidly induced transactivation of the epidermal growth factor receptor (EGFR) and activation of ERK1/2 via the EP2 receptor. Preincubation of cells with chemical inhibitors of protein kinase A, c-Src, and EGFR kinase abolished the EP2-induced activation of EGFR and ERK1/2. PGE2 signaling via the EP2 receptor also promoted the mRNA expression and secretion of vascular endothelial growth factor protein in Ishikawa cells. This effect was inhibited by preincubation with chemical inhibitors of EGFR kinase, ERK1/2 signaling, and small inhibitory RNA molecules targeted against the EGFR. Therefore, we have demonstrated that elevated EP2 receptor expression may facilitate the PGE2-induced release of proangiogenic factors in reproductive tumor cells via intracellular cAMP-mediated transactivation of the EGFR and ERK1/2 pathways.

Schmitt, J. F., D. S. Millar, et al. (2002). "Hypermethylation of the Inhibin {alpha}-Subunit Gene in Prostate Carcinoma." *Mol. Endocrinol.* **16**(2): 213-220.

<http://mend.endojournals.org/cgi/content/abstract/16/2/213>

Inhibin is composed of an {alpha}- and a {beta}-subunit. Transgenic studies assigned a tumor-suppressive role to the inhibin {alpha}-subunit, and in human prostate cancer inhibin {alpha}-subunit gene expression was down-regulated. This study examined the inhibin {alpha}-subunit gene promoter and gene locus to determine whether promoter hypermethylation or LOH occurred in DNA from prostate cancer. The 5'-untranslated region of the human inhibin {alpha}-subunit gene was sequenced and shown to be highly homologous to the bovine, rat, and mouse inhibin {alpha}-subunit promoter sequences. A 135-bp region of the human promoter sequence that contained a cluster of CpG sites was analyzed for hypermethylation. Significant ($P < 0.001$) hypermethylation of the inhibin {alpha}-subunit gene promoter occurred in DNA from Gleason pattern 3, 4, and 5 carcinomas compared with nonmalignant tissue samples. A subset of the carcinomas with a cribriform pattern were unmethylated. LOH at 2q32-36, the chromosomal region harboring the inhibin {alpha}-subunit gene, was observed in 42% of prostate carcinomas. These data provide the first demonstration that promoter hypermethylation and LOH are associated with the inhibin {alpha}-subunit gene and gene locus in prostate cancer.

Sciacchitano, S., A. Orecchio, et al. (2002). "Cloning of the Mouse Insulin Receptor Substrate-3 (mIRS-3) Promoter, and Its Regulation by p53." Mol. Endocrinol. **16**(7): 1577-1589.

<http://mend.endojournals.org/cgi/content/abstract/16/7/1577>

The insulin receptor substrate-3 (IRS-3) is a member of a family of intermediate adapter proteins that function as major intracellular targets for phosphorylation by the activated insulin and IGF-I receptors. Among the four IRS proteins identified so far, IRS-3 exhibits a rather peculiar expression pattern during both the embryonic development and adult life, suggesting a different mechanism of regulation of its expression. In this study, we cloned the 5' flanking region of the mIRS-3 gene and analyzed its promoter activity. The mIRS-3 promoter is inhibited by wild-type p53, and this effect is completely abolished by cotransfection of a dominant negative p53. Tumor-derived p53 mutants show variable, but lower suppressing capability than wt p53. In addition, treatment with doxorubicin inhibits endogenous expression of mIRS-3 mRNA in C2C12 and 3T3-L1 cells. The DNA region spanning from nucleotides -287 and -178 in the mIRS-3 promoter is responsible for a 32.2% reduction of the mouse double minute 2 (MDM2) promoter activity, suggesting its involvement in the p53-mediated inhibitory effect. In conclusion, our study demonstrates that the mIRS-3 promoter is regulated by p53 at the transcriptional level. The inhibition of mIRS-3 promoter by wild-type p53, and its de-repression by tumor-derived p53 mutants, appears to be similar to that previously reported for the IGF-I receptor promoter, suggesting a common role of these two genes in p53-mediated cell growth and differentiation.

Shiota, M., T. Tanihiro, et al. (2003). "Protein Tyrosine Phosphatase PTP20 Induces Actin Cytoskeleton Reorganization by Dephosphorylating p190 RhoGAP in Rat Ovarian Granulosa Cells Stimulated with Follicle-Stimulating Hormone." Mol. Endocrinol. **17**(4): 534-549.

<http://mend.endojournals.org/cgi/content/abstract/17/4/534>

We identified 25 protein tyrosine phosphatases (PTPs) expressed in rat ovarian granulosa cells. Of these PTPs, the expression levels of at least PTP20, PTP-MEG1, PTP{epsilon}M, and PTP{epsilon}C significantly changed during the estrous cycle. We examined the cellular functions of PTP20 in granulosa cells by expressing the wild type, a catalytically inactive CS mutant in which Cys229 of PTP20 was changed to Ser, or a substrate-trapping DA mutant in which Asp197 was mutated to Ala, using an adenovirus vector. Overexpression of the wild type, but not of the CS mutant, induced retraction of the cell body with the extension of long, dendritic-like processes after stimulation with FSH, a critical factor for the survival and differentiation of these cells. In addition, cell adhesion to the substratum decreased in an FSH-dependent manner. Inhibiting Rho GTPase activity with C3 botulinum toxin caused similar morphological changes. The FSH-enhanced phosphotyrosine (p-Tyr) level of p190 RhoGAP was selectively reduced by the overexpressed wild type, but not by mutated PTP20. Although p190 RhoGAP is tyrosine phosphorylated by c-Src via the tyrosine kinase Pyk2, wild-type PTP20 had little effect on p-Tyr418 of c-Src and no effect on p-Tyr402 of Pyk2, which are required for full c-Src activity and for interacting between Pyk2 and c-Src, respectively. The CS and DA mutants as well as the wild type reduced the formation of p190 RhoGAP-p120 RasGAP complexes. Confocal microscopy analysis revealed that PTP20 intracellularly colocalizes with p190 RhoGAP. These results demonstrate that PTP20 regulates the functions of granulosa cells in an FSH-dependent manner by dephosphorylating p190 RhoGAP and subsequently inducing reorganization of the actin cytoskeleton. Moreover, our data suggest that PTPs play significant roles in controlling the dynamics of ovarian functions.

U, H. S., W. Alilain, et al. (2002). "Fetal Brain Progenitor Cells Transdifferentiate to Fates Outside the Nervous System." Mol. Endocrinol. **16**(11): 2645-2656.

<http://mend.endojournals.org/cgi/content/abstract/16/11/2645>

Central nervous system stem cells give rise to neurons and glia when exposed to specific trophic factors. In our studies with rat fetal brain-derived stem cells (RSCs), we showed that they could be induced to express the developmentally regulated transcription factors and cell markers characteristic of cells derived from another germ layer, e.g. pituitary cells. Therefore, rat fetal brain-derived stem cells do not seem to be restricted to a defined developmental fate. They may retain pluripotentiality and can be redirected to develop into other cell types not found in the brain provided the correct set of stimuli is present. This multipotent developmental behavior also suggests that instructive signals are operative.

Mol. Hum. Reprod. (23)

Akbar, F., S. Heinonen, et al. (2005). "Haplotypic association of DDAH1 with susceptibility to pre-eclampsia." Mol. Hum. Reprod. **11**(1): 73-77.

<http://molehr.oupjournals.org/cgi/content/abstract/11/1/73>

Association between pre-eclampsia (PEE1) and the dimethylarginine dimethylaminohydrolase (DDAH) 1 and 2 genes, which play a role in the regulation of nitric oxide synthesis and release, was studied. In a case-control study design single nucleotide polymorphisms (SNPs) were determined at eight sites in the DDAH1 gene and at one site (Pro231Pro) in the DDAH2 gene from 132 women with pre-eclampsia and 112 healthy controls. Three SNPs in the DDAH1 gene were associated with pre-eclampsia, showing complete linkage disequilibrium with each other, but none of the associations in the allele or genotype data reached statistical significance in either of the genes after the correction for multiple testing. Haplotype frequencies were estimated using a population based on a maximum likelihood method (EM algorithm). Four common DDAH1 haplotypes were present and a significant association of haplotypes H2 and H3 with pre-eclampsia ($P=0.03$) was found. The risk of pre-eclampsia was greatest in individuals (odds ratio: 3.93; 95% confidence interval: 1.54-9.99) who had two copies of the high-risk haplotypes (H2 or H3). The observed haplotypic association provides the first evidence of the importance of DDAH1 polymorphisms in pre-eclampsia susceptibility.

Cauffman, G., H. Van de Velde, et al. (2005). "Oct-4 mRNA and protein expression during human preimplantation development." Mol. Hum. Reprod. **11**(3): 173-181.

<http://molehr.oupjournals.org/cgi/content/abstract/11/3/173>

The transcription factor OCT-4 is regarded as a critical factor in controlling mammalian early embryonic development because of its role in toti-/pluripotency. In human preimplantation embryos, OCT-4 studies are limited to RNA analysis of abnormally developing embryos. This study thoroughly investigated the expression pattern of OCT-4 throughout the human preimplantation development. Expression was examined by single-cell RT-PCR or indirect immunocytochemistry in 36 single oocytes of various maturity and 112 normally developing preimplantation embryos at the level of single blastomeres, morulas, blastocysts, or inner cell mass (ICM) and trophectoderm (TE) samples. Oocytes and cleavage stage embryos revealed a variable OCT-4 expression pattern, concomitant with a pure cytoplasmic localization of the

protein. During compaction, the variability in expression faded away indicating embryonic OCT-4 expression and the protein appeared in the nucleus implying biological activity. In blastocysts, OCT-4 transcripts and proteins were present in the ICM and the TE. At protein level, blastocysts displayed different spatial expression patterns within a cell for the splice variants of OCT-4, which may endow them with different functional properties. As OCT-4 transcripts were also found in various differentiated cells, the presence of OCT-4 transcripts or proteins may not be sufficient for identifying undifferentiated cell lines in humans. Further, we suggest to examine the localization of OCT-4 proteins within a cell rather than to look for the presence and/or amount of transcripts.

Dalrymple, A., D. M. Slater, et al. (2002). "Molecular identification and localization of Trp homologues, putative calcium channels, in pregnant human uterus." Mol. Hum. Reprod. **8**(10): 946-951.

<http://molehr.oupjournals.org/cgi/content/abstract/8/10/946>

The mechanisms underlying the switch from uterine quiescence to contractile activity in labour are not clearly understood. Increasing evidence suggests that pathways of myometrial calcium homeostasis, including store-operated calcium entry (SOCE), may play an important role. The molecular basis of the membrane-associated calcium channels contributing to SOCE in pregnant human myometrium is not known, but they are likely to be hetero- or homo-oligomeric assemblies of transient receptor potential channel (TrpC) proteins, encoded by the mammalian homologues of *Drosophila* Trp genes. This study has therefore determined Trp gene expression and also TrpC protein expression and localization in term pregnant human myometrial tissue and primary cultured human myometrial smooth muscle (HMSM) cells. RT-PCR amplified fragments of Trp1, Trp3, Trp4, Trp6 and Trp7. PCR products were 100% homologous to published human sequences. Western blot analysis detected TrpC1, TrpC3, TrpC4 and TrpC6 proteins, which were of expected size. Immunolocalization revealed TrpC1, TrpC3, TrpC4 and TrpC6 protein expression in myometrial tissue and HMSM cells. TrpC protein immunostaining in HMSM cells was distributed in a distinct reticular fashion. TrpC proteins may be candidate proteins forming SOCE channels in term pregnant human myometrium.

De Vos, A., K. Sermon, et al. (2003). "Preimplantation genetic diagnosis for Charcot-Marie-Tooth disease type 1A." Mol. Hum. Reprod. **9**(7): 429-435.

<http://molehr.oupjournals.org/cgi/content/abstract/9/7/429>

Charcot-Marie-Tooth (CMT) disease is the common name for a range of hereditary peripheral neuropathies. CMT1 is the most common form and is transmitted in an autosomal dominant manner. CMT1A maps to chromosome 17p11.2 and is caused, in the majority of cases, by a 1.5 Mb DNA duplication, that includes the peripheral myelin protein 22 (PMP) gene. This paper reports on preimplantation genetic diagnosis (PGD) for CMT1A in five couples. The CMT1A duplication was detected by fluorescent PCR analysis using polymorphic (CA)_n markers localized within the duplication. Single-cell PCR on blastomeres allowed genetic analysis of embryos obtained after ICSI. Only healthy unaffected embryos were transferred to the uterus. PCR experiments with single EBV-transformed lymphoblasts or with research blastomeres allowed the evaluation of amplification efficiencies, as well as contamination and allele drop-out (ADO) rates for each PCR protocol. Three simplex PCR protocols (using one primer pair) and two duplex PCR protocols (using two primer pairs) were developed for CMT1A. Additionally, a protocol using all three primer pairs in triplex was also established. Thirteen clinical ICSI-PGD cycles were performed for five couples (12 simplex PCR cycles and one duplex PCR cycle), resulting in seven embryo transfers. Three singleton pregnancies ensued in two couples and three healthy babies were delivered. This report describes different fluorescent PCR-based tests which allow efficient and accurate single-cell level detection of the CMT1A duplication. On the basis of the presence of

the healthy allele of the affected parent-to-be (and/or absence of the affected one), healthy embryos can be selected for transfer. The assays are suitable for PGD for other couples who present with the same CMT1A duplication [depending on their informativity for the (CA)_n markers available] as described here.

Driver, P. M., S. Rauz, et al. (2003). "Characterization of human trophoblast as a mineralocorticoid target tissue." Mol. Hum. Reprod. **9**(12): 793-798.

<http://molehr.oupjournals.org/cgi/content/abstract/9/12/793>

In mineralocorticoid target tissues, 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) confers mineralocorticoid receptor selectivity by metabolizing hormonally active cortisol to inactive cortisone, allowing aldosterone access to the receptor. This enzyme is also expressed in high abundance in fetal tissues, particularly in placental trophoblast, where a role has been proposed in regulating fetal growth and development by protecting the fetus from maternal hypercortisolaemia and modulating local glucocorticoid receptor (GR), rather than mineralocorticoid receptor-mediated responses. As such the placenta has not been considered a mineralocorticoid target tissue. We have used conventional RT-PCR and real-time quantitative RT-PCR to demonstrate that primary cultures of term human cytotrophoblast express the mineralocorticoid-responsive genes Na/K-ATPase (α 1 and β 1 subunits), epithelial sodium channel (ENaC, α and γ subunits) and the serum and glucocorticoid-inducible kinase (SGK). SGK expression was found to be rapidly and strongly induced by corticosteroids (24- and 38-fold by 10⁻⁷ mol/l aldosterone and 10⁻⁷ mol/l dexamethasone respectively after 1 h). Dexamethasone-, but not aldosterone-stimulated SGK induction was inhibited by GR antagonist (RU38486), confirming the presence of a functional mineralocorticoid receptor and suggesting that placental trophoblast expresses a functional mineralocorticoid receptor, which is in part responsible for the corticosteroid regulation of SGK expression. Placental 11 β -HSD2 may protect the MR in a fashion analogous to classical mineralocorticoid tissues to modulate trophoblast sodium transport.

Fiorentino, F., A. Biricik, et al. (2004). "Development and clinical application of a strategy for preimplantation genetic diagnosis of single gene disorders combined with HLA matching." Mol. Hum. Reprod. **10**(6): 445-460.

<http://molehr.oupjournals.org/cgi/content/abstract/10/6/445>

Preimplantation HLA matching has recently emerged as a tool for couples desiring to conceive a potential donor progeny for transplantation in a sibling with a life-threatening disorder. In this paper we describe a strategy optimized for preimplantation genetic diagnosis (PGD) of haemoglobinopathies combined with HLA matching. This procedure involves a minisequencing-based genotyping of HLA regions A, B, C and DRB combined with mutation analysis of the gene regions involved by mutation. Analysis of at least eight polymorphic short tandem repeat (STR) markers scattered through the HLA complex has also been included to detect potential contamination and crossing-over occurrences between HLA genes. The above assay can also be used for preimplantation HLA matching as a primary indication. The strategy was clinically applied for HLA matching in 17 cycles (14 for β -thalassaemia, one for Wiscott-Aldrich syndrome and two for leukaemia). A reliable HLA genotype was achieved in 255/266 (95.9%) of the blastomeres. In total, 22 (14.8%) embryos were obtained that were HLA-matched with the affected siblings, 14 (9.4%) of which were unaffected and transferred back to the patients. Four clinical pregnancies were obtained, three of which (one twin, two singletons) are ongoing and were confirmed as healthy and HLA-identical with the affected children. Minisequencing-based HLA typing combined with HLA STR haplotyping has been shown to be a reliable strategy for

preimplantation HLA matching. The major advantage of this approach is that the validation of a single assay can be done once and then used for the majority of the patients, reducing notably time needed for preclinical set-up of each case.

Fiorentino, F., M. C. Magli, et al. (2003). "The minisequencing method: an alternative strategy for preimplantation genetic diagnosis of single gene disorders." Mol. Hum. Reprod. **9**(7): 399-410.

<http://molehr.oupjournals.org/cgi/content/abstract/9/7/399>

We have applied a new method of genetic analysis, called minisequencing', to preimplantation genetic diagnosis (PGD) of monogenic disorders from single cells. This method involves computer-assisted mutation analysis, which allows exact base identity determination and computer-assisted visualization of the specific mutation(s), and thus facilitates data interpretation and management. Sequencing of the entire PCR product is unnecessary, yet the same qualitative characteristics of sequence analysis are maintained. The main benefit of the minisequencing strategy is the use of a mutation analysis protocol based on a common procedure, irrespective of the mutations involved. To evaluate the reliability of this method for subsequent application to PGD, we analysed PCR products from 887 blastomeres including 55 PGD cases of different genetic diseases, such as cystic fibrosis, {beta}-thalassaemia, sickle cell anaemia, haemophilia A, retinoblastoma, and spinal muscular atrophy. Minisequencing was found to be a useful technique in PGD analysis, due to its elevated sensitivity, automation, and easy data interpretation. The method was also efficient, providing interpretable results in 96.5% (856/887) of the blastomeres tested. Fifteen clinical pregnancies resulted from these PGD cases; conventional prenatal diagnosis confirmed all the PGD results, and 10 healthy babies have already been born. Its applicability to PGD could be helpful, particularly in cases in which the mutation(s) involved are difficult to assess by restriction analysis or other commonly used methods.

Horcajadas, J. A., A. Riesewijk, et al. (2005). "Effect of controlled ovarian hyperstimulation in IVF on endometrial gene expression profiles." Mol. Hum. Reprod. **11**(3): 195-205.

<http://molehr.oupjournals.org/cgi/content/abstract/11/3/195>

Controlled ovarian hyperstimulation (COH) used in IVF produces lower implantation rates per embryo transferred compared to natural cycles utilized in ovum donation, suggesting a suboptimal endometrial development. Endometrial receptivity has recently been investigated in natural menstrual cycles with the aid of microarray technology. The aim of this study is to investigate the impact of COH using urinary gonadotrophins with a long protocol with GnRH agonists without progesterone supplementation (similar to the natural cycle) on endometrial gene expression profiles during the window of implantation by comparing the profiles at day hCG+7 of COH versus LH+7 of a previous natural cycle in the same women. For this purpose we have used microarray technology by Affymetrix (GeneChip HG_U133A), which allows more than 22 000 genes to be tested simultaneously. Results were validated by semi-quantitative PCR and quantitative PCR experiments. We found that more than 200 genes showed a differential expression of more than 3-fold when COH and normal cycles were compared at hCG+7 versus LH+7. We simultaneously re-analysed the LH+2 versus LH+7 endometrial gene expression profiles in previous natural cycles in the same subject using this specific GeneChip, the results obtained were consistent with our own published results. This is the first time that gene expression profiles of the endometrium during COH are reported. The large degree of gene expression disturbance is surprising and highlights the need for further efforts to optimize COH protocols.

Jaleel, M. A., A. C. Tsai, et al. (2004). "Stromal cell-derived factor-1 (SDF-1) signalling regulates human placental trophoblast cell survival." *Mol. Hum. Reprod.* **10**(12): 901-909.

<http://molehr.oupjournals.org/cgi/content/abstract/10/12/901>

Stromal cell-derived factor-1 (SDF-1 or CXCL12) is the physiologic ligand for the chemokine receptor CXCR4. CXCR4-mediated signalling regulates cell migration and apoptosis in certain haematopoietic and neuronal cells. Using gene profiling, we determined that CXCR4 is the only chemokine receptor for which mRNA expression is regulated during trophoblast differentiation in vitro. Based on the known effects of CXCR4 ligation, we hypothesized that CXCR4 activation may regulate placental trophoblast cell survival (i.e. protection from apoptosis), an important mechanism for the establishment and maintenance of the uteroplacental barrier. Human cytotrophoblasts (CTBs) were cultured in defined media and treated with graded doses of SDF-1 (10-100 ng/ml) or with an anti-CXCR4 neutralizing antibody. Exposure to anti-CXCR4 antibody reduced CTB cell numbers by 25-40%. Treatment with SDF-1 decreased the proportions of apoptotic terminal deoxynucleotidyl transferase-mediated dUTP-FITC nick-end labelling(+) cells (apoptotic index [AI] of 2.79{+/-}0.61% [control] versus 1.88{+/-}0.56% [SDF-1]; $P<0.05$) and caspase-activated cells (AI of 7.95{+/-}2.49% [control] versus 3.81{+/-}1.49% [SDF-1]; $P<0.05$). We determined that SDF-1 also activated the triple MAP Kinase isoforms ERK1/2 and p38 in trophoblasts. Immunocytochemistry confirmed SDF-1-induced nuclear translocation of phosphorylated ERK1/2. Blocking of ERK1/2 signalling with the specific inhibitor PD98059 reversed SDF-1-mediated inhibition of apoptosis (AI of 1.65{+/-}0.34 [SDF-1] versus 3.50{+/-}0.5 [SDF-1 + PD98059]; $P<0.05$), suggesting that SDF-1 acts through this pathway as a trophoblast survival factor. These results indicate that SDF-1/CXCR4 signalling stimulates anti-apoptotic pathways in cultured trophoblasts. This chemotactic ligand/receptor system may promote trophoblast survival during pregnancy. Alterations in SDF-1 and/or CXCR4 expression or function may be associated with specific pregnancy disorders.

Kurahashi, N., F. Sata, et al. (2005). "Maternal genetic polymorphisms in CYP1A1, GSTM1 and GSTT1 and the risk of hypospadias." *Mol. Hum. Reprod.* **11**(2): 93-98.

<http://molehr.oupjournals.org/cgi/content/abstract/11/2/93>

Hypospadias is one of the most common congenital anomalies. Increased exposure to environmental factors (endocrine-disrupting chemicals and smoking) or maternal endogenous estrogen may cause hypospadias because male sexual differentiation is dependent on normal androgen homeostasis. Moreover, interactions between genetic factors and cigarette smoking and other chemicals have been suggested. It has been demonstrated that the CYP1A1 metabolizes not only environmental chemicals but also estrogens, and glutathione-S-transferases (GSTs) are detoxification enzymes that protect cells from toxicants by conjugation with glutathione. In this study, to investigate the association of CYP1A1 (MspI), GSTM1 and GSTT1 polymorphisms with hypospadias, a case-control study of 31 case mothers who had boys with hypospadias and 64 control mothers was performed in Japan. These polymorphisms were investigated by PCR-based methods using DNA from peripheral lymphocytes. We found that the heterozygous CYP1A1 and heterozygous and homozygous CYP1A1 were less frequent in the case mothers than in the control mothers [adjusted odds ratio (OR)=0.17, 95% confidence interval (CI)=0.04-0.74, OR = 0.28, 95% CI = 0.08-0.97, respectively]. We found no effect of maternal smoking on the hypospadias risks among the gene polymorphisms. The results suggest that mothers with the CYP1A1 MspI variant allele may have a decreased risk for hypospadias.

Loudon, J. A. Z., S. R. Sooranna, et al. (2004). "Mechanical stretch of human uterine smooth muscle cells increases IL-8 mRNA expression and peptide synthesis." Mol. Hum. Reprod. **10**(12): 895-899.

<http://molehr.oupjournals.org/cgi/content/abstract/10/12/895>

Labour is associated with increased synthesis of interleukin-8 (IL-8) by the fetal membranes and myometrium, which leads to an inflammatory infiltrate. Stretch has been shown to increase the expression of contraction-associated proteins in animal models of labour and in human myocytes in vitro. In this study, we tested the hypothesis that mechanical stretch of human myometrial cells increases IL-8 messenger ribonucleic acid (mRNA) expression. We isolated myocytes from non-pregnant women undergoing hysterectomy and pregnant women undergoing Caesarean section before and after the onset of labour. Myocytes in culture were subjected to stretch of varying intensity (6-16%) and duration (1 or 6 h) using the Flexercell system. IL-8 mRNA expression was lowest in myocytes from pregnant women not in labour, intermediate in those from non-pregnant women and greatest in those from pregnant women in labour. Stretch increased IL-8 mRNA expression independent of reproductive state. The stretch-induced increase in IL-8 mRNA expression was associated with higher IL-8 levels in the culture supernatant and enhanced promoter activity. These data suggest that stretch contributes to the increase in myometrial IL-8 synthesis associated with the onset of labour in humans.

Lundwall, A., A. Giwercman, et al. (2003). "A frequent allele codes for a truncated variant of semenogelin I, the major protein component of human semen coagulum." Mol. Hum. Reprod. **9**(6): 345-350.

<http://molehr.oupjournals.org/cgi/content/abstract/9/6/345>

Human semen coagulum predominantly consists of high molecular mass complexes of the seminal vesicle secreted semenogelin I (SgI) and semenogelin II (SgII). Here we describe a previously unknown variant of the SgI gene that is present at an allele frequency of [~]3% in the Swedish population. It gives rise to a protein with a molecular mass of 43 kDa, SgI43, which compared with the 50 kDa variant, SgI50, is lacking a tandem repeat of 60 amino acid residues that was probably deleted by homologous recombination. In spite of the size difference, SgI43 has many properties in common with SgI50, such as a very high iso-electric point and susceptibility to proteolytic degradation by prostate-specific antigen. Heterozygous carriers of the SgI43 allele neither show impaired fertility nor do they significantly differ from individuals homozygous for SgI50 with respect to sperm parameters such as semen volume, sperm count and fraction of motile spermatozoa.

Makela, S., R. Eklund, et al. (2005). "Mutational analysis of the human SLC26A8 gene: exclusion as a candidate for male infertility due to primary spermatogenic failure." Mol. Hum. Reprod. **11**(2): 129-132.

<http://molehr.oupjournals.org/cgi/content/abstract/11/2/129>

SLC26A8 is an anion transporter that is solely expressed in the testes. It interacts with MgcRacGAP that shows strong structural similarity with the Drosophila protein RotundRacGAP, which is established to have an essential role for male fertility in the fruit fly. To explore whether the SLC26A8 gene has a role in human male infertility, we performed mutational analysis in the coding region of the SLC26A8 gene in 83 male infertility patients and two groups of controls using single-strand conformational polymorphism and direct sequencing methods. We found six novel coding sequence variations, of which five lead to amino acid substitutions. All variants were found with similar frequencies in both patients and controls, thus suggesting that none of them may be

causally associated with infertility. We conclude that the SLC26A8 mutations are not a common cause of male infertility.

Piyamongkol, W., M. G. Bermudez, et al. (2003). "Detailed investigation of factors influencing amplification efficiency and allele drop-out in single cell PCR: implications for preimplantation genetic diagnosis." Mol. Hum. Reprod. **9**(7): 411-420.

<http://molehr.oupjournals.org/cgi/content/abstract/9/7/411>

Preimplantation genetic diagnosis (PGD) of single gene disorders relies on PCR-based tests performed on single cells (polar bodies or blastomeres). Despite the use of increasingly robust protocols, allele drop-out (ADO; the failure to amplify one of the two alleles in a heterozygous cell) remains a significant problem for diagnosis using single cell PCR. In extreme cases ADO can affect >40% of amplifications and has already caused several PGD misdiagnoses. We suggest that an improved understanding of the origins of ADO will allow development of more reliable PCR assays. In this study we carefully varied reaction conditions in >3000 single cell amplifications, allowing factors influencing ADO rates to be identified. ADO was found to be affected by amplicon size, amount of DNA degradation, freezing and thawing, the PCR programme, and the number of cells simultaneously amplified. Factors found to have little or no effect on ADO were local DNA sequence, denaturing temperature (94 or 96{degrees}C) and cell type. Consideration of the causal factors identified during this study should permit the design of PGD protocols that experience little ADO, thus improving the accuracy of PGD for single gene disorders.

Ray, P. F., N. Frydman, et al. (2002). "Birth of healthy female twins after preimplantation genetic diagnosis of cystic fibrosis combined with gender determination." Mol. Hum. Reprod. **8**(7): 688-694.

<http://molehr.oupjournals.org/cgi/content/abstract/8/7/688>

Two healthy sisters with a familial history of mental retardation were referred to our centre for preimplantation genetic diagnosis (PGD). Their two brothers showed severe mental retardation. The molecular basis for their disorder could not be identified, but one of the sisters and the mother presented a highly skewed pattern of X-inactivation reinforcing the likelihood of an X-linked mode of inheritance. Both sisters requested PGD to avoid the abortion of potentially affected male fetuses. PGD for sex by fluorescent in-situ hybridization was carried out for the first sister and resulted in the birth of a female child. The second sister and her partner, whose niece had cystic fibrosis (CF), were tested for CF mutations, and were both found to be $\Delta F508$ heterozygous. We developed an efficient single cell PCR protocol for the simultaneous amplification of the CF ($\Delta F508$) locus as well as the X-linked amelogenin gene and its highly homologous pseudogene on the Y chromosome. Two PGD cycles were carried out to screen against male and $\Delta F508$ homozygous deleted embryos. In each case several embryos could be selected for transfer and the second cycle resulted in a twin pregnancy followed by the birth of two healthy female infants.

Sales, K. J., A. A. Katz, et al. (2002). "Seminal plasma activates cyclooxygenase-2 and prostaglandin E2 receptor expression and signalling in cervical adenocarcinoma cells." Mol. Hum. Reprod. **8**(12): 1065-1070.

<http://molehr.oupjournals.org/cgi/content/abstract/8/12/1065>

Enhanced cyclooxygenase (COX) expression and prostaglandin E2 (PGE2) synthesis are regarded as promoters of neoplastic cell proliferation and angiogenesis. Expression of COX-2 and synthesis of PGE2 are up-regulated in cervical carcinomas. In sexually active women, growth and invasiveness of neoplastic cervical epithelial cells may be also under the direct influence of PGE2 present in seminal plasma. The aims of this study were to investigate the effect of seminal plasma and PGE2 on the expression of COX-2 and expression and signalling of the PGE2 receptor subtypes (EP1-EP4) in HeLa (cervical adenocarcinoma) cells. Treatment of HeLa cells with seminal plasma or PGE2 resulted in up-regulation of COX-2 expression ($P < 0.05$). In addition, seminal plasma induced the mRNA expression of EP1, EP2 and EP4 receptors, whilst PGE2 treatment of HeLa cells induced the expression of the EP4 receptor ($P < 0.05$). This was coincident with a rapid accumulation of adenosine 3',5'-cyclic monophosphate (cAMP) in HeLa cells stimulated with seminal plasma or PGE2, which was greater in seminal plasma stimulated cells compared with PGE2 stimulated cells ($P < 0.05$). Subsequently, we investigated whether the effect of seminal plasma on cAMP signalling in HeLa cells was mediated via the cAMP-linked EP2/EP4 receptors. Stimulation of HeLa cells with seminal plasma or PGE2 resulted in an augmented cAMP accumulation in cells transfected with the EP2 or EP4 receptor cDNA compared with control transfected cells ($P < 0.05$). These data suggest that, in sexually active women, seminal plasma may play a role in modulating neoplastic cell function and cervical tumorigenesis.

Sata, F., H. Yamada, et al. (2003). "Glutathione S-transferase M1 and T1 polymorphisms and the risk of recurrent pregnancy loss." *Mol. Hum. Reprod.* **9**(3): 165-169.

<http://molehr.oupjournals.org/cgi/content/abstract/9/3/165>

The aetiology of recurrent pregnancy loss (RPL) remains unclear, but it may be related to a possible genetic predisposition together with involvement of environmental factors. We examined the relation between RPL and polymorphisms in two genes, glutathione S-transferases (GST) M1 and T1, which are involved in the metabolism of a wide range of environmental toxins and carcinogens. A case-control study of 115 cases with RPL and 160 controls was conducted. All cases and controls were women resident in Sapporo, Japan and the surrounding area. They were genotyped for polymorphisms of GSTM1 and GSTT1 using PCR-based methods. We found that 65.2% of the cases with RPL and 45.6% of the controls had the GSTM1 null genotype [odds ratio (OR) = 2.23, 95% confidence interval (CI) = 1.36-3.66]. On the other hand, 47.0% of the cases and 49.4% of the controls had the GSTT1 null genotype (OR = 0.95; 95% CI = 0.58-1.55). The results suggest that women with GSTM1 null polymorphism may therefore have an increased risk of RPL.

Sata, F., H. Yamada, et al. (2003). "A polymorphism in the CYP17 gene relates to the risk of recurrent pregnancy loss." *Mol. Hum. Reprod.* **9**(11): 725-728.

<http://molehr.oupjournals.org/cgi/content/abstract/9/11/725>

The CYP17 gene encodes the enzyme cytochrome P450c17{ α }, which mediates both 17{ α }-hydroxylase and 17,20-lyase activity in the steroid biosynthesis pathway. A T[-&gt]C polymorphism in the 5' promoter region of CYP17 has been described. To examine the association between recurrent pregnancy loss (RPL) and a polymorphism in CYP17, a case-control study of 117 cases with RPL and 164 controls was conducted. This polymorphism was investigated by PCR/restriction fragment length polymorphism using DNA from peripheral

lymphocytes. The T[→]C transition in the variant allele (A2) creates a new recognition site for the restriction enzyme MspA1, which permits designation of the wildtype allele (A1) and A2. Women with the A2 allele of CYP17 had an increased risk of RPL [A1/A1 genotype (reference); A1/A2 genotype: odds ratio (OR), 1.68; 95% confidence interval (CI), 0.94-3.01; A2/A2 genotype: OR, 2.37; 95% CI, 1.16-4.83; P trend, 0.016]. Additionally, there was a similar tendency for the increased risk of primary RPL [A1/A1 genotype (reference); A1/A2 genotype: OR, 2.14; 95% CI, 1.14-4.01; A2/A2 genotype: OR, 2.50; 95% CI, 1.16-5.41; P trend, 0.015]. These results suggest that possession of the A2 variant of CYP17 may predispose to an increased risk of RPL with a gene dosage effect.

Shahid, M., V. S. Dhillon, et al. (2004). "Two new novel point mutations localized upstream and downstream of the HMG box region of the SRY gene in three Indian 46,XY females with sex reversal and gonadal tumour formation." *Mol. Hum. Reprod.* **10**(7): 521-526.

<http://molehr.oupjournals.org/cgi/content/abstract/10/7/521>

The Y chromosome-specific gene SRY is one of the key genes involved in human sex determination. The SRY gene encodes a testis-specific transcription factor that plays a key role in sexual differentiation and development in males and is located on the distal region of the short arm of the Y chromosome. Mutations in SRY gene result in XY sex reversal and pure gonadal dysgenesis. SRY expression initiates a network of gene activity that transforms the undifferentiated gonad, genital ridge into testis. Mutations in the SRY gene have been considered to account for only 10-15% of 46,XY gonadal dysgenesis cases, whereas the majority of the remaining cases may have mutation(s) in the SRY regulatory elements or other genes involved in the sex differentiation pathway. Patients both with gonadal dysgenesis and Y-chromosome presence are at high risk of developing gonadoblastoma. Using PCR, single strand conformational polymorphism (SSCP) and automated DNA sequencing, we analysed the mutations in the SRY gene in three 46,XY sex reversal patients. Two patients demonstrated nucleotide substitution (A[→]G) within the open reading frame just outside and upstream of the conserved DNA-binding motif called the high-mobility group (HMG) box, replacing glutamine at codon 57 with arginine. Altered SSCP patterns were also observed in these patients. Histological examination of gonads in patient 1 revealed the formation of gonadoblastoma. Patient 3 demonstrated A[→]T substitution which replaces serine at codon 143 with cysteine, just outside but downstream of the HMG box. Results suggest the involvement of SRY gene in sex reversal which further supports the relationship between SRY alterations, gonadal dysgenesis and/or primary infertility.

Sun, W. S., J. Fujimoto, et al. (2003). "Clinical implications of coexpression of growth arrest-specific gene 6 and receptor tyrosine kinases Axl and Sky in human uterine leiomyoma." *Mol. Hum. Reprod.* **9**(11): 701-707.

<http://molehr.oupjournals.org/cgi/content/abstract/9/11/701>

The expression of Gas6, the protein product of the growth arrest-specific gene 6 (gas6), a member of the vitamin K-dependent protein family, and the receptor tyrosine kinases Axl and Sky and their mRNAs in uterine leiomyoma and normal uterine myometrium tissues were investigated by competitive RT-PCR-Southern blot analysis using recombinant RNA and immuno histochemical analysis respectively. There was no significant difference between the histoscores and levels of Sky mRNA in uterine leiomyoma and normal uterine myometrium, although the levels of Gas6 and Axl mRNAs in uterine leiomyoma were significantly higher than in normal uterine myometrium in each case. It is suggested that Gas6 and Axl signal transduction is aberrantly stimulated in uterine leiomyoma, possibly related to its growth.

Sun, W. S., R. Misao, et al. (2002). "Coexpression of growth arrest-specific gene 6 and receptor tyrosine kinases, Axl and Sky, in human uterine endometrium and ovarian endometriosis." Mol. Hum. Reprod. **8**(6): 552-558.

<http://molehr.oupjournals.org/cgi/content/abstract/8/6/552>

We demonstrated the expression of Gas6, the protein product of the growth arrest-specific gene 6 (gas6) and a member of the vitamin K-dependent protein family, and its receptor tyrosine kinases, Axl and Sky, in human uterine and ovarian endometriotic endometria using RT-PCR-Southern blot analysis and immunohistochemistry. Gas6, Axl and Sky mRNA were detected in all samples analysed. There was no significant difference between the levels of Sky mRNA in normal uterine and endometriotic endometria; however, the levels of Gas6 and Axl mRNA in endometriotic endometria were significantly higher than in normal endometria. These mRNA levels showed no significant alteration during the menstrual cycle. In the immunohistochemical study, Gas6, Axl and Sky were found in endometrial glandular cells and stromal cells in all samples analysed. This study demonstrates the coexpression of receptor tyrosine kinases and their ligand, Gas6, in normal uterine and ovarian endometriotic endometria, and the overexpression of Axl and Gas6 in endometriotic endometria. It is suggested that Gas6 and Axl signal transduction is aberrantly stimulated in endometriotic endometria, and is plausibly related to its growth potential.

Wieser, F., C. Schneeberger, et al. (2002). "Endometrial nuclear receptor co-factors SRC-1 and N-CoR are increased in human endometrium during menstruation." Mol. Hum. Reprod. **8**(7): 644-650.

<http://molehr.oupjournals.org/cgi/content/abstract/8/7/644>

Steroid hormone receptor co-factors are abundantly expressed in the uterus in order to modify steroid hormone receptor action, either leading to activation or repression of transcription in the endometrium. However, the role of co-factors in remodelling of the human endometrium has not been established. We therefore endeavoured to evaluate the presence of the co-activator SRC (steroid receptor co-activator)-1 and the co-repressors N-CoR (nuclear receptor co-repressor) and steroid co-repressor SMRT (silencing mediator of retinoid and thyroid) receptors in the human endometrium during the different phases of the menstrual cycle. By using a real-time RT-PCR assay, we showed that SRC-1, N-CoR and SMRT mRNA are expressed in human endometrium during all phases of the menstrual cycle, as well as in inactive endometrium. Moreover, endometrial expression of SRC-1 and N-CoR mRNA increased during menstruation when compared with the other phases of the menstrual cycle ($P < 0.001$). Immunohistochemistry demonstrated that SRC-1 and N-CoR stain positive in the glandular epithelium and stroma in menstrual phase endometrium. The staining was weak in proliferative and secretory endometrium and absent in inactive endometrium. Our results suggest that differential expression of endometrial steroid receptor co-factors probably play a role in the regulation of human endometrium remodelling.

Yamada, H., F. Sata, et al. (2004). "A polymorphism in the CYP17 gene and intrauterine fetal growth restriction." Mol. Hum. Reprod. **10**(1): 49-53.

<http://molehr.oupjournals.org/cgi/content/abstract/10/1/49>

Intrauterine fetal growth restriction is a multifactorial disorder, and its aetiology includes both

environmental and genetic components. We aimed to investigate whether maternal genetic polymorphisms of metabolic enzymes affects fetal growth and pregnancy duration. Genomic DNA was obtained from 134 women who experienced singleton deliveries beyond 24 weeks of gestation. Maternal age, birth weight, gestational age at birth and frequencies of fetal growth restriction, prematurity and pregnancy-induced hypertension were compared among genotypic subgroups of cytochrome P450 (CYP) and glutathione S-transferase (GST) genes. The polymorphisms of CYP1A1 (MspI), CYP17 (MspAI) and GSTP1 (BsmAI) genotypes, and the presence or absence of GSTM1 and GSTT1 genes were analysed by PCR-based methods. The frequency of fetal growth restriction (<10th percentile/<-1.5 SD; 22.7%/11.4%) in 44 women who were homozygous for the A1 allele (A1A1) of CYP17 was significantly higher than that (7.8%/2.2%) in 90 women who carried the A2 allele (A1A2/A2A2) of CYP17 ($P < 0.05$), with an odds ratio =3.41 (95% confidence interval = 1.18-9.84). The gestational age at birth (mean {+/-} SD, 37.5 {+/-} 3.1 weeks) in 67 women with GSTM1 null genotype was significantly lower than that (38.5 {+/-} 2.4 weeks) in 67 women who carried GSTM1 ($P < 0.05$). The polymorphism of CYP17 that encodes the cytochrome P450c17{alpha} enzyme might be associated with the pathophysiology underlying fetal growth restriction.

Mol. Pathol. (3)

Davids, V., S. H. Kidson, et al. (2003). "Accurate molecular detection of melanoma nodal metastases: an assessment of multimarker assay specificity, sensitivity, and detection rate." Mol. Pathol. **56**(1): 43-51.

<http://mp.bmjournals.com/cgi/content/abstract/56/1/43>

Background: The application of lymphoscintigraphy followed by sentinel lymph node (SN) biopsy to patients with primary melanoma has revolutionised the ability to identify accurately, yet conservatively, those patients who harbour occult nodal metastases. The molecular detection of SN micrometastases facilitates the cost effective analysis of the entire SN using multiple markers. Currently, a lack of marker specificity is the main barrier preventing the molecular evaluation of SN tissue from becoming clinically applicable. **Aims:** To develop a reproducible multimarker reverse transcription-polymerase chain reaction (RT-PCR) assay, with the emphasis on achieving high specificity for the accurate detection of melanoma metastases in nodal tissue. **Methods:** Three pigment cell specific (PCS) markers--tyrosinase, Pmel-17, and MART-1--and one cancer testis antigen (CTA)--MAGE-3--were selected for use in a multimarker RT-PCR assay. The conditions for this assay were optimised. **Results:** High specificity was achievable for each marker by optimising the PCR cycle number such that unwanted transcripts (that is, illegitimate transcripts and/or specific transcripts from other low abundance nodal cell types) remained undetectable in appropriate controls (normal visceral nodes). Tyrosinase was 100% specific at 40 PCR cycles, MAGE-3 and MART-1 at 35 PCR cycles, and Pmel-17 at 30 PCR cycles. Tyrosinase proved to be the most sensitive marker, detecting 10 melanoma cells in 0.1 g of nodal tissue. **Conclusions:** Excellent reproducibility of the entire nodal processing and RT-PCR protocol for the detection of very low numbers of melanoma cells in nodal tissue was shown, although there is a risk of false positives using the PCS markers alone, because of an approximate 4-8.5% incidence rate of nodal nevi in melanoma draining SNs (these nevi being absent in all other normal nodes). MAGE-3 was shown to be the only marker that is not expressed by melanocytes. However, because not all melanomas express MAGE-3, it is recommended that more emphasis should be placed on the development of a panel of CTA markers to ensure a zero false positive rate and to provide optimum detection.

Jung, A., S. Ruckert, et al. (2002). "7-Deaza-2'-deoxyguanosine allows PCR and sequencing reactions from CpG islands." Mol. Pathol. **55**(1): 55-57.

<http://mp.bmjjournals.com/cgi/content/abstract/55/1/55>

CpG islands are GC rich sequences that are found in the promoters of many genes in higher eukaryotes. They contain a high frequency of CG dinucleotides, which are substrates for DNA methylases. Methylation leads to transcriptional silencing of promoters. Owing to their high GC content CpG islands exhibit strong base-base interactions, which lead to superstructures and consequently to regions with higher melting temperatures. Therefore, Taq polymerases (especially sequenases) fall off their templates, causing premature termination of the polymerase chain reaction (PCR) or sequencing reactions. The results from such reactions are thus insufficient for further analysis. Therefore, we have evaluated the use of 7-deaza-2'-deoxyguanosine for PCR amplification of the human p16INK4A promoter and sequencing of HUMARA exon 1 PCR products. Our results show that the addition of 7-deaza-2'-deoxyguanosine significantly improves results, particularly when small amounts of poor quality DNA are available as starting material.

Lenze, D., A. Greiner, et al. (2003). "Receptor revision of immunoglobulin heavy chain genes in human MALT lymphomas." Mol. Pathol. **56**(5): 249-255.

<http://mp.bmjjournals.com/cgi/content/abstract/56/5/249>

Background/Aims: Rearrangement of immunoglobulin gene segments, leading to B cells with functional receptors, is thought to be largely restricted to developing immature B cells in bone marrow. However, accumulating evidence suggests that mature B cells occasionally modify their antigen specificity by VH segment replacements during the germinal centre reaction to enhance antigen affinity, or to overcome self reactive antigen receptors. Although malignant B cells maintain the features of their normal counterparts in most instances, to date, such replacements have not been described for human B cell lymphomas. **Methods:** Rearranged immunoglobulin heavy chain genes from two extranodal marginal zone B cell lymphomas were amplified, cloned, and sequenced. Sequences with identical CDR3 regions were selected and aligned to each other and public databases. **Results:** VH replacements were seen in two extranodal marginal zone B cell lymphomas. In line with the hypothesis that in mature B cells these replacements are associated with active somatic hypermutation, in addition to VH replacement, different mutation patterns were seen in the revised VH portions. In the remaining common 3'-VH regions, these mutations could be used to establish a phylogenetic relation between the sequences, rendering the possibility of artefactual chimaeric polymerase chain reaction products very unlikely. **Conclusions:** These results support the view that VH replacements are a further mechanism for reshaping antigen affinity and specificity, and indicate that these receptor modifications are not restricted to normal and reactive germinal centre B cells, but may also occur in close association with the development of malignant B cell lymphomas.

Baudhuin, L. M., K. L. Cristina, et al. (2002). "Akt Activation Induced by Lysophosphatidic Acid and Sphingosine-1-phosphate Requires Both Mitogen-Activated Protein Kinase Kinase and p38 Mitogen-Activated Protein Kinase and Is Cell-Line Specific." Mol. Pharmacol. **62**(3): 660-671.

<http://molpharm.aspetjournals.org/cgi/content/abstract/62/3/660>

The signaling pathways that lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) use to activate Akt in ovarian cancer cells are investigated here. We show for the first time, with the use of both pharmacological and genetic inhibitors, that the kinase activity and S473 phosphorylation of Akt induced by LPA and S1P requires both mitogen-activated protein (MAP) kinase kinase (MEK) and p38 MAP kinase, and MEK is likely to be upstream of p38, in HEY ovarian cancer cells. The requirement for both MEK and p38 is cell type- and stimulus-specific. Among 12 cell lines that we tested, 11 respond to LPA and S1P and all of the responsive cell lines require p38 but only nine of them require MEK. Among different stimuli tested, platelet-derived growth factor stimulates S473 phosphorylation of Akt in a MEK- and p38-dependent manner. However, epidermal growth factor, thrombin, and endothelin-1-stimulated Akt S473 phosphorylation require p38 but not MEK. Insulin, on the other hand, stimulates Akt S473 phosphorylation independent of both MEK and p38 in HEY cells. T308 phosphorylation stimulated by LPA/S1P requires MEK but not p38 activation. MEK and p38 activation were sufficient for Akt S473 but not T308 phosphorylation in HEY cells. In contrast to S1P and PDGF, LPA requires Rho for Akt S473 phosphorylation, and Rho is upstream of phosphatidylinositol 3-kinase (PI3-K). LPA/S1P-induced Akt activation may be involved in cell survival, because LPA and S1P treatment in HEY ovarian cancer cells results in a decrease in paclitaxel-induced caspase-3 activity in a PI3-K/MEK/p38-dependent manner.

Boverhof, D. R., E. Tam, et al. (2004). "2,3,7,8-Tetrachlorodibenzo-p-dioxin Induces Suppressor of Cytokine Signaling 2 in Murine B Cells." Mol. Pharmacol. **66**(6): 1662-1670.

<http://molpharm.aspetjournals.org/cgi/content/abstract/66/6/1662>

The B cell, a major component of humoral immunity, is a sensitive target for the immunotoxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), possibly by rendering cells less responsive to antigenic or mitogenic stimulation. Potential mechanisms of TCDD action on B cells were examined in murine B cell lymphoma cells (CH12.LX) treated with 3 nM TCDD or dimethyl sulfoxide vehicle using sequence-verified cDNA microarrays. One transcript that was significantly induced by TCDD was suppressor of cytokine signaling 2 (Socs2). Changes in Socs2 mRNA levels paralleled that of Cyp1a1 with a maximal 3-fold induction observed at 4 h, as determined by quantitative real-time polymerase chain reaction. Socs2 induction seems B cell-specific, because no induction was observed in TCDD-responsive mouse hepatoma cells or human breast cancer cells. TCDD-mediated induction of Socs2 mRNA was dose-dependent and exhibited the characteristic structure-activity relationships observed for the aryl hydrocarbon receptor (AhR) ligands 3,3',4,4',5-pentachlorobiphenyl (PCB-126), indolo[3,2-b]-carbazole, and {beta}-naphthoflavone. Experiments with cycloheximide and AhR-deficient B cells indicated that Socs2 mRNA induction is a primary effect that is AhR-dependent. Western blot analysis confirmed that Socs2 and Cyp1a1 protein levels were also induced in CH12.LX cells. Promoter analysis revealed the presence of four dioxin-response elements within 1000 base pairs upstream of the Socs2 transcriptional start site, and a reporter gene regulated by the Socs2 promoter was inducible by TCDD. Promoter activity was also dependent on a functional AhR signaling pathway. These results indicate that Socs2 is a primary TCDD-inducible gene that may represent a novel mechanism by which TCDD elicits its immunosuppressive effects.

Briz, O., R. I. R. Macias, et al. (2003). "Usefulness of Liposomes Loaded with Cytostatic Bile Acid

Derivatives to Circumvent Chemotherapy Resistance of Enterohepatic Tumors." Mol. Pharmacol. **63**(3): 742-750.

<http://molpharm.aspetjournals.org/cgi/content/abstract/63/3/742>

We have investigated the sensitivity of the cisplatin-resistant enterohepatic tumor cell lines LS174T/R (human colon adenocarcinoma), WIF-B9/R (rat hepatoma-human fibroblast hybrid), and Hepa 1-6/R (mouse hepatoma) to free and liposome-encapsulated cytostatic bile acid derivatives Bamet-R2 and bamet-UD2. Expression of resistance associated genes was measured by quantitative reverse transcription-polymerase chain reaction or Western blotting. Drug uptake was determined by atomic absorption spectrophotometry. In resistant cells, overexpression of MRP1 and MRP2 was accompanied by reduced accumulation of cisplatin. The expression of MDR1 and GST-P was only enhanced in LS 174T/R. A higher expression of p53 was seen in LS 174T/R and Hepa 1-6/R cell lines but not in WIF-B9/R cells. In wild-type counterparts, uptake and cytostatic ability of Bamets were markedly higher (UD2 > R2) than that of cisplatin. Both effects were further enhanced by liposome formulation. Bamets were able to overcome cisplatin resistance in all cell lines. Cisplatin prolonged the survival time of nude mice in whose livers a Hepa 1-6 tumor had been implanted, but failed to exert a beneficial effect when the tumor was Hepa 1-6/R. In both cases, tissue distribution of cisplatin was: kidney [IMG] liver > tumor. Survival was markedly longer in animals receiving Bamet-UD2, even if the implanted tumor was resistant. The accumulation of Bamet-UD2 in tissues was: liver > tumor > kidney. Liposome formulation further enhanced the beneficial properties of Bamet-UD2. Thus, the amount of drug in the tumor was increased and that in liver and kidney was reduced (tumor > liver > kidney), and life span was prolonged. In conclusion, liposomal Bamet-UD2 may be a useful tool to circumvent resistance to chemotherapy, particularly in tumors of the enterohepatic circuit.

Cagetti, E., J. Liang, et al. (2003). "Withdrawal from Chronic Intermittent Ethanol Treatment Changes Subunit Composition, Reduces Synaptic Function, and Decreases Behavioral Responses to Positive Allosteric Modulators of GABAA Receptors." Mol. Pharmacol. **63**(1): 53-64.

<http://molpharm.aspetjournals.org/cgi/content/abstract/63/1/53>

One of the pharmacological targets of ethanol is the GABAA receptor (GABAR), whose function and expression are altered after chronic administration of ethanol. The details of the changes differ between experimental models. In the chronic intermittent ethanol (CIE) model for alcohol dependence, rats are exposed to intermittent episodes of intoxicating ethanol and withdrawal, leading to a kindling-like state of behavioral excitability. This is accompanied by presumably causal changes in GABAR expression and physiology. The present study investigates further the effect of CIE on GABAR function and expression. CIE is validated as a model for human alcohol withdrawal syndrome (AWS) by demonstrating increased level of anxiety; diazepam improved performance in the test. In addition, CIE rats showed remarkably reduced hypnotic response to a benzodiazepine and a steroid anesthetic, reduced sensitivity to a barbiturate, but not propofol. Immunoblotting revealed decrease in [alpha]1 and [delta] expression and increase in [gamma]2 and [alpha]4 subunits in hippocampus of CIE rats, confirmed by an increase in diazepam-insensitive binding for ethyl-8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo(1,5-[alpha])(1,4)benzodiazepine-3-carboxylate (Ro15-4513). Elevated mRNA levels were shown for the [gamma]2S and [gamma]1 subunits. Recordings in hippocampal slices from CIE rats revealed that the decay time of GABAR-mediated miniature inhibitory postsynaptic currents (mIPSCs) in CA1 pyramidal cells was decreased, and potentiation of mIPSCs by positive modulators of GABAR was also reduced compared with control rats. However, mIPSC potentiation by the [alpha]4-preferring benzodiazepine ligands bretazenil and Ro15-4513 was maintained, and increased, respectively. These data suggest that specific alterations in GABAR occur after CIE and may underlie the development of hyperexcitability and ethanol dependence.

Christian, K., M. Lang, et al. (2004). "Interaction of Heterogeneous Nuclear Ribonucleoprotein A1 with Cytochrome P450 2A6 mRNA: Implications for Post-Transcriptional Regulation of the CYP2A6 Gene." *Mol. Pharmacol.* **65**(6): 1405-1414.

<http://molpharm.aspetjournals.org/cgi/content/abstract/65/6/1405>

The human xenobiotic-metabolizing enzyme cytochrome P450, CYP2A6, catalyzes the bioactivation of a number of carcinogens and drugs and is overexpressed in cases of liver diseases, such as cirrhosis, viral hepatitis, and parasitic infestation, and in certain tumor cells. This suggests that CYP2A6 may be a major liver catalyst in pathological conditions. In the present study, we have addressed molecular mechanisms underlying the regulation of the CYP2A6 gene. We present evidence of several proteins present in human hepatocytes that interact specifically with the 3'-untranslated region (UTR) of CYP2A6 mRNA. Biochemical and immunological evidence show that the RNA-protein complex of highest intensity contains the heterogeneous nuclear ribonucleoprotein (hnRNP) A1 or a closely related protein. Mapping of the hnRNP A1 binding site within CYP2A6 3'-UTR reveals that the smallest portion of RNA supporting significant binding consists of 111 central nucleotides of the 3'-UTR. Our studies also indicate that hnRNPA1 from HepG2 cancer cells exhibits modified binding characteristics to the CYP2A6 3'-UTR compared with primary hepatocytes. We found that the level of CYP2A6 mRNA remains high in conditions of impaired transcription in primary human hepatocytes, showing that CYP2A6 expression can be affected post-transcriptionally in conditions of cellular stress. Our results indicate that the post-transcriptional regulation involves interaction of the hnRNP A1 protein with CYP2A6 mRNA. The present data suggest that hnRNPA1 is a critical regulator of expression of the human CYP2A6 gene and support the notion that this P450 isoform may be of particular significance in stressed human liver cells.

Dobelis, P., M. J. Marks, et al. (2002). "A Polymorphism in the Mouse Neuronal alpha 4 Nicotinic Receptor Subunit Results in An Alteration in Receptor Function." *Mol. Pharmacol.* **62**(2): 334-342.

<http://molpharm.aspetjournals.org/cgi/content/abstract/62/2/334>

Nicotine-stimulated 86Rb⁺ efflux and [3H]cytisine binding, both of which seem to measure the nicotinic acetylcholine receptor, composed of [alpha]4 and [beta]2 subunits, were assessed in eight brain regions obtained from 14 inbred mouse strains. The potential role of a single nucleotide polymorphism (SNP) in the nicotinic receptor [alpha]4 subunit gene (Chrna4) on nicotinic receptor binding and function in mice was also evaluated. This SNP leads to an alanine-to-threonine variation at amino acid position 529 of the nascent [alpha]4 subunit polypeptide. Both nicotine-stimulated 86Rb⁺ efflux and [3H]cytisine binding were found to vary across brain regions and among mouse strains. Variability in nicotine-stimulated 86Rb⁺ efflux was positively correlated ($r > 0.9$) within each strain with the number of [3H]cytisine binding sites. However, the number of [3H]cytisine binding sites was not correlated with nicotine-stimulated 86Rb⁺ efflux across mouse strains. In contrast, the Chrna4 polymorphism was associated with receptor function across mouse strains: 86Rb⁺ efflux was greater in seven of the eight brain regions studied in those mouse strains that carry the Ala-529 variant of Chrna4. The Chrna4 SNP did not seem to influence the number of [3H]cytisine binding sites across mouse strains. These data indicate that inbred mouse strains exhibit differences in receptor function that cannot be attributed to variation in receptor expression but may be explained, at least in part, by the missense polymorphism in the [alpha]4 subunit.

Gautam, D., T. S. Heard, et al. (2004). "Cholinergic Stimulation of Salivary Secretion Studied with M1 and M3 Muscarinic Receptor Single- and Double-Knockout Mice." *Mol. Pharmacol.* **66**(2): 260-267.

<http://molpharm.aspetjournals.org/cgi/content/abstract/66/2/260>

Identification of the specific muscarinic acetylcholine receptor (mAChR) subtypes mediating stimulation of salivary secretion is of considerable clinical interest. Recent pharmacological and molecular genetic studies have yielded somewhat confusing and partially contradictory results regarding the involvement of individual mAChRs in this activity. In the present study, we re-examined the roles of M1 and M3 mAChRs in muscarinic agonist-mediated stimulation of salivary secretion by using M1 and M3 receptor single-knockout (KO) mice and newly generated M1/M3 receptor double-KO mice. When applied at a low dose (1 mg/kg, s.c.), the muscarinic agonist pilocarpine showed significantly reduced secretory activity in both M1 and M3 receptor single-KO mice. However, when applied at higher doses, pilocarpine induced only modestly reduced (5 mg/kg, s.c.) or unchanged (15 mg/kg, s.c.) salivation responses, respectively, in M1 and M3 receptor single-KO mice, indicating that the presence of either M1 or M3 receptors is sufficient to mediate robust salivary output. Quantitative reverse transcriptase-polymerase chain reaction studies with salivary gland tissue showed that the inactivation of the M1 or M3 mAChR genes did not lead to significantly altered mRNA levels of the remaining mAChR subtypes. Strikingly, the sialagogue activity of pilocarpine was abolished in M1/M3 receptor double-KO mice. However, salivary glands from M1/M3 receptor double-KO mice remained responsive to stimulation by the β -adrenergic receptor agonist, (S)-isoproterenol. Taken together these studies support the concept that a mixture of M1 and M3 receptors mediates cholinergic stimulation of salivary flow.

Gray, T., P. Nettesheim, et al. (2004). "Interleukin-1 β -Induced Mucin Production in Human Airway Epithelium Is Mediated by Cyclooxygenase-2, Prostaglandin E2 Receptors, and Cyclic AMP-Protein Kinase A Signaling." *Mol. Pharmacol.* **66**(2): 337-346.

<http://molpharm.aspetjournals.org/cgi/content/abstract/66/2/337>

We reported recently that interleukin (IL)-1 β exposure resulted in a prolonged increase in MUC5AC mucin production in normal, well differentiated, human tracheobronchial epithelial (NHTBE) cell cultures, without significantly increasing MUC5AC mRNA (Am J Physiol 286:L320-L330, 2004). The goal of the present study was to elucidate the signaling pathways involved in IL-1 β -induced MUC5AC production. We found that IL-1 β increased cyclooxygenase-2 (COX-2) mRNA expression and prostaglandin (PG) E2 production and that the COX-2 inhibitor celecoxib suppressed IL-1 β -induced MUC5AC production. Addition of exogenous PGE2 to NHTBE cultures also increased MUC5AC production and IL-1 β -induced Muc5ac hypersecretion in tracheas from wild-type but not from COX-2 $^{-/-}$ mice. NHTBE cells expressed all four E-prostanoid (EP) receptor subtypes and misoprostol, an EP2 and EP4 agonist, increased MUC5AC production, whereas sulprostone, an EP1 and EP3 agonist, did not. Furthermore, specific protein kinase A (PKA) inhibitors blocked IL-1 β and PGE2-induced MUC5AC production. However, neither inhibition of epidermal growth factor receptor (EGFR) activation with the tyrosine kinase inhibitor 4-(3-chloroanilino)-6,7-dimethoxyquinazoline HCl (AG-1478) or EGFR blocking antibody nor inhibition of extracellular signal-regulated kinase/P-38 mitogen activated protein kinases with specific inhibitors blocked IL-1 β stimulation of MUC5AC mucin production. We also observed that tumor necrosis factor (TNF)- α , platelet activating factor (PAF), and lipopolysaccharide (LPS) induced COX-2 and increased MUC5AC production that was blocked by celecoxib, suggesting a common signaling pathway of inflammatory mediator-induced MUC5AC production in NHTBE cells. We conclude that the induction of MUC5AC by IL-1 β , TNF- α , PAF, and LPS involves COX-2-generated PGE2, activation of EP2 and/or EP4 receptor(s), and cAMP-PKA-mediated signaling.

Jayanthi, S., M. T. McCoy, et al. (2002). "Methamphetamine Causes Coordinate Regulation of Src, Cas, Crk, and the Jun N-Terminal Kinase-Jun Pathway." Mol. Pharmacol. **61**(5): 1124-1131.

<http://molpharm.aspetjournals.org/cgi/content/abstract/61/5/1124>

The clinical abuse of methamphetamine (METH) is a major concern because it can cause long-lasting neurodegenerative effects in humans. Current concepts of the molecular mechanisms underlying these complications have centered on the formation of reactive oxygen species. Herein, we provide cDNA microarray evidence that METH administration caused the induction of c-Jun and of other members involved in the pathway leading to c-Jun activation [stress-activated protein kinase/Jun N-terminal kinase (JNK3), Crk-associated substrate-Cas and c-Src] after environmental stresses or cytokine stimulation. Reverse transcription-polymerase chain reaction analysis confirmed these increases and also showed that the expression of JNK1 and JNK3 but not JNK2 was also increased in the METH-treated mice. Western blot analysis showed that METH increased the expression of c-Jun phosphorylated at serine-63 and serine-73 residues. Other upstream members of the JNK pathway, including phosphorylated JNKs, mitogen-activated protein kinase kinase 4, mitogen-activated protein kinase kinase 7, Crk II, Cas, and c-Src were also increased at the protein level. These values returned to baseline by 1 week after drug treatment. These results are discussed in terms of their support for a possible role of the activation of the JNK/Jun pathway in the pathophysiological effects of METH.

Kennedy, J. C., S. Memet, et al. (2004). "Antisense Evidence for Nuclear Factor- κ B-Dependent Embryopathies Initiated by Phenytoin-Enhanced Oxidative Stress." Mol. Pharmacol. **66**(3): 404-412.

<http://molpharm.aspetjournals.org/cgi/content/abstract/66/3/404>

Endogenous and xenobiotic-enhanced oxidative stress may initiate embryonic death and birth defects via reactive oxygen species (ROS) signaling pathways involving nuclear transcription factor- κ B (NF- κ B). Using embryo culture and a transgenic mouse engineered with a NF- κ B-dependent β -galactosidase reporter gene, we employed NF- κ B antisense oligonucleotide therapy to determine whether NF- κ B signaling contributes to the embryopathic effects of the ROS-initiating teratogen phenytoin. Phenytoin selectively increased NF- κ B activity in target tissues and caused embryopathies, both of which were blocked by NF- κ B antisense oligonucleotides but not by sense and nonsense oligonucleotide controls. NF- κ B signaling may therefore contribute to the mechanism of ROS-mediated embryopathies.

King, L. M., J. Ma, et al. (2002). "Cloning of CYP2J2 Gene and Identification of Functional Polymorphisms." Mol. Pharmacol. **61**(4): 840-852.

<http://molpharm.aspetjournals.org/cgi/content/abstract/61/4/840>

CYP2J2 is abundant in cardiovascular tissue and active in the metabolism of arachidonic acid to eicosanoids that possess potent anti-inflammatory, vasodilatory, and fibrinolytic properties. We cloned and sequenced the entire CYP2J2 gene (~40.3 kb), which contains nine exons and eight introns. We then sequenced the CYP2J2 exons and intron-exon boundaries in 72 healthy persons representing African, Asian, and European/white populations as part of the National Institutes of Health/National Institute of Environmental Health Sciences Environmental Genome Single Nucleotide Polymorphism Program. A variety of polymorphisms were found, four of which resulted in coding changes (Arg158Cys, Ile192Asn, Asp342Asn, and Asn404Tyr). A fifth variant

(Thr143Ala) was identified by screening a human heart cDNA library. All five variant cDNAs of CYP2J2 were generated by site-directed mutagenesis and expressed in Sf9 insect cells by using a baculovirus system. The recombinant wild-type and variant CYP2J2 proteins immunoreacted with peptide-based antibodies to CYP2J2 and displayed typical cytochrome P450 (P450) CO-difference spectra; however, the Asn404Tyr and Ile192Asn variants also had prominent spectral peaks at 420 nm. The ability of these variants to metabolize arachidonic acid and linoleic acid was compared with that of wild-type CYP2J2. Three variants (Asn404Tyr, Arg158Cys, and Thr143Ala) showed significantly reduced metabolism of both arachidonic acid and linoleic acid. The Ile192Asn variant showed significantly reduced activity toward arachidonic acid only. The Asp342Asn variant showed similar metabolism to wild-type CYP2J2 for both endogenous substrates. Based on these data, we conclude that allelic variants of the human CYP2J2 gene exist and that some of these variants result in a P450 protein that has reduced catalytic function. Insofar as CYP2J2 products have effects in the cardiovascular system, we speculate that these variants may be functionally relevant.

Levresse, V., L. Marek, et al. (2002). "Regulation of Platinum-Compound Cytotoxicity by the c-Jun N-Terminal Kinase and c-Jun Signaling Pathway in Small-Cell Lung Cancer Cells." Mol. Pharmacol. **62**(3): 689-697.

<http://molpharm.aspetjournals.org/cgi/content/abstract/62/3/689>

Cytotoxic platinum compounds including cisplatin are standard cancer chemotherapeutics and are also activators of stress-signaling pathways. In this study, we tested the role of the c-Jun N-terminal kinase (JNK) family of mitogen-activated protein kinases and their transcription factor target, c-Jun, in the cytotoxic response of small-cell lung cancer (SCLC) cells to cisplatin and its less effective trans-isomer, transplatin. Both agents stimulated JNK activity; the transplatin response was rapid and transient, whereas JNK activation by cisplatin was delayed and sustained. Despite the differential kinetics of JNK activation, expression of nonphosphorylatable JNK mutants sensitized the SCLC cells to killing by cisplatin or transplatin, suggesting that JNK activation in response to these agents signals a protective response. Consistent with this finding, overexpression of the JNK target, c-Jun, significantly protected SCLC cells from platinum compounds, whereas expression of a c-Jun mutant encoding only the DNA binding domain increased the sensitivity of the SCLC cells to these drugs. These findings support the hypothesis that activation of the JNKs by platinum compounds controls c-Jun-dependent transcriptional events that promote a protective response in SCLC cells. Oligonucleotide array analysis identified genes encoding a variety of signaling proteins whose expression was reciprocally changed by c-Jun and c-Jun-DBD (c-Jun-DNA binding domain). It is noteworthy that genes whose products are involved in DNA repair, glutathione synthesis, or drug accumulation did not exhibit altered expression by c-Jun or c-Jun-DBD. The findings indicate that inhibition of the JNK pathway is a potential means to enhance the sensitivity of SCLC cells to platinum compounds.

Lin, C. W., M. Nakane, et al. (2002). "trans-Activation and Repression Properties of the Novel Nonsteroid Glucocorticoid Receptor Ligand 2,5-Dihydro-9-hydroxy-10-methoxy-2,2,4-trimethyl-5-(1-methylcyclohexen-3-yl)-1H-[1]benzopyrano[3,4-f]quinoline (A276575) and Its Four Stereoisomers." Mol. Pharmacol. **62**(2): 297-303.

<http://molpharm.aspetjournals.org/cgi/content/abstract/62/2/297>

Glucocorticoids are potent anti-inflammatory and immunosuppressant agents. However, they also produce serious side effects that limit their usage. It has been proposed that anti-inflammatory properties of glucocorticoids are caused mostly by repression of activator protein 1- and nuclear factor [kappa][beta]-stimulated synthesis of inflammatory mediators, whereas most of their

adverse effects are associated with trans-activation of genes involved with metabolic processes. Our laboratories have sought to discover novel glucocorticoid receptor (GR) ligands that have high repression but low trans-activation activities. We describe here cellular properties of 2,5-dihydro-9-hydroxy-10-methoxy-2,2,4-trimethyl-5-(1-methylcyclohexen-3-yl)-1H-[1]benzopyrano[3,4-f]quinoline (A276575) and its four enantiomers. Similar to dexamethasone, A276575 exhibited high affinity for GR and potently repressed interleukin (IL) 1[β]-stimulated IL-6 production in human skin fibroblasts, prostaglandin (PG) E₂ production in A549 human lung epithelial cells, and concanavalin A-induced monocyte proliferation. In contrast to dexamethasone, A276575 caused smaller induction of aromatase activity in human skin fibroblasts and antagonized dexamethasone-induced activation of an mouse mammary tumor virus-glucocorticoid-response element (GRE) reporter gene construct. Among the four enantiomers of A276575, the two ([-])-enantiomers showed 10- to 30-fold higher affinities for GR than their respective (+)-enantiomers. Both ([-])-Syn and ([-])-Anti enantiomers of A276575 were potent inhibitors of IL-1[β]-stimulated PGE₂ production in A549 lung epithelial cells; unexpectedly, however, only the ([-])-Anti enantiomer inhibited regulated on T-cell activation, normal T-cell expressed and secreted (RANTES) production in A549 cells. In summary, A276575 is a novel, nonsteroidal GR ligand that possesses high repression activities against inflammatory mediator production but has lower GRE trans-activation activities than traditional steroids. Differential repression of RANTES and PGE₂ production in a cell by the two ([-])-enantiomers of A276575 illustrates the complexity of repression by GR.

Ma, J., J. Graves, et al. (2004). "Regulation of Mouse Renal CYP2J5 Expression by Sex Hormones." Mol. Pharmacol. **65**(3): 730-743.

<http://molpharm.aspetjournals.org/cgi/content/abstract/65/3/730>

Mouse CYP2J5 is abundant in kidney and active in the metabolism of arachidonic acid to epoxyeicosatrienoic acids. Western blots of microsomes prepared from mouse kidneys demonstrate that after puberty, CYP2J5 protein is present at higher levels in male mice than in female mice. Northern analysis reveals that CYP2J5 transcripts are more abundant in adult male versus female kidneys, indicating that gender differences in renal CYP2J5 expression are regulated at a pretranslational level. Castration of male mice results in decreased renal CYP2J5 expression, and treatment of castrated male mice or female mice with 5 α -dihydrotestosterone increases expression to levels that approximate those in intact male mice. In contrast, treatment of ovariectomized female mice or castrated male mice with 17 β -estradiol causes a further reduction in CYP2J5 expression. Growth hormone-deficient (lit/lit) mice respond similarly to castration and 5 α -dihydrotestosterone treatment, indicating that the androgen effects are not mediated by alterations in the growth hormone secretory pattern. Mice that lack a functional androgen receptor (Tfm hemizygous) have reduced levels of renal CYP2J5 and do not respond to 5 α -dihydrotestosterone treatment. Similarly, wild-type male mice treated with flutamide, an androgen antagonist, exhibit reduced renal CYP2J5 levels. Female estrogen receptor- α knockout (α ERKO) mice, which are known to have elevated circulating testosterone levels, have significantly increased renal CYP2J5 expression compared with wild-type female mice, and these differences are abrogated by ovariectomy or treatment with flutamide. Based on these data, we conclude that the renal expression of CYP2J5 is up-regulated by androgen and down-regulated by estrogen.

Peeters, P. J., H. W. Gohlmann, et al. (2004). "Transcriptional Response to Corticotropin-Releasing Factor in AtT-20 Cells." Mol. Pharmacol. **66**(5): 1083-1092.

<http://molpharm.aspetjournals.org/cgi/content/abstract/66/5/1083>

Corticotropin-releasing factor (CRF) plays a central role in the regulation of the hypothalamic-pituitary-adrenal axis, mediating endocrine and behavioral responses to various stressors. Two high-affinity receptors for CRF have been described. Although many of the intracellular signaling pathways activated by CRF have been studied extensively, our knowledge of transcriptional responses downstream of the CRF receptor 1 (CRFR1) is still limited. To elucidate gene networks regulated by CRF and CRFR1, we applied microarray technology to explore transcriptional response to CRF stimulation. Therefore, mouse pituitary-derived AtT-20 cells were exposed continuously to CRF either in the presence or absence of the specific CRFR1 antagonist R121919. Transcriptional responses to different treatments were studied in a time course ranging from 0.5 to 24 h. Microarray data were analyzed using classic microarray data analysis tools such as correspondence factor analysis, cluster analysis, and fold-change filtering. Furthermore, spectral map analysis was applied, a recently introduced unsupervised multivariate analysis method. A broad and transient transcriptional response to CRF was identified that could be blocked by the antagonist. This way, several known CRF-induced target genes and novel CRF responsive genes were identified. These include transcription factors such as cAMP-responsive element modulator (7x increased), secreted peptides such as cholecystokinin (1.5x), and proteins involved in modulating intracellular signaling, such as regulator of G-protein signaling 2 (11x). Up-regulation of many of these genes can be explained as negative feedback, attenuating CRF-activated pathways. In addition, spectral map analysis proved to be a promising new tool for microarray data analysis.

Siwkowski, A. M., L. A. Madge, et al. (2004). "Effects of Antisense Oligonucleotide-Mediated Depletion of Tumor Necrosis Factor (TNF) Receptor 1-Associated Death Domain Protein on TNF-Induced Gene Expression." Mol. Pharmacol. **66**(3): 572-579.

<http://molpharm.aspetjournals.org/cgi/content/abstract/66/3/572>

Tumor necrosis factor (TNF) receptor 1-associated death domain protein (TRADD) is an adaptor protein known to be involved in the TNF signaling pathway as well as signaling of other members of the TNF receptor superfamily, including DR3, DR6, p75NTR, and the Epstein-Barr virus latent membrane protein 1. Current knowledge of the function of the adaptor protein has been derived from studies examining its over-expression in either wild-type or mutated forms. In this study, we analyzed the consequences of antisense oligonucleotide (ASO)-mediated depletion of endogenous TRADD on TNF induction of inflammation-related gene products, such as intercellular adhesion molecule-1, and associated kinase signaling pathways in human umbilical vein endothelial cells. A broader perspective of TRADD's role in TNF signaling was indicated by microarray gene expression analysis, where 20 of 24 genes that showed a 5-fold or greater increase in TNF-induced mRNA expression levels displayed a reduction in TNF-induced expression as a consequence of ASO-mediated knockdown of TRADD. Reduced activation of the nuclear factor- κ B and c-Jun NH₂-terminal kinase pathways, as measured by I κ B- α protein levels and the extent of c-Jun phosphorylation, was also observed. These results indicate usage of antisense inhibitors of TRADD expression for modulating diseases associated with TRADD-dependent signal transduction pathways.

Soderberg, M., F. Raffalli-Mathieu, et al. (2002). "Inflammation Modulates the Interaction of Heterogeneous Nuclear Ribonucleoprotein (hnRNP) I/Polypyrimidine Tract Binding Protein and hnRNP L with the 3'Untranslated Region of the Murine Inducible Nitric-Oxide Synthase mRNA." Mol. Pharmacol. **62**(2): 423-431.

<http://molpharm.aspetjournals.org/cgi/content/abstract/62/2/423>

Interaction of two members of the heterogeneous nuclear ribonucleoprotein (hnRNP) family with

the 3'untranslated region (UTR) of the murine inducible nitric-oxide synthase (iNOS) mRNA is demonstrated in this study. An iNOS RNA-protein complex is formed using protein extracts from untreated and septic shock treated mouse liver. UV cross-linking reveals that the complex consists of at least two proteins, with apparent molecular masses of 60 and 70 kDa, respectively. The 60-kDa protein binding site lies within a 112-nt pyrimidine-rich sequence, approximately 160 nt from the coding sequence, and the RNA-protein complex can be precipitated by a monoclonal antibody directed against hnRNP I [also named polypyrimidine tract binding protein (PTB)]. The 70-kDa protein binds a 43-nt sequence near the 3'end of the 3'UTR and is immunoprecipitated by a monoclonal antibody against hnRNP L. A computer-simulated conformation of the 3'UTR suggests that both binding sites reside in regions easily accessible for a protein. Supershifts of the native RNA-protein complex could only be achieved with anti-hnRNP L, suggesting that within this multiprotein RNA complex, only hnRNP L is exposed to the antibodies, whereas the hnRNP I/PTB is mainly responsible for its interaction with the mRNA. Up-regulation of iNOS by septic shock reduces the RNA-protein complex formation, thus showing that hnRNP I/PTB and hnRNP L binding to the iNOS mRNA is modulated by inflammation. This suggests a novel function for the two previously described proteins as regulators of the iNOS gene.

Struckmann, N., S. Schwering, et al. (2003). "Role of Muscarinic Receptor Subtypes in the Constriction of Peripheral Airways: Studies on Receptor-Deficient Mice." Mol. Pharmacol. **64**(6): 1444-1451.

<http://molpharm.aspetjournals.org/cgi/content/abstract/64/6/1444>

In the airways, increases in cholinergic nerve activity and cholinergic hypersensitivity are associated with chronic obstructive pulmonary disease and asthma. However, the contribution of individual muscarinic acetylcholine receptor subtypes to the constriction of smaller intrapulmonary airways that are primarily responsible for airway resistance has not been analyzed. To address this issue, we used videomicroscopy and digital imaging of precision-cut lung slices derived from wild-type mice and mice deficient in either the M1 (mAChR1^{-/-} mice), M2 (mAChR2^{-/-} mice), or M3 receptor subtype (mAChR3^{-/-} mice) or lacking both the M2 and M3 receptor subtypes (mAChR2/3^{-/-} double-knockout mice). In peripheral airways from wild-type mice (mAChR^{+/+} mice), muscarine induced a triphasic concentration-dependent response, characterized by an initial constriction, a transient relaxation, and a sustained constriction. The bronchoconstriction was diminished by up to 60% in mAChR3^{-/-} lungs and was completely abolished in mAChR2/3^{-/-} lungs. The sustained bronchoconstriction was reduced in mAChR2^{-/-} bronchi, and, interestingly, the transient relaxation was absent; the bronchoconstriction in response to 10⁻⁸ M muscarine was increased by 158% in mAChR1^{-/-} mice. Quantitative reverse transcriptase-polymerase chain reaction analysis revealed that the disruption of specific mAChR genes had no significant effect on the expression levels of the remaining mAChR subtypes. These results demonstrate that cholinergic constriction of murine peripheral airways is mediated by the concerted action of the M2 and M3 receptor subtypes and suggest the existence of pulmonary M1 receptor activation, which counteracts cholinergic bronchoconstriction. Given the important role of muscarinic cholinergic mechanisms in pulmonary disease, these findings should be of considerable therapeutic relevance.

Molecular and Cellular Endocrinology(61)

Aavik, E., D. d. Toit, et al. (2001). "Estrogen receptor beta dominates in baboon carotid after endothelial

denudation injury." Molecular and Cellular Endocrinology **182**(1): 91.

<http://www.sciencedirect.com/science/article/B6T3G-44GGB9K-B/2/3f4f3a86f3f70012be86a160d3ddf1c8>

Increasing evidence, mainly from rodents, suggests that the predominant estrogen receptor (ER) in arteries is the newly-described ERbeta. We have investigated the expression of the two ERs in baboon carotid artery before and after denudation injury. Prior to denudation, both full length receptors were detected in semiquantitative RT-PCR; in addition two ERalpha but no ERbeta splicing variants were found. After denudation, ERbeta mRNA increased five-fold and declined, whereas ERalpha mRNA expression remained low. Prior to and after denudation, two ERalpha-specific antibodies showed no reaction with the vessel wall. Instead, two affinity purified antisera to ERbeta demonstrated a weak but distinct reaction over vascular smooth muscle cells with predenudation specimens, escalating post-denudation and declining thereafter. The results suggest that selective targeting to ERbeta should be attempted when designing estrogen-based vasculoprotective drug therapies devoid of uterotrophic side effects.

Ando, M., S. Kol, et al. (1999). "Non-steroidal anti-inflammatory drugs (NSAIDs) block the late, prostanoid-dependent/ceramide-independent component of ovarian IL-1 action: implications for the ovulatory process." Molecular and Cellular Endocrinology **157**(1-2): 21.

<http://www.sciencedirect.com/science/article/B6T3G-3XT71FT-3/2/538a578d88e486eachba7322391866333>

The therapeutic efficacy and antiovarian properties of non-steroidal anti-inflammatory drugs (NSAIDs) is attributed to their ability to suppress prostaglandin endoperoxide synthase (PGS) activity. Given the likely role of interleukin (IL)-1 in the inflammatory (and probably the ovulatory) process, we set out to evaluate whether the antiovarian property of NSAIDs is attributable, in part, to the inhibition of ovarian IL-1 action. Whole ovarian dispersates from immature rats were cultured under serum-free conditions in the absence or presence of the indicated agents. At the conclusion of the culture period, total RNA was extracted and probed for transcripts corresponding to PGS-1, PGS-2, IL-1[beta], IL-1 receptor antagonist (IL-1RA) or type I IL-1 receptor (IL-1R) by a solution hybridization/ribonuclease protection assay. Treatment with indomethacin was without significant effect on the early (1 h) response to IL-1[beta]; however, it led to complete and highly significant dose-dependent blockade of the late (48 h) response to IL-1[beta] as assessed in terms of PGS-2 transcripts, proteins and activity. The addition of PGE2 to cells augmented the ability of IL-1[beta] to upregulate PGS-2 transcripts. Moreover, the addition of PGE2 to indomethacin-treated cells all but reversed the ability of indomethacin to suppress the IL-1[beta] effect at both the PGS-2 transcript and protein levels. The upregulation by IL-1 of IL-1[beta], IL-1R and IL-1RA transcripts was similarly inhibited by indomethacin. Taken together, these observations suggest that the anti-ovulatory property of NSAIDs may be due, in part, to blockade of the late, prostanoid-dependent component of ovarian IL-1 action.

Autelitano, D. J., L. Snyder, et al. (1989). "Dopamine D2-receptor messenger RNA is differentially regulated by dopaminergic agents in rat anterior and neurointermediate pituitary." Molecular and Cellular Endocrinology **67**(1): 101.

<http://www.sciencedirect.com/science/article/B6T3G-47GGRXR-F/2/7811f0f6159449df21a8cfbd1a964734>

Hypothalamic dopamine (DA), acting at DA D2-receptors (D2-R) on pituitary target cells,

mediates peptide release and biosynthesis of rat pituitary anterior lobe (AL) prolactin, and neurointermediate lobe (NIL) pro-opiomelanocortin (POMC). We were interested in determining if dopamine agonists and antagonists were capable of modifying D2-R gene expression in these pituitary cells. Utilizing the recently published sequence of the rat D2-R, we isolated a rat D2-R cDNA clone by polymerase chain reaction, and have synthesized RNA probes to quantitate levels of D2-R mRNA by solution hybridization/nuclease protection assay. We report here that 5-day administration of the DA antagonist haloperidol led to significant increase in both D2-R mRNA and POMC mRNA in the NIL; the DA agonist bromocriptine caused a significant decrease in NIL POMC mRNA with no parallel change in D2-R mRNA. In contrast, no significant changes in D2-R mRNA in AL were observed following treatment with either the DA agonist or antagonist. These data provide evidence for tissue-specific regulation of D2-R mRNA in response to dopaminergic manipulation.

Bauer, M. P., J. T. Bridgham, et al. (2000). "Conservation of steroidogenic acute regulatory (StAR) protein structure and expression in vertebrates." Molecular and Cellular Endocrinology **168**(1-2): 119.

<http://www.sciencedirect.com/science/article/B6T3G-41JM913-D/2/623b23032e74b10b54a473808932ba74>

Complementary DNAs for the open reading frames of the chicken, Xenopus and zebrafish StAR homologs were cloned along with a partial cDNA of the zebrafish homolog to MLN64, a StAR-related protein. A comparison of the amino acid sequences of piscine, amphibian, avian and mammalian StARs, indicates strong conservation of the protein across divergent vertebrate groups. On Northern blots probed with species specific StAR cDNAs, expression of StAR transcripts was observed in the ovary and adrenal of chicken, and the ovary, testis, kidney and head of zebrafish. The expression of StAR mRNA in various compartments of the hen ovary was consistent with the results of past studies on steroidogenesis; expression was first observed in follicles selected into the preovulatory hierarchy and was greatest in the largest preovulatory follicle. The expression of StAR mRNA was also consistent with aromatase expression in zebrafish ovaries. The conserved deduced protein sequence and expression pattern of StAR transcripts in chicken and zebrafish tissues, strongly suggest that StAR is also involved in the regulation of steroidogenesis in nonmammalian vertebrates.

Belanger, G., M. Beaulieu, et al. (1995). "Expression of transcripts encoding steroid UDP-glucuronosyltransferases in human prostate hyperplastic tissue and the LNCaP cell line." Molecular and Cellular Endocrinology **113**(2): 165.

<http://www.sciencedirect.com/science/article/B6T3G-3XWRSY9-23/2/4c2e3d7c67c0ec4449759ac8c6511f76>

The UDP-glucuronosyltransferase (EC 2.4.1.17) enzymes transform many lipophilic compounds to more water-soluble products via conjugation with glucuronic acid. This conversion is responsible for enhancing the excretion of endogenous aglycones such as steroids. To date, several distinct isoforms of steroid UDP-glucuronosyltransferases (UGTs) have been isolated in the human liver. Among these UGTs, UGT2B7 is specific for estriol and 3,4-catechol estrogens, UGT2B15 glucuronidates 17[beta]-hydroxy-C19 steroids while UGT2B10 has as yet an undescribed activity. To further demonstrate the presence of UGTs in peripheral tissues we studied the expression of these enzymes in human prostate hyperplastic tissue and the LNCaP cell line. Metabolism studies using intact LNCaP cells in culture indicate the presence of UGT activities involved in the glucuronidation of 3[alpha]-hydroxysteroids (androsterone) and 17[beta]-hydroxysteroids (testosterone and dihydrotestosterone). Northern blot analysis of poly(A+) RNA from LNCaP cells and prostate using a UGT2B15 cDNA probe revealed two bands of 2.0 and 2.3

kb. In order to identify more specifically the mRNAs detected in Northern blot analysis we used RNase protection and RT-PCR assays. The relatively high expression of UGT2B10 and UGT2B15 in LNCaP cells was confirmed by RNase protection and RT-PCR, although, these approaches did not allow detection of UGT2B7 transcripts. Our studies demonstrate the presence of two UGT activities and at least two types of UGT transcripts in both the human prostate and the LNCaP cells.

Bowman, C. J., K. J. Kroll, et al. (2002). "Estradiol-induced gene expression in largemouth bass (*Micropterus salmoides*)."
Molecular and Cellular Endocrinology **196**(1-2): 67.

<http://www.sciencedirect.com/science/article/B6T3G-46THXCK-5/2/3ba70e8424422115541d89cf6e2f0ccc>

Vitellogenin (Vtg) and estrogen receptor (ER) gene expression levels were measured in largemouth bass to evaluate the activation of the ER-mediated pathway by estradiol (E2). Single injections of E2 ranging from 0.0005 to 5 mg/kg up-regulated plasma Vtg in a dose-dependent manner. Vtg and ER mRNAs were measured using partial cDNA sequences corresponding to the C-terminal domain for Vtg and the ligand-binding domain of ER[alpha] sequences. After acute E2-exposures (2 mg/kg), Vtg and ER mRNAs and plasma Vtg levels peaked after 2 days. The rate of ER mRNA accumulation peaked 36-42 h earlier than Vtg mRNA. The expression window for ER defines the primary response to E2 in largemouth bass and that for Vtg a delayed primary response. The specific effect of E2 on other estrogen-regulated genes was tested during these same time windows using differential display RT-PCR. Specific up-regulated genes that are expressed in the same time window as Vtg were ERp72 (a membrane-bound disulfide isomerase) and a gene with homology to an expressed gene identified in zebrafish. Genes that were expressed in a pattern that mimics the ER include the gene for zona radiata protein ZP2, and a gene with homology to an expressed gene found in winter flounder. One gene for fibrinogen [gamma] was down-regulated and an unidentified gene was transiently up-regulated after 12 h of exposure and returned to basal levels by 48 h. Taken together these studies indicate that the acute molecular response to E2 involves a complex network of responses over time.

Breen, J. J., N. J. Hickok, et al. (1997). "The rat TSH[beta] gene contains distinct response elements for regulation by retinoids and thyroid hormone."
Molecular and Cellular Endocrinology **131**(2): 137.

<http://www.sciencedirect.com/science/article/B6T3G-3R7B1MD-2/2/f59e4986d3415a349ffa4ae587cdf40b>

We have previously shown that thyroid stimulating hormone-[beta] (TSH[beta]) mRNA levels are modulated by vitamin A status in vivo and using transient transfection, that suppression of rat TSH[beta] gene promoter activity by all-trans retinoic acid (RA) requires RA receptor (RAR) and retinoid X receptor (RXR). In this paper we have used deletion analysis to delineate the sequences of the rTSH[beta] gene involved in RA regulation, their relationship to the rTSH[beta] gene negative thyroid hormone response elements and the retinoid receptor species that interact with these sequences. Using transient transfection in CV-1 cells, we found that the -204/+9 region of the rat TSH[beta] gene, when fused to a luciferase reporter, was sufficient for suppression by all-trans-RA in the presence of RAR/RXR. Thus, regulation by RA did not involve the major rTSH[beta] negative TRE located between +15 and +43. Mutational analysis also showed that the minor rTSH[beta] negative TRE between -11 and +5 was not required by suppression by RA. However, in a heterologous promoter this sequence element acted as a strong positive RARE. The combination of RA and T3 treatment caused synergistic inhibition of rat TSH[beta] gene expression in the presence of RAR/RXR and TR. EMSA analysis demonstrated that the -204/-79 sequence binds RAR/RXR heterodimer. Therefore, we conclude that there are separate response

elements for RA and T3 on the rat TSH[beta] gene, that the RARE binds RAR/RXR heterodimer and that RA and T3 interact functionally via these elements in the negative regulation of rat TSH[beta] gene expression.

Brevini-Gandolfi, T. A. L., F. Cillo, et al. (2001). "Somatostatin up-regulates topoisomerase II alpha expression and affects LNCaP cell cycle." Molecular and Cellular Endocrinology **176**(1-2): 103.

<http://www.sciencedirect.com/science/article/B6T3G-430NR88-F/2/3dc4d72b66fc7cb61108dacbbd42f59e>

mRNA differential display-PCR analysis was used to perform a systematic screening of Somatostatin (SS)-regulated genes in the human prostatic carcinoma cell line LNCaP (Lymph Node Carcinoma of the Prostate). A 170 bp fragment was shown to be up-regulated by SS. Sequence analysis of this fragment revealed its homology with the human Topoisomerase II Alpha gene. Up-regulation of Topoisomerase II Alpha was confirmed by Northern blot hybridisation and was induced by the same dose of SS (1 nM) earlier demonstrated to inhibit LNCaP cell growth. Furthermore, SS possible effects on timing, as well as concentration of Topoisomerase II Alpha along the different phases of the cell cycle were investigated. To this purpose changes in the enzyme protein concentration in response to SS were assessed in synchronised LNCaP cells. The hormone was shown to exert a perturbing effect on both parameters considered, possibly related to its inhibitory action on LNCaP cell replication.

Celano, M., F. Arturi, et al. (2003). "Expression of adenylyl cyclase types III and VI in human hyperfunctioning thyroid nodules." Molecular and Cellular Endocrinology **203**(1-2): 129.

<http://www.sciencedirect.com/science/article/B6T3G-481N1T0-3/2/b7bbafd099c208fc10751e81385a14a7>

Hyperfunctioning thyroid nodules are characterized by the presence of spontaneous somatic mutations responsible for constitutive activation of the cAMP pathway. However, alterations affecting other elements of the cAMP signaling system may counteract the effects of the mutations. In this study, the expression of the adenylyl cyclase (AC) types III and VI was investigated by Western blot in 18 hyperfunctioning thyroid nodules; in 12 samples, we also assessed the presence of TSH receptor (TSHR) or gsp mutations and levels of AC VI and III mRNA. We found that the expression of nodular AC VI (but not AC III) was significantly lower (85.1% of normal, $P=0.014$) than the expression of both adenylyl cycles types of perinodular tissue from the same patients. Slightly, but not significant differences were detected in nodules with or without mutations and AC protein levels generally showed correlation with the levels of the transcripts detected by RT-PCR. In addition, AC III and AC VI expression levels within a given nodule were characterized by a significant positive correlation. These findings indicate that a diminished expression of AC type VI may be part of the mechanisms occurring in the hyperfunctioning nodules, independently of the presence of TSHR or gsp mutations, which influence the resulting phenotype.

Celi, F. S., G. Canettieri, et al. (1998). "Genomic characterization of the coding region of the human type II 5'-deiodinase gene." Molecular and Cellular Endocrinology **141**(1-2): 49.

<http://www.sciencedirect.com/science/article/B6T3G-3TGVJSC-8/2/5fcb2875c10aea84df387c9083beffb6>

Type II 5'-Deiodinase (5'DII) is a key element in the maintenance of peripheral thyroid hormone homeostasis through the regulation of local T4 to T3 conversion in pituitary, brain, brown adipose tissue and placenta. The cDNA containing the coding region of the human 5'DII (HDII) has been recently cloned from infant brain. In the present paper we report the genomic structure, chromosomal localization and restriction map of the coding region of HDII. The presence of a single intron located at codon 75 was demonstrated using a PCR-based strategy; the exon-intron junctions were then cloned and partially sequenced. Chromosomal localization was performed by radiation hybrid mapping. This study demonstrated that the entire coding region of the HDII gene is contained in two exons spliced at codon 75 by a 7.4 Kb intron and that the HDII chromosomal location is 14q24.3. These data will allow further studies of the role of HDII in the pathophysiology of thyroid homeostasis.

Chatterjee, A., Y. L. Hsieh, et al. (2001). "Molecular cloning of cDNA encoding thyroid stimulating hormone [beta] subunit of bighead carp *Aristichthys nobilis* and regulation of its gene expression." Molecular and Cellular Endocrinology **174**(1-2): 1.

<http://www.sciencedirect.com/science/article/B6T3G-42SGHHC-1/2/88d709127f65a8b7c23d0ea865782b78>

The complementary DNA (cDNA) encoding pituitary thyroid stimulating hormone beta subunit (TSH-[beta]) of bighead carp was cloned and regulation of its gene expression was investigated for understanding phylogenetic divergence and evolution of TSH molecule. The cDNA was obtained from bighead carp pituitary total RNA by reverse transcription and polymerase chain reaction. Oligonucleotide primers were designed from the sequence of common carp. The full length sequence was then obtained by 3' and 5' rapid amplification of cDNA ends (RACE). The full-length sequence consisting of 3' and 5' untranslated regions was 585 bp long. The predicted amino acid sequence consisted of a signal peptide of 19 amino acid residues and a mature TSH [beta] subunit protein of 131 residues. The coding sequences of the cDNAs showed variable percentage homologies with those of other teleosts and vertebrate species. The predicted amino acid sequence shared 71% identity with rainbow trout and salmon, 90% with goldfish, 50% with eel and 94% with common carp in the mature protein region. The percentages of identity in the same region in comparison with bovine, porcine, rat, mouse, human and chicken were only 39, 42, 41, 40, 45 and 46%, respectively. TSH [beta] mRNA expression was found only in the pituitary tissue out of other tissues tested as testis, muscle, brain and heart. For the first time, thyrotropin releasing hormone (TRH) and thyroxine (T4) effects on pituitary TSH mRNA expression were tested in teleosts under in vitro conditions. TRH treatment on pituitary cells increased TSH [beta] mRNA level, while T4 treatment decreased TSH [beta] mRNA level. The present study provides a direct evidence, for the first time that TRH directly upregulates TSH [beta] gene expression in teleosts.

Cheng, J. and J. R. Malayer (1999). "Responses to stable ectopic estrogen receptor-beta expression in a rat fibroblast cell line." Molecular and Cellular Endocrinology **156**(1-2): 95.

<http://www.sciencedirect.com/science/article/B6T3G-3XM2M1R-C/2/be232578da87b1a8294f1275adeb9fb4>

To examine activity of estrogen receptor-beta (ER[beta]) independently of estrogen receptor-alpha (ER[alpha]), retrovirus-mediated gene transfer was used to insert rat ER[beta] into a rat fibroblast cell line (rat-1) that does not ordinarily express ER. Stable expression of ER[beta] in rat-1 cells was validated and then characterized by reverse-transcription polymerase chain-reaction (RT-PCR) analysis to examine the effects of estradiol (E2) treatment on expression of specific target mRNAs. Results were compared with rat-1 cells and a previously constructed rat-

1+ER[alpha] cell line. Progesterone receptor mRNA was not detected in rat-1 cells and was induced by E2 in both rat-1+ER[alpha] and rat-1+ER[beta] cells. Treatment with E2 resulted in an increased rate of cell proliferation ($P<0.05$) in rat-1+ER[alpha] cells, but not in rat-1 or rat-1+ER[beta] cells. Data confirm studies using transient ER expression demonstrating that ER[alpha] and ER[beta] have both discrete and overlapping activity within the same cell type in the presence of the same ligand.

Drews, R. T., R. A. Gravel, et al. (1992). "Identification of G protein [alpha] subunit mutations in human growth hormone (GH)- and GH/prolactin-secreting pituitary tumors by single-strand conformation polymorphism (SSCP) analysis." Molecular and Cellular Endocrinology **87**(1-3): 125.

<http://www.sciencedirect.com/science/article/B6T3G-47MCKGT-N0/2/5303840e55504f7a11c9cdae3daefaca>

We have applied the polymerase chain reaction (PCR) and single-strand conformation polymorphism analysis (SSCP) to detect activating mutations in the Gs[alpha] subunit gene, amplifying genomic DNA extracted from growth hormone (GH)- and GH/prolactin (PRL)-secreting human pituitary tumors. Of 15 tumors tested six contained mutations in the analyzed regions of the Gs[alpha]. SSCP analysis revealed band shift in exon 8 in four GH- and in one GH/PRL-secreting tumors, and in exon 9 in one GH/PRL-secreting tumor. Direct sequencing of PCR reaction products identified the mutations as R201-H, R201-S and R201-C in exon 8 and Q227-L in exon 9. These results show the efficacy of PCR/SSCP analysis in the detection of G protein mutations and extend the generalization that these sites are hot spots in tumor-inducing mutations.

Drury, K. C., M. C. Liu, et al. (2001). "Results on single cell PCR for Huntington's gene and WAVE(TM) product analysis for preimplantation genetic diagnosis." Molecular and Cellular Endocrinology **183**(Supplement 1): S1.

<http://www.sciencedirect.com/science/article/B6T3G-441N4PC-3/2/bb2f89492c2a90dbc09b03dbac3e71fc>

Triple repeat base pair amplification is the basis for a number of prevalent genetic diseases such as Huntington's, Fragile X, Myotonic Dystrophy and others. We have chosen to investigate the use of PCR to amplify a portion of the Huntington's gene in single cells in order to develop a clinical test system for preimplantation genetic diagnosis (PGD). Amplification of CAG triple repeat sequences poses difficulties due to resistance of GC melting for amplification. Special PCR modifications are necessary to carry out the amplification of GC rich areas found in most triple base pair expansions. We have used a modified polymerase chain reaction (PCR) protocol to amplify the expanded repeat sequence of the Huntington's gene with satisfactory efficiency. Detection of the amplified expanded CAG repeats is shown to be possible using both agarose gel electrophoresis and high definition denaturing high pressure liquid (DHPLC) chromatography. The incidence of allele dropout (ADO) is documented.

Faria, T. N., G. J. LaRosa, et al. (1998). "Characterization of genes which exhibit reduced expression during the retinoic acid-induced differentiation of F9 teratocarcinoma cells: involvement of cyclin D3 in RA-mediated growth arrest." Molecular and Cellular Endocrinology **143**(1-2): 155.

<http://www.sciencedirect.com/science/article/B6T3G-3TX5WGR->

In the presence of retinoic acid (RA), F9 murine teratocarcinoma cells differentiate into cells resembling the extra-embryonic endoderm of the early mouse embryo. Using differential hybridization, we have cloned and characterized six cDNAs corresponding to mRNAs that exhibit reduced expression in F9 cells following RA treatment. Two of these cDNAs encode novel genes (REX-2 and REX-3). The other isolated cDNAs encode genes that have been previously described in other contexts: 1-4 (cyclin D3); 2-10 (pyruvate kinase); 2-12 (glutathione S-transferase); and 2-17 (GLUT 3). The mRNA levels of these genes are reduced by RA or RA plus theophylline and cAMP (RACT) only after 48 h of treatment, and continue to decrease at 96 h. The half-lives of these mRNAs are not changed by RA treatment, indicating that these mRNAs may be regulated through a transcriptional mechanism. In isoleucine-deprived cells, which are growth arrested but do not differentiate, the steady state mRNA levels of genes Rex 2, Rex 3, pyruvate kinase and GLUT 3 are not reduced, in contrast to cyclin D3 and glutathione S-transferase. The expression of the REX-2, REX-3, pyruvate kinase, glutathione S-transferase and GLUT 3 genes is reduced by RACT to the same extent in F9 RAR[gamma]^{-/-} and RAR[alpha]^{-/-} lines as in F9-Wt. In contrast, cyclin D3 exhibits lower mRNA expression in F9 RAR[gamma]^{-/-} and RAR[alpha]^{-/-} stem cells, and this mRNA is not decreased by RACT treatment. Overexpression of cyclin D3 blocks the RA-induced growth arrest of F9 cells, indicating that the downregulation of this gene following RA treatment may constitute a necessary step in the cascade of events leading to growth inhibition by RA.

Findlay, I., P. L. Matthews, et al. (2001). "Using MF-PCR to diagnose multiple defects from single cells: implications for PGD." Molecular and Cellular Endocrinology **183**(Supplement 1): S5.

<http://www.sciencedirect.com/science/article/B6T3G-441N4PC-4/2/53ea46b097ff2d67319fceb84769d555>

Single cell genetic analysis is generally performed using PCR and FISH. Until recently, FISH has been the method of choice. FISH however is expensive, has significant misdiagnosis rates, can result in interpretation difficulties and is labour intensive making it unsuitable for high throughput processing. Recently fluorescent PCR reliability has increased to levels at or surpassing FISH whilst maintaining low cost. However, PCR accuracy has been a concern due to allelic dropout. Multiplex PCR can now increase accuracy by using multiple markers for each chromosome to firstly provide diagnosis if markers fail and/or secondly confirm diagnosis. We compare a variety of diagnostic methods and demonstrate for the first time a multiplex PCR system providing simultaneous diagnosis and confirmation of the major aneuploidy chromosomes (21, 18, 13) and sex as well as DNA fingerprint in single cells. We also discuss the implications of using PCR for aneuploidy screening in preimplantation genetic diagnosis.

Funkenstein, B., C. J. Bowman, et al. (2000). "Contrasting effects of estrogen on transthyretin and vitellogenin expression in males of the marine fish, *Sparus aurata*." Molecular and Cellular Endocrinology **167**(1-2): 33.

<http://www.sciencedirect.com/science/article/B6T3G-417ND93-4/2/6305660ecd7eae390a1bd41917c7fb26>

A partial cDNA encoding for the C-terminus of vitellogenin (VTG) was cloned from liver of *Sparus aurata* male treated with 17[beta]-estradiol (E2). E2 treatment of *S. aurata* males resulted in increased synthesis and secretion of VTG protein into the plasma, determined by a specific enzyme-linked immunosorbent assay (ELISA) in a time-dependent manner. While VTG mRNA

was induced by E2 treatment, transthyretin (TTR) mRNA levels were reduced. These data provide the first demonstration that estrogen exhibits contrasting effect on VTG and on TTR gene expression in teleosts.

Griswold, M. D., J.-S. Kim, et al. (2001). "Mechanisms involved in the homologous down-regulation of transcription of the follicle-stimulating hormone receptor gene in Sertoli cells." Molecular and Cellular Endocrinology **173**(1-2): 95.

<http://www.sciencedirect.com/science/article/B6T3G-42D2C93-9/2/b163d07c83f0dd8bb843999c3b1bf580>

The action of follicle-stimulating hormone (FSH) in spermatogenesis is regulated at a fundamental level by controlling the number of competent receptors present at the surface of Sertoli cells. By controlling the number of receptors, the cell is able to modulate the timing and magnitude of subsequent signal transduction in response to FSH. One mechanism of control is the down-regulation of the steady state levels of the FSH receptor gene after exposure to FSH or agents that stimulate or prolong the cAMP signal transduction cascade (homologous down-regulation) in Sertoli cells. The goals of this study were to examine possible mechanisms involved in the down-regulation of mRNA levels of this gene. Analysis of transcription and processing by a PCR-based assay showed that treatment of Sertoli cells with FSH caused at least a 50% reduction of hnRNA for the FSH receptor gene. Reporter genes controlled by 5' flanking sequences of the FSH receptor gene that were transiently transfected into Sertoli cells were not down-regulated. In electrophoretic mobility shift assays (EMSA), cAMP-inducible nuclear protein complex containing c-Fos formed on the activator protein-1/cAMP responsive element-like site located at -216 to -210 in the promoter of the rat FSH receptor gene. We concluded from this study that there was no evidence for the putative role of ICER in the down-regulation of the FSH receptor promoter. In addition, the FSH-induced down-regulation of the transcription of the FSH receptor gene in Sertoli cells was prevented by the treatment of Sertoli cells with trichostatin A prior to the addition of FSH. This experiment coupled with other observations suggested that the down-regulation may be mediated by changes in chromatin structure.

Haidan, A., S. R. Bornstein, et al. (2000). "Expression of adrenocortical steroidogenic acute regulatory (StAR) protein is influenced by chromaffin cells." Molecular and Cellular Endocrinology **165**(1-2): 25.

<http://www.sciencedirect.com/science/article/B6T3G-40XNWF2-4/2/4b0ba37ce58b6c2d8bf1b514467f77b3>

Cell-cell interactions are crucial role for the proper functioning of endocrine glands. We recently demonstrated that interactions of chromaffin and cortical cells are important for adrenocortical steroidogenesis. However, the molecular mechanisms have not been elucidated and it is unclear if this involves acute and/or chronic processes. By Northern analysis and the quantitative technique of TaqMan PCR we investigated whether chromaffin cells influence the regulation of StAR and the peripheral benzodiazepine receptor (PBR), both required for the rate-limiting step, the delivery of cholesterol to the inner mitochondrial membrane. StAR mRNA levels in bovine adrenocortical cells were increased by incubation with chromaffin cell-conditioned medium (CCM). Short-term treatment for 4 h resulted in a greater stimulation (229 $\pm$ 29% of basal, mean $\pm$ SEM) than did longer incubation times of 8 h and 5 days (159 $\pm$ 13 and 177 $\pm$ 24%). Neither short nor a long-term treatment affected PBR expression. Consistently, the major secretion of chromaffin cells, epinephrine dose-dependently stimulated StAR expression with no effect on PBR mRNA. In conclusion, adrenomedullary secretory products are not necessary for the maintenance of PBR expression but facilitate steroid biosynthesis by increasing StAR mRNA

expression and therefore can account for an ACTH-independent regulation of the rate-limiting step in steroidogenesis.

Hakola, K., P. Van der Boogaart, et al. (1997). "Recombinant rat follicle-stimulating hormone; production by Chinese hamster ovary cells, purification and functional characterization." Molecular and Cellular Endocrinology **127**(1): 59.

<http://www.sciencedirect.com/science/article/B6T3G-3RSG33W-7/2/aa6cb501a8cc437099d41b002908aefe>

In order to obtain homologous follicle-stimulating hormone (FSH) for in vivo and in vitro studies in the rat, rat recombinant (rec) FSH was produced in Chinese Hamster Ovary (CHO) cells. The synthesized rat recFSH was purified and subjected to physico-chemical and biological characterization, including a comparison with two rat pituitary (pit) and reference preparations (NIDDK-rFSH I-8 and NIDDK-rFSH-RP2) as well as with human recFSH (Org 32489). The molecular masses of rat recFSH and human recFSH were determined by SDS-polyacrylamide (SDS-PAGE) and were found to be similar, about 40 kD. The pI distribution of rat recFSH is similar to rat pitFSH, and slightly more acidic than human recFSH (3.6-5.6 vs 3.9-5.5, respectively) as determined by isoelectric focussing in immobilized pH gradients. Rat recFSH displayed dose-response curves parallel and in the same dose range as the rat pitFSH in receptor binding and in vitro bioassays. However, the in vivo activities of rat recFSH and rat pitFSH were 8824 and 3051 IU/mg, respectively, determined by the Steelman Pohley assay. Rat (pit and rec) and human FSH are very different. Human recFSH bound to both calf testicular membranes and CHO cells expressing the human FSH receptor (CHO hFSH-R) with about 10-fold higher affinity (K_a) than pituitary and recombinant rat FSH. In in vitro bioassays with immature rat Sertoli cells and CHO hFSH-R cells human recFSH was also about 10-fold more potent than the rat FSH preparations. In the in vitro bioassays with immature rat granulosa cells the difference was about 5-10-fold. These studies indicate that the receptor binding and in vitro activities of rat pitFSH and rat recFSH are similar. The differences in in vivo activity are probably due to the differences in glycosylation. The biological behaviour of rat FSH (pit and rec) is different from that of human FSH. Therefore, if the rat is used as a model for physiology of gonadotropic action, the results may be greatly influenced by the type (species) of hormone preparation used. The availability of homologous hormone preparations is therefore crucial.

Hofslis, E., L. Thommesen, et al. (2002). "Expression of chromogranin A and somatostatin receptors in pancreatic AR42J cells." Molecular and Cellular Endocrinology **194**(1-2): 165.

<http://www.sciencedirect.com/science/article/B6T3G-45V716K-3/2/db2ec71553ddcd75b5a876da3cdf987>

The exocrine pancreatic cell line AR42J is also known to display some neuroendocrine (NE) features. We have extended this fact by showing that AR42J cells express mRNA of chromogranin A (CgA), display immunoreactivity (IR) to CgA, and secrete its cleavage product pancreastatin. A sparse occurrence of typical NE secretion granules, together with only a faint IR to conventional NE markers, indicates that the NE cells are of a poorly differentiated type. CgA promoter reporter plasmid experiments showed that gastrin, epidermal growth factor, and phorbol 12-myristate 13-acetate, induce upregulation of CgA after 24 h. By RT-PCR, it was found that AR42J expresses all of the five subtypes of the somatostatin (SST) receptor (SSTR) family, except SSTR4. The existence of functional SSTRs was confirmed by showing that the SST analog octreotide could inhibit gastrin-induced proliferation. Thus, the AR42J cell line may function as a valuable experimental model to study the regulation of CgA and SSTRs in poorly differentiated NE tumor cells.

Ikeda, M., M. Taga, et al. (1997). "Gene expression of gonadotrophin-releasing hormone, but not its receptor, in human endometrium and decidua." Molecular and Cellular Endocrinology **135**(2): 165.

<http://www.sciencedirect.com/science/article/B6T3G-3S0DFD5-8/2/96dd212255b4864156bd06da63cc68a7>

Gonadotropin-releasing hormone (GnRH) has been reported to exist in extrahypothalamic tissues such as the placenta, gonads and mammary glands. While we have reported the presence of GnRH-mRNA in the rodent uterus, there have been no reports concerning gene expression of GnRH and its receptor (GnRH-R) in human endometrial tissue. In order to investigate the role of GnRH as a local regulator in the human endometrium, we examined the gene for GnRH and GnRH-R in non-pregnant endometrium and decidua of early pregnancy. Using reverse transcriptase-polymerase chain reaction (RT-PCR) and Southern blot analysis we found GnRH-mRNA but not GnRH-R-mRNA transcripts in the human endometrium and decidua at 7-9 weeks gestation. This is the first report that suggests GnRH gene expression in the human endometrium/decidua.

Jansen, E., P. H. Steenbergh, et al. (1991). "Identification of multiple transcription start sites in the human insulin-like growth factor-I gene." Molecular and Cellular Endocrinology **78**(1-2): 115.

<http://www.sciencedirect.com/science/article/B6T3G-47NWG0N-9K/2/f6720eacf0c9e1756cb77eb884c5a1de>

We have localized four transcription initiation sites in the human insulin-like growth factor-I (IGF-I) gene. Two transcription start sites were identified which result in a longer and shorter version of the leader derived from the known exon 1 of the IGF-I gene. Transcription starting at the upstream transcription initiation site results in a leader exon 1 of about 1155 nucleotides (nt), whereas transcription starting at the downstream initiation site results in a leader of about 240 nt. The majority of the transcripts initiate at the latter site. We further identified a region in the human IGF-I gene between exons 1 and 2, which shows a high degree of homology with the rat IGF-I leader exon 1B. By means of the polymerase chain reaction (PCR) we detected human IGF-I mRNAs containing this novel leader. The corresponding exon was designated exon 1B according to the rat IGF-I gene terminology. PCR and RNase protection analyses identified two transcription start sites within this alternative leader exon 1B. Transcription initiated at the most upstream start site results in a leader of about 750 nt, whereas transcription starting at the downstream site is heterogeneous, resulting in leaders of 65-75 nt long. No consensus TATA-box or AT-rich regions are present immediately upstream of all four transcription start sites identified, nor are these regions particularly GC-rich. The IGF-I gene is known to be expressed differentially in a tissue- and development-specific fashion. Differential activation of multiple promoters could very well play a crucial role in IGF-I gene regulation.

Kim, S., H.-J. Shin, et al. (2004). "Genistein enhances expression of genes involved in fatty acid catabolism through activation of PPAR[alpha]." Molecular and Cellular Endocrinology **220**(1-2): 51.

<http://www.sciencedirect.com/science/article/B6T3G-4CHGD3V-3/2/60694f877fd81e42df72e93a40040744>

Although evidences are emerging that dietary isoflavones have beneficial effects in treatment of hyperlipidemia and cardiovascular diseases, the underlying molecular mechanism has not yet been extensively characterized. In this report, we showed that genistein, one of the major isoflavones, increased expression of genes involved in lipid catabolism such as carnitine palmitoyltransferase 1, liver form (CPT1L) in HepG2 cells, when assayed by real-time reverse-transcriptase polymerase chain reactions as well as Western blotting analysis. The increase in mRNA-level of CPT1L after genistein treatment was not changed in the presence of ICI182780, a potent inhibitor of estrogen receptor, suggesting that this effect of genistein was estrogen receptor-independent. Since these genes involved in fatty acid catabolism are considered putative downstream target genes of peroxisome proliferators-activated receptor [alpha] (PPAR[alpha]), we examined whether expression of PPAR[alpha] was modulated by genistein treatment. Interestingly, genistein induced expression of PPAR[alpha] at both mRNA- and protein-level. Further, genistein activated transcriptional activity of PPAR[alpha], when determined by reporter gene analysis, suggesting genistein as a potential ligand for PPAR[alpha]. Taken together, this study provides a picture of the regulatory action of genistein, as an activator of PPAR[alpha] in fatty acid catabolism and potential use of genistein as lipid-lowering agent.

Koedam, J. A., J. J. Smink, et al. (2002). "Glucocorticoids inhibit vascular endothelial growth factor expression in growth plate chondrocytes." Molecular and Cellular Endocrinology **197**(1-2): 35.

<http://www.sciencedirect.com/science/article/B6T3G-46WWBFP-1/2/6a112916f6846868ff41484b949a66d8>

Vascular endothelial growth factor (VEGF) plays an essential role in angiogenesis in the growth plate and ultimately in regulating endochondral ossification. Since longitudinal bone growth is often disturbed in children who are treated with glucocorticoids, we investigated the effects of dexamethasone on VEGF expression by epiphyseal chondrocytes. Cells were cultured from tibial growth plates of neonatal piglets. Using Northern blotting and RT-PCR techniques, the chondrocyte-specific markers aggrecan, collagen II and CD-RAP were detected. Also the glucocorticoid receptor (GR) was expressed. VEGF protein secreted from these cells was examined by ELISA and Western immunoblotting. The VEGF121 and VEGF165 isoforms were detected in the supernatant. As determined by RT-PCR, all three major mRNA splice variants were produced, including the species encoding VEGF189. Dexamethasone (100 nM) inhibited both protein and mRNA expression by approximately 45%. Hydrocortisone (cortisol) and prednisolone also inhibited VEGF secretion, but they were less active than dexamethasone. The inhibitory actions of dexamethasone were almost completely blocked by the GR antagonist Org34116, indicating that the GR mediates these actions. Degradation of the VEGF mRNA was not accelerated by dexamethasone. Therefore, a transcriptional mechanism seems likely. Downregulation of this important growth factor could lead to disruption of the normal invasion of blood vessels in the growth plate, which could contribute to disturbed endochondral ossification and growth.

Koehorst, S. G. A., J. J. Cox, et al. (1994). "Functional analysis of an alternatively spliced estrogen receptor lacking exon 4 isolated from MCF-7 breast cancer cells and meningioma tissue." Molecular and Cellular Endocrinology **101**(1-2): 237.

<http://www.sciencedirect.com/science/article/B6T3G-47NWGNK-JP/2/bfaf52e828405223ab0ccf311aea2ba2>

An alternatively spliced mRNA coding for a variant estrogen receptor (ER) missing exon 4 (ER [Delta]4) was detected in the breast tumor cell line MCF7 and meningioma tissue by using the reversed transcriptase PCR technique. The trans-activational properties of this mutant ER were

assessed in embryo carcinoma P19EC and human choriocarcinoma JEG3 cells by co-transfection of the ER [Delta]4 expression vector with an oxytocin promoter construct containing an estrogen-responsive element. ER [Delta]4 did not trans-activate the oxytocin promoter in either a hormone-dependent or -independent manner. Co-transfection of ER [Delta]4 together with the wtER did not show any interference of ER /gDA4 on the stimulation of the oxytocin promoter by the wtER. ER [Delta]4 was translated in vitro. Its capacity to bind estradiol, and the binding of the variant to a synthetic estrogen-responsive element were compared to those of the wild-type receptor. ER [Delta]4 did not bind to a synthetic estrogen-responsive element, nor did it bind estradiol. Hence, ER [Delta]4 appears to be a silent variant and we speculate that it is without any role in tumor progression.

Krisinger, J., T. Setoyama, et al. (1994). "Expression of calbindin-D9k in the early pregnant rat uterus: Effects of RU 486 and correlation to estrogen receptor mRNA." Molecular and Cellular Endocrinology **102**(1-2): 15.

<http://www.sciencedirect.com/science/article/B6T3G-47MCKW8-TX/2/ddf86b897f940cc8bc6e67b966a5fe39>

Calbindin-D9k (CaBP-9k) is a calcium binding protein expressed at high levels in the rat uterus. The CaBP-9k gene carries an estrogen response element which is involved in the steroid hormone regulation of the gene during the estrous cycle and gestation. The present study was aimed at determining expression of the gene during the first half of pregnancy and to assess the role of progesterone (P4) and the estrogen receptor (ER). Expression of CaBP-9k mRNA was determined by Northern blot analysis during the first 10 days of pregnancy. On pregnancy day 1 (P1), CaBP-9k mRNA levels were relatively high. On P2, 3 and 5 CaBP-9k mRNA decreased to the detection limit using 10 [mu]g total RNA probed with a random primed cDNA. On P10, CaBP-9k transcripts began to reappear at levels of about 30% of P1. Expression of [beta]-actin mRNA displayed a continuous increase during this period with a rapid rise of 240% between P2 and P3. The typical increase of P4 accompanied by moderate changes of estradiol (E2) was determined in serum of experimental groups. When RU 486 at 10 mg/kg was administered as a single s.c. injection on P3, the CaBP-9k down-regulation was rapidly interrupted and mRNA expression became extremely high. The effect was seen maximally at 24 h post injection and was maintained at 48 and 72 h. Expression of [beta]-actin mRNA was increased only moderately at 24 h and was unchanged at 48 and 72 h. Serum P4 remained unaffected by the treatment and E2 displayed a slight increase. When ER mRNA was quantified by reverse transcription/PCR techniques a more than 300% increase was detected in the RU 486 treated rats. These data implicate that P4 in early pregnancy is responsible for down regulation of the CaBP-9k gene in uterus via an indirect effect involving the expression of the estrogen receptor.

Lethimonier, C., M. Tujague, et al. (2002). "Peptide insertion in the DNA-binding domain of fish glucocorticoid receptor is encoded by an additional exon and confers particular functional properties." Molecular and Cellular Endocrinology **194**(1-2): 107.

<http://www.sciencedirect.com/science/article/B6T3G-46HBR4P-1/2/d5f55fa3e8600c50a139216cecb7b947>

The trout glucocorticoid receptor (rtGR) contains an additional sequence of nine amino acids located between the two zinc fingers of the DNA-binding domain (DBD) (Endocrinology 136 (1995) 3774). Polymerase chain reaction on trout genomic DNA and sequencing were performed in the DBD region, demonstrating that this peptide is encoded by an additional exon of 27 nucleotides between the two exons encoding the two zinc fingers of other nuclear receptors. This additional sequence in the rtGR confers a better binding affinity of the receptor to a single GRE,

as shown by gel shift experiments with GST-DBDrtGR fusion proteins, deleted or not of the nine amino acids ([Delta]9). This higher affinity is correlated with a higher constitutive transcriptional activity of the receptor on a reporter gene driven by a single GRE, but not with the ligand-induced transcriptional activity. Nevertheless, on a double GRE, the wild type and rtGR-[Delta]9 are equally active on both constitutive or dexamethasone-induced transcriptional activity. This original DBD structure could have emerged during evolution such as to allow better regulation of glucocorticoid dependent genes in relation to the large spectrum of cortisol physiological functions in fish.

Ma, J. F., L. Nonn, et al. (2004). "Mechanisms of decreased Vitamin D 1[alpha]-hydroxylase activity in prostate cancer cells." Molecular and Cellular Endocrinology **221**(1-2): 67.

<http://www.sciencedirect.com/science/article/B6T3G-4CDS5V6-2/2/665db4b9a84f85b9b341edb60a80fe6f>

Vitamin D 1[alpha]-hydroxylase (1[alpha](OH)ase), which converts the circulating prohormone 25-hydroxyvitamin-D3 (25(OH)D3) to the active 1[alpha]-25-dihydroxyvitamin-D3 (1,25(OH)2D3), is present in normal prostatic epithelium. However, prostate cancer cells, both primary cultured cells and cell lines, have greatly decreased activity of 1[alpha](OH)ase and are therefore resistant to the tumor suppressor activity of circulating 25(OH)D3. We quantitated 1[alpha](OH)ase mRNA and protein levels to investigate mechanism(s) responsible for decreased 1[alpha](OH)ase enzymatic activity in prostate cancer. Prostate cancer cell lines had low 1[alpha](OH)ase mRNA levels. Primary prostate cell cultures derived from normal and cancer tissues had equivalent levels of 1[alpha](OH)ase RNA and protein. Equivalent 1[alpha](OH)ase protein levels were observed in prostate tissue sections containing normal and malignant cells. The protein levels of hsc70, whose homolog intracellular Vitamin D binding protein (IDBP-1) facilitates delivery of 25(OH)D3 to 1[alpha](OH)ase in monkey cells, were equivalent in the normal and cancer cells. Equivalent activity in normal and cancer cells of Vitamin D 24-hydroxylase, a mitochondrial enzyme that also uses 25(OH)D3 as a substrate, further ruled out lack of access to substrate as a basis for low activity of 1[alpha](OH)ase in cancer cells. We conclude that diminished 1[alpha](OH)ase activity in prostate cancer cell lines is through decreased gene expression, whereas decreased activity in primary cultures and tissues is post-translational.

Madsen, M. W., B. E. Reiter, et al. (1995). "Differential expression of estrogen receptor mRNA splice variants in the tamoxifen resistant human breast cancer cell line, MCF-7/TAMR-1 compared to the parental MCF-7 cell line." Molecular and Cellular Endocrinology **109**(2): 197.

<http://www.sciencedirect.com/science/article/B6T3G-3XVH3H7-B/2/0c777ad193865591daf3c2b56d1d5b9f>

Breast cancer patients with an estrogen receptor (ER) positive tumor can be treated with the anti-estrogen tamoxifen, but development of anti-estrogen resistance is a serious problem. We have analyzed a tamoxifen resistant human breast cancer cell line MCF-7/TAMR-1 for alterations in ER which might explain the tamoxifen resistance. The MCF-7/TAMR-1 cells expressed both wildtype ER mRNA and protein, and by RT-PCR we were able to clone ER cDNAs corresponding to the following mRNA splice variants: ER[Delta]E2, ER[Delta]E4, ER[Delta]E5, ER[Delta]E7 and a new double splice variant lacking both exon 4 and 7 (ER[Delta]E4,7). The existence of the ER[Delta]E4,7 variant was confirmed by RNase protection assay. Semi-quantitative RT-PCR revealed that ER[Delta]E2 mRNA was expressed at a higher level in MCF-7/TAMR-1 cells, whereas the ER[Delta]E5 mRNA was expressed at a significantly lower level in MCF-7/TAMR-1 cells compared with MCF-7 cells. The differential expression of the two ER mRNA splice variants indicates that they may be involved in anti-estrogen resistance, although the present knowledge

of their biological function does not provide us with an explanation.

Mantovani, G., S. Corbetta, et al. (2001). "Absence of thyroid transcription factor-1 expression in human parathyroid and pituitary glands." Molecular and Cellular Endocrinology **182**(1): 13.

<http://www.sciencedirect.com/science/article/B6T3G-44GGB9K-2/2/bc435508e32da8b816c81ce744d54dc4>

Thyroid transcription factor-1 (TTF-1), a tissue-specific nuclear transcription factor involved in the embryogenesis and differentiation of human thyroid, lung and brain, has been recently identified in other rat tissues, including parafollicular C cells and parathyroid chief cells. Based on this distribution, a possible role for this factor in calcium homeostasis has been suggested. This study investigated the presence of TTF-1 transcripts and protein in human tissues expressing the calcium sensing receptor (CaSR). Using a RT-PCR technique, complemented by Southern blot analysis, TTF-1 expression was detected in human C cells (two medullary thyroid carcinomas), but not in normal and adenomatous (four adenomas and three hyperplasia) parathyroid, and normal and adenomatous (six adenomas) pituitary tissues. CaSR was expressed in all samples. The absence of expression was confirmed by Western blot. In contrast to previous studies in the rat, this study demonstrates the absence of TTF-1 transcripts in the human adult parathyroid and pituitary glands, although a role for this factor during the ontogeny of these organs cannot be excluded.

Miki, Y., T. Suzuki, et al. (2005). "Steroid and xenobiotic receptor (SXR), cytochrome P450 3A4 and multidrug resistance gene 1 in human adult and fetal tissues." Molecular and Cellular Endocrinology **231**(1-2): 75.

<http://www.sciencedirect.com/science/article/B6T3G-4F9SY7K-1/2/292d2858cd639edba9114f6329864bec>

The steroid and xenobiotic receptor (SXR) has been demonstrated to play an important role in the regulation of the cytochrome P450 3A4 gene (CYP3A4) and multidrug resistance gene 1 (MDR1) by both endogenous and xenobiotic substrates. SXR and its rodent ortholog PXR exhibit marked differences in their ability to be activated by xenobiotic inducers. This suggests that results obtained by rodent models may not always accurately predict responses to the same compounds in humans. SXR expression was demonstrated in the human liver and intestine, but its systemic distribution remains unknown. Therefore in this study, we first characterized the expression of SXR and its target genes CYP3A4, and MDR1 in human adult and fetal tissues using quantitative RT-PCR, immunoblotting, and combined laser capture microscopy and RT-PCR analysis. SXR mRNA and protein are expressed in adult and fetal liver, lung, kidney, and intestine. There is a close association between the expression of SXR and its target genes in all of the tissues examined. The amounts of SXR mRNA in the liver and intestine reached maximal levels in young adults (15-38 years old) and then subsequently decreased to less than half of the maximal levels with aging. These findings demonstrated age-related differences in the body's capacity to metabolize steroids and xenobiotic compounds and suggest an important role for SXR and its target genes, CYP3A4 and MDR1 in this process.

Muthukumar, G. and J. Lenard (1991). "A preproinsulin-like pseudogene from *Neurospora crassa*." Molecular and Cellular Endocrinology **82**(2-3): 275.

<http://www.sciencedirect.com/science/article/B6T3G-47MCJDT-50/2/f59d95f4dd3cd08bdd07c320e55251a4>

A segment of DNA was amplified from the *Neurospora crassa* genome by the polymerase chain reaction using several oligonucleotides coding for highly conserved domains in proinsulin as primers and probe. A genomic clone corresponding to this segment was isolated and the nucleotide sequence was determined. The deduced amino acid sequence of a part of this segment bears remarkable resemblance to preproinsulin, but lacks several requirements for transcription or translation and must therefore be considered to be a pseudogene.

Nicod, J., B. Dick, et al. (2004). "Mutation analysis of CYP11B1 and CYP11B2 in patients with increased 18-hydroxycortisol production." *Molecular and Cellular Endocrinology* **214**(1-2): 167.

<http://www.sciencedirect.com/science/article/B6T3G-4BBHDY4-7/2/05a261e86d2a04511fcf29425a720bce>

Background: In patients with glucocorticoid remediable aldosteronism (GRA), a rare hypertensive disorder caused by the presence of a chimeric aldosterone synthase (CYP11B2) and 11[beta]-hydroxylase (CYP11B1) gene, high level of urinary 18-hydroxycortisol (18OHF) excretion are observed. In some patients with hypertension, increased urinary 18OHF secretion is also found in the absence of the hybrid CYP11B1/CYP11B2 gene. We hypothesised that gene variants of CYP11B1 or CYP11B2 may be linked to this abnormal glucocorticoid production. Methods: The urinary steroid profile was analysed by gas chromatography/mass spectrometry in 429 hypertensive patients and 98 (23%) thereof tested positive for increased 18OHF excretion. After correction for total cortisol excretion, 12 subjects showed an abnormally high 18OHF excretion. For genotyping DNA was obtained from six of these patients. All were tested negative for the hybrid CYP11B1/CYP11B2 gene and were further analysed for mutations in all exons and promoter regions of both CYP11B1 and CYP11B2 by single strand conformation polymorphism (SSCP) and sequencing when appropriate. Results: The genetic analysis of the two genes revealed the presence of nine molecular variants in CYP11B2 and three in CYP11B1. In addition to published polymorphic sites, we identified two new variants in CYP11B2 but no new variants in CYP11B1. The newly identified CYP11B2 mutations are a C/T single nucleotide exchange located in the first intron and a double nucleotide exchange at the 3'-splice site of exon 8. The mutated sequence corresponds to the sequence of CYP11B1 indicating a gene conversion. This suggests that the mutant is not likely to affect splicing. Thus, none of the genetic variants identified explains the high urinary excretion of 18OHF. Conclusions: We present here a complete method for the genetic analysis of the CYP11B1 and CYP11B2 genes. By this method we could not identify genetic variants responsible for a GRA-like phenotype. The presence of high levels of 18OHF should not be used alone as a diagnosis tool for GRA.

Nilsson, E., V. Doraiswamy, et al. (2001). "Expression and action of transforming growth factor beta (TGF[beta]1, TGF[beta]2, TGF[beta]3) in normal bovine ovarian surface epithelium and implications for human ovarian cancer." *Molecular and Cellular Endocrinology* **182**(2): 145.

<http://www.sciencedirect.com/science/article/B6T3G-43YSY4C-1/2/5db8b392819a86b3a0abdfc59c8e159b>

The majority of ovarian tumors are derived from the single layer of epithelial cells on the surface of the ovary termed the ovarian surface epithelium (OSE). Stromal cell-OSE interactions are postulated to be an important aspect of normal OSE biology and the biology of ovarian cancer. Transforming growth factor beta (TGF[beta]) has been shown to often be a mesenchymal cell-

derived growth factor that mediates stromal cell-epithelial cell interactions in a variety of different tissues. The current study investigates the expression and action of TGF[β] isoforms (TGF[β]1, TGF[β]2, and TGF[β]3) in OSE and the underlying stroma in both normal bovine and human tumor tissues. Normal bovine ovaries are similar to human ovaries and are used as a model system to investigate normal OSE and stromal cell functions. All three TGF[β] isoforms and their receptor, transforming growth factor β receptor type II (TGF[β]RII), proteins were found to be detected in the OSE from normal bovine ovaries using immunohistochemistry. Ovarian stromal tissue also contained positive immunostaining for TGF[β] isoforms and TGF[β]RII. RNA was collected from normal bovine OSE and ovarian stromal cells to examine TGF[β] gene expression. TGF[β]1, TGF[β]2, and TGF[β]3 transcripts were detected in both freshly isolated and cultured bovine OSE and stromal cells by a sensitive quantitative polymerase chain reaction assay. TGF[β]1 and TGF[β]2 mRNA levels were found to be present at similar levels in freshly isolated OSE and stroma. Interestingly, TGF[β]3 mRNA levels were significantly higher in freshly isolated OSE than stromal cells. All but TGF[β]3 mRNA in OSE increased when the cells were cultured. Observations indicate that normal bovine OSE and stroma cells express the three TGF[β] isoforms in vivo and in vitro. Human ovarian tumors from stage II, stage III and stage IV cases were found to express TGF[β]1, TGF[β]2, TGF[β]3 and TGF[β]RII protein primarily in the epithelial cell component by immunohistochemistry analysis. The stromal cell component of the human ovarian tumors contained little or no TGF[β] or TGF[β]RII immunostaining. TGF[β] actions on bovine OSE and stromal cells were also investigated. TGF[β] was found to inhibit the growth of OSE, but not stromal cells. To further examine the actions of TGF[β] on OSE, the expression of two growth factors previously shown to be expressed by OSE were analyzed. TGF[β]1 was found to stimulate the expression of both keratinocyte growth factor (KGF) and kit ligand/stem cell factor (KL) by bovine OSE. Therefore, TGF[β] actions on OSE will likely promote a cascade of cell-cell interactions and cellular responses involving multiple growth factors. The effects of regulatory agents on TGF[β] expression by the bovine OSE were examined. Transforming growth factor α (TGF[α]) stimulated TGF[β]1 expression, TGF[β]1 stimulated TGF[β]2 expression, and follicle stimulating hormone (FSH) stimulated TGF[β]3 expression. These results demonstrate that TGF[β] isoforms are regulated differently by the regulatory agents tested. In summary, all the TGF[β] isoforms are differentially expressed by the OSE and TGF[β] appears to have an important role in regulating OSE and possibly stromal-OSE interactions. A complex network of endocrine and paracrine interactions appears to influence the expression and actions of TGF[β] on OSE. Abnormal expression and/or action of TGF[β] is postulated to in part be involved in the onset and progression of ovarian cancer.

Nilsson, E. E., P. Kezele, et al. (2002). "Leukemia inhibitory factor (LIF) promotes the primordial to primary follicle transition in rat ovaries." *Molecular and Cellular Endocrinology* **188**(1-2): 65.

<http://www.sciencedirect.com/science/article/B6T3G-44PVPVK-3/2/c32f6ea6ce39c2dec46ef5d86a13a33b>

In a sexually mature female, primordial follicles continuously leave the arrested pool and undergo the primordial to primary follicle transition. The oocytes increase in size and the surrounding squamous pre-granulosa cells become cuboidal and proliferate to form a layer of cuboidal cells around the growing oocyte. This development of the primordial follicle commits the follicle to undergo the process of folliculogenesis. When the available pool of primordial follicles is depleted reproductive function ceases and humans enter menopause. The current study examines whether leukemia inhibitory factor (LIF) promotes the primordial to primary follicle transition that initiates follicular development. Ovaries from 4 day-old rats were cultured in the absence or presence of LIF or neutralizing antibody to LIF. LIF treatment increased the proportion of follicles that initiated the primordial to primary follicle transition to 59%, compared to 45% in untreated cultured ovaries. The ability of LIF to induce primordial follicle development was enhanced to

greater than 75% by the presence of insulin in the culture medium. Anti-LIF neutralizing antibody reduced the proportion of spontaneous developing primordial follicles. Immunocytochemical studies demonstrated higher levels of LIF protein in the granulosa and surrounding somatic cells of primordial and primary follicles compared to the oocyte. In contrast, later pre-antral and antral stage follicles showed LIF expression primarily in the oocyte. In granulosa and theca cell cultures LIF had no effect on cell proliferation. However, LIF treatment did increase expression of Kit ligand (KL) mRNA in cultured granulosa cells. KL has been shown to promote ovarian cell growth and induce primordial follicle development. LIF induction of KL expression may be involved in the actions of LIF to promote primordial to primary follicle transition. In summary, LIF treatment increased the primordial to primary follicle transition in cultured ovaries and LIF may interact with KL to promote primordial follicle development.

Nilsson, E. E. and M. K. Skinner (2004). "Kit ligand and basic fibroblast growth factor interactions in the induction of ovarian primordial to primary follicle transition." Molecular and Cellular Endocrinology **214**(1-2): 19.

<http://www.sciencedirect.com/science/article/B6T3G-4BDM4B2-7/2/e91c96ad2af742c2a4c6aa16f7046f49>

Ovulated eggs during a female's reproductive life are derived from a pool of primordial follicles arrested in prophase of the first meiotic division. When follicles leave the resting pool they undergo a primordial to primary follicle transition and will grow and develop until either ovulation occurs or follicles undergo atresia. Several growth factors have been implicated as acting locally within the ovary to regulate the primordial to primary follicle transition. How these growth factors may interact and cooperate to perform this vital function remains to be elucidated. The objective of the current study is to investigate interactions between kit ligand (KL) (i.e. stem cell factor) and basic fibroblast growth factor (bFGF) that promote the primordial to primary follicle transition in rat ovaries. Ovaries were removed from 4-day-old rat pups and cultured for 2 weeks with KL alone or with KL and a neutralizing antibody against bFGF. The ability of KL treatment to increase primordial follicle transition was blocked with a bFGF neutralizing antibody. In addition, ovary cultures were treated with bFGF alone or with bFGF and an anti-c-kit receptor antibody which blocks KL signaling. The ability of bFGF treatment to increase primordial follicle transition was blocked with an anti-c-kit receptor antibody. Observations indicate that both KL and bFGF must be active in order to optimally promote the changes that occur in oocytes, granulosa cells, and stromal/interstitial cells when primordial follicles initiate development. Cultured ovaries were treated with either KL or bFGF for 3 days and then bFGF and KL mRNA expression levels in the whole ovary were measured. KL was not found to regulate bFGF expression. In contrast, bFGF treatment was found to increase KL mRNA expression in cultured ovaries. These observations suggest that one function of the oocyte-derived bFGF is to increase the granulosa derived KL expression and that both KL and bFGF are required to optimally promote primordial to primary follicle transition. Elucidating the cell-cell interactions that mediate this network of specific locally derived growth factors is critical to understanding the physiology of the primordial to primary follicle transition.

Nogues, N., E. Magnan, et al. (1998). "Expression of a fusion gene consisting of the mouse growth hormone-releasing hormone gene promoter linked to the SV40 T-antigen gene in transgenic mice." Molecular and Cellular Endocrinology **137**(2): 161.

<http://www.sciencedirect.com/science/article/B6T3G-3T14BKT-8/2/289e12d7bb6e2c9477eeec20189f618f>

Limited information is available concerning the regulation of growth hormone-releasing hormone

(GHRH) gene expression in the hypothalamus, largely because of the lack of a suitable cellular model. In an attempt to immortalize hypothalamic GHRH-producing neurons, we have generated a transgenic mouse model which expresses the simian virus 40 (SV40) T-antigen gene (Tag) under the control of the GHRH gene promoter. The transgene contains [ap]5 kb of mouse GHRH gene sequences, including 3.5 kb of the 5'-flanking region, the entire hypothalamic exon 1 and 1.5 kb of intron 1, fused to the SV40 Tag gene. This construct was microinjected into fertilized oocytes. Fourteen of 96 mice born had integrated the transgene. These mice were fertile and showed no signs of central or peripheral tumors. The pattern of expression of the SV40 Tag gene was analyzed in four different transgenic lines by RT-PCR. The tissues tested include: hypothalamus, pituitary, cortex, cerebellum, spinal cord, adrenal, testis, spleen and lung. Transgene expression was consistently detected in the hypothalamus of all lines. In addition, SV40 Tag expression was also detected in the hypothalamus by Northern blot analysis in two of the transgenic lines. SV40 Tag expression was also detected in the testis of all transgenic lines by RT-PCR. This result was not expected since the GHRH gene sequences present in the transgene do not include the testis-specific transcription initiation site previously described. This suggests that GHRH gene expression in the mouse testis can be directed by regulatory sequences located downstream of the testis specific transcription start site. We conclude that the promoter region of the GHRH gene included in this construct contains the regulatory elements necessary to drive hypothalamic and testis expression in vivo. In addition, all mice from one of the transgenic lines developed cataracts in both eyes. SV40 Tag expression was detected not only in eyes with cataracts, but also, to a lesser extent, in eyes from other transgenic lines. Furthermore, the endogenous GHRH gene was found to be expressed in the eyes of normal mice.

Okazaki, T., M. Nishibori, et al. (2003). "LH reduces proliferative activity of cumulus cells and accelerates GVBD of porcine oocytes." Molecular and Cellular Endocrinology **209**(1-2): 43.

<http://www.sciencedirect.com/science/article/B6T3G-49SFKB2-2/2/cbf44e02924441edf68c87c37594aa4b>

It has been reported that LH receptor (LHR) mRNA is not detected in cumulus cells of porcine cumulus-oocyte complexes (COCs) just after collection from small antral follicles. The present study showed that the formation of LHR in cumulus cells was up-regulated by the cultivation with 20 ng/ml FSH. When the newly synthesized receptors were stimulated by 1.0 [mu]g/ml LH, significantly higher levels of cAMP and progesterone production in cumulus cells were observed as compared with those of COCs cultured with FSH. A loss of proliferative activity of cumulus cells was induced by the additional LH to FSH-containing medium; however, the inhibitory effect was overcome by progesterone receptor antagonist RU486. Furthermore, the addition of LH also accelerated ongoing GVBD in cumulus cells-enclosed oocytes. These results revealed that during in vitro meiotic maturation of porcine COCs, progesterone secreted by FSH- and LH-stimulated cumulus cells reduced proliferative activity of cumulus cells; the changes of cumulus cells might be involved in inducing meiotic resumption of porcine oocytes.

Pan, B., K. Sengoku, et al. (2004). "Differential expression of heparin-binding epidermal growth factor-like growth factor in the rat ovary." Molecular and Cellular Endocrinology **214**(1-2): 1.

<http://www.sciencedirect.com/science/article/B6T3G-4BHCSPV-5/2/7320a52c8d11166f511f6aa8a62e4c6b>

We investigated the expression of heparin-binding epidermal growth factor-like growth factor (HB-EGF) and its receptors in the rat ovary to define the role of HB-EGF in the ovarian function. The expression pattern of HB-EGF mRNA and protein were studied by semi-quantitative RT-PCR and immuno-histochemistry using an antibody that was specifically stained for the precursor form of

HB-EGF in naturally cycling rats and immature pseudo-pregnant rat models. The immuno-histochemical study showed that in naturally cycling rats, HB-EGF was expressed in most granulosa cells of early follicles and all the developing follicles but not in preovulatory follicles. This was supported by the semi-quantitative RT-PCR results in that the lowest level of HB-EGF mRNA during the estrous cycle was found in the evening of proestrous when the HB-EGF negative preovulatory follicles were most prominent. The results suggest that HB-EGF might be a mitogen for granulosa cells and down regulation of its expression may be necessary for the final maturation of follicles. In corpora lutea, luteal cells of older generation stained stronger than those of younger generation. Moreover, luteal cells of late luteal phase stained stronger than those of the mid and early luteal phases in the immature pseudo-pregnant rat models, indicating that the precursor form may be associated with death of luteal cells. Finally, of the two cognate receptors for HB-EGF, erbB1 was expressed in the rat ovary, but erbB4 was specifically not expressed in this organ. The spatial and temporal pattern of HB-EGF expression suggest that HB-EGF may an important local regulator of ovarian function and structure.

Parrott, J. A., V. Doraiswamy, et al. (2001). "Expression and actions of both the follicle stimulating hormone receptor and the luteinizing hormone receptor in normal ovarian surface epithelium and ovarian cancer." Molecular and Cellular Endocrinology **172**(1-2): 213.

<http://www.sciencedirect.com/science/article/B6T3G-427JWGY-1W/2/019fab2cefdb0e017de6b393c2921817>

The ability of gonadotropins to act on and regulate normal ovarian surface epithelial (OSE) cells and ovarian cancer cells was investigated. Bovine OSE was used as a model to study normal OSE. Results demonstrate that follicle stimulating hormone (FSH) and the luteinizing hormone (LH) like molecule, human chorionic gonadotropin (hCG), can both stimulate (3H)-thymidine incorporation into DNA in normal OSE cells. Similar results were obtained using either purified hormones or recombinant human hormones. A human ovarian cancer cell-line OCC1 was also stimulated to grow in response to FSH and hCG, but the growth of a different human ovarian cancer cell-line SKOV3 was not affected. In addition to effects on cell growth, gonadotropins also stimulated growth factor expression. Both FSH and hCG stimulated steady state levels of keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), and kit ligand (KL) mRNA in OSE cells. Previously, KGF, HGF, and KL have been shown to stimulate OSE growth. Both follicle stimulating hormone receptor (FSHR) and luteinizing hormone receptor (LHR) were observed in OSE cells by Northern blot analysis. Reverse transcription polymerase chain reaction (RT-PCR) analysis was performed on fresh and cultured OSE cells. Normal OSE was found to express FSHR and LHR both in vivo and in vitro. The PCR reaction products were sequenced and found to provide a 100% homology with the bovine gonadotropin receptor sequences previously reported. FSHR and LHR transcripts were also detected in gonadotropin responsive OCC1 cells, but not in the gonadotropin insensitive SKOV3 cells. Observations support the hypothesis that gonadotropins may influence some ovarian cancers. In summary, the current study demonstrates the novel observation that both the FSHR and LHR are expressed by bovine OSE and selected ovarian cancers. Interestingly, the actions of FSH and LH to promote OSE growth may in part be mediated indirectly through an elevation in the expression of autocrine growth factors (KGF, HGF, and KL). Ovarian cancer is more common in conditions with elevated gonadotropins such as post-menopausal women. Therefore, gonadotropin actions on the OSE are postulated to be a potential factor in the onset and progression of some ovarian cancers.

Parrott, J. A., G. Kim, et al. (2000). "Expression and action of keratinocyte growth factor (KGF) in normal ovarian surface epithelium and ovarian cancer." Molecular and Cellular Endocrinology **167**(1-2): 77.

<http://www.sciencedirect.com/science/article/B6T3G-417ND93-8/2/7f5c9c567b7ee804149815b5eaf57aa9>

The current study investigates the expression and action of keratinocyte growth factor (KGF) in normal ovarian surface epithelium (OSE) and ovarian cancer tissues. Ovarian tumors are primarily derived from the OSE. KGF is a mesenchymal cell-derived growth factor that mediates stromal cell-epithelial cell interactions in a variety of different tissues. Human ovarian tumors from borderline, stage I and stage III cases were found to express KGF protein in the epithelial cell component by immunocytochemical analysis. The stromal cell component of human ovarian tumors contained little or no KGF immunostaining. Normal bovine ovaries have similarities to human ovaries and are used as a model system to investigate normal OSE functions. KGF protein was detected in the OSE from normal human and bovine ovaries by immunocytochemistry. Ovarian stromal tissue contained light but positive KGF immunostaining. RNA was collected from normal bovine OSE and ovarian stromal cells to examine KGF gene expression. KGF transcripts were detected in cultured OSE and stromal cells by Northern blot analysis. In order to examine and quantitate KGF gene expression in freshly isolated versus cultured tissues, a sensitive quantitative RT-PCR assay for KGF was utilized. KGF gene expression was found to be high in freshly isolated OSE, but very low in freshly isolated stroma. Levels of KGF gene expression after culture of OSE and stromal cells increased. Observations indicate that normal OSE express high levels of KGF in vivo and in vitro. Expression of KGF by normal epithelial cells versus stromal cells was unexpected and suggests KGF may be an important autocrine stimulator of OSE. KGF actions on bovine OSE cells were investigated. KGF was found to stimulate the growth of normal OSE cells to the same level as epidermal growth factor. Two ovarian cancer cell lines, SKOV3 and OCC1, were also stimulated to proliferate in response to KGF. Current results demonstrate the production and action of KGF on normal OSE cells and ovarian cancer cells. Observations can be interpreted to suggest that KGF may in part be involved in the growth of ovarian tumors. This appears to be one of the first reports of KGF production by an epithelial cell. The autocrine stimulation of OSE growth by the local production and action of KGF provides insight into how the OSE may develop abnormal growth characteristics involved in the onset and progression of ovarian cancer.

Roy, R. N., A. Cecutti, et al. (1997). "Endometrial transcripts of human insulin-like growth factors arise by differential promoter usage." Molecular and Cellular Endocrinology **135**(1): 11.

<http://www.sciencedirect.com/science/article/B6T3G-3RSN3V8-2/2/95396995e11007bcd62a80b11c2ea2d2>

By the application of RT-PCR, we have demonstrated that in the human endometrium mRNAs for insulin-like growth factors, IGF-I and II, and their receptors are expressed not only in the intact endometrium, but also in the freshly isolated stromal and epithelial cells. The expression of multiple transcript forms of the IGF-I and II at various phases of the menstrual cycle, occurs by differential use of all four IGF-I transcriptional start sites, and two of the four known promoter sites of the IGF-II gene. The complete spectrum of transcripts is displayed by the proliferative phase and the menstrual phase endometrium. During the secretory phase, the exon 1 upstream start site of the IGF-I gene and the P2 promoter of the IGF-II gene are not used. Irrespective of the phase of the menstrual cycle, the stromal cells always display the same transcriptional patterns of both growth factor genes as those of the intact endometrium. In contrast, the epithelial cells do not express IGF-I transcript originating from the exon 2 upstream initiation site. These results indicate that the expressions of the IGF-I and II genes in the intact endometrium and stromal and epithelial cells are modulated at the transcriptional level during the menstrual cycle by differential usage of promoters and start sites.

Roy, R. N., A. H. Gerulath, et al. (1999). "Discordant expression of insulin-like growth factors and their receptor messenger ribonucleic acids in endometrial carcinomas relative to normal endometrium." Molecular and Cellular Endocrinology **153**(1-2): 19.

<http://www.sciencedirect.com/science/article/B6T3G-3WWDH35-3/2/a4b6e34802c7bd57fd54c69d3c06f604>

The inappropriate expressions of insulin-like growth factors (IGF-I and II) and IGF-I receptor (IGF-IR) are implicated in the malignant growth of many cancers. To determine changes, if any, in the levels of expression of IGFs and IGF receptor genes in neoplastic endometrium, relative to normal endometrium, the mRNA levels of IGF-I and II and of IGF-IR and IIR were measured in samples of endometrial carcinomas (EC) and normal endometrium, through all phases of the menstrual cycle, by semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) assays. In normal endometrium, the mRNA levels of IGF-I were elevated in the proliferative and early secretory phases. The IGF-II mRNAs were relatively high in the proliferative phase, but unaltered through early and late secretory phases. Significantly elevated levels of IGF-II transcripts were observed during the menstrual phase, suggesting a possible role of IGF-II in endometrial regeneration. A positive correlation between the levels of IGF-I and IGF-IR mRNAs, apparent in the samples of normal endometrium, was not observed in endometrial carcinomas. The IGF-IR and IIR mRNA levels were elevated in endometrial carcinoma samples. On the other hand, the IGF-I and II mRNA levels were conspicuously low in many carcinoma samples, which were not associated with hyperplasia (type II EC), but relatively elevated in two other carcinoma samples, associated with adenomatous hyperplasia (type I EC). These results albeit with few samples suggest the possibility that the overexpressed receptor, IGF-IR, could be activated differently in two types of endometrial carcinomas, namely ligand-dependently in type I ECs and ligand-independently in type II ECs.

Rudolph, A. E., E. R. Blasi, et al. (2000). "Tissue-specific corticosteroidogenesis in the rat." Molecular and Cellular Endocrinology **165**(1-2): 221.

<http://www.sciencedirect.com/science/article/B6T3G-40XNWF2-V/2/fe5cbf222279fd98a755e70b656acb>

The steroid aldosterone plays a major role in the maintenance of total body sodium homeostasis and also contributes to cardiovascular pathophysiology by mediating cardiac hypertrophy and fibrosis. In addition to classical adrenal production of aldosterone, endogenous tissue production of aldosterone has been observed in various organs; aldosterone biosynthesis in cardiac tissues, however, remains highly controversial. The current study provides a comprehensive evaluation of steroid hormone biosynthetic capabilities in multiple tissues from two distinct rat strains under unstimulated and stimulated conditions. Panels of tissues from Wistar and Sprague-Dawley rats were probed for 11 [beta]-hydroxylase (P45011[beta]) and aldosterone synthase (P450aldo) by reverse transcriptase-polymerase chain reaction (RT-PCR). Under unstimulated conditions, cardiac P45011[beta] and P450aldo were detected only in Wistar rats. Angiotensin II (100 [mu]g/day) stimulated myocardial expression of both enzymes in both strains. Cerebral cortex and mesenteric artery message levels in both strains was reduced by angiotensin II. These data demonstrate the potential for local steroid synthesis in vascular, cardiac, renal, and neuronal tissues, and that biosynthesis of non-adrenal aldosterone may be differentially regulated between strains. This variability may thus resolve in part or whole the current controversy over the existence of non-adrenal steroidogenic systems.

Sabo-Attwood, T., K. J. Kroll, et al. (2004). "Differential expression of largemouth bass (*Micropterus salmoides*) estrogen receptor isotypes alpha, beta, and gamma by estradiol." Molecular and

Cellular Endocrinology **218**(1-2): 107.

<http://www.sciencedirect.com/science/article/B6T3G-4BV484D-1/2/e7c0b4fe059dbd9dfc82ef7c70360463>

The expression levels of three estrogen receptor (ER) isotypes alpha, beta, and gamma were quantified in female largemouth bass (*Micropterus salmoides*) (LMB) liver, ovary, brain, and pituitary tissues. ER alpha and beta expression predominated in the liver, while ERs beta and gamma predominated in the other tissues. Temporally in females, ER alpha was highly up-regulated, ER gamma was slightly up-regulated, and ER beta levels remained unchanged in the liver when plasma 17-[beta] estradiol (E2) and vitellogenin (Vtg) levels were elevated in the spring. In ovarian tissue from these same fish, all three ERs were maximally expressed in the fall, during early oocyte development and prior to peak plasma E2 levels. When males were injected with E2, ER alpha was highly inducible, ER gamma was moderately up-regulated, and ER beta levels were not affected. None of the ER isotypes were induced by E2 in gonadal tissues. These results combined suggest that the ERs themselves are not regulated in the same manner by E2, and furthermore, do not contribute equally to the transcriptional regulation of genes involved in fish reproduction such as Vtg.

Salameh, W., M. Choucair, et al. (2005). "Leydig cell hypoplasia due to inactivation of luteinizing hormone receptor by a novel homozygous nonsense truncation mutation in the seventh transmembrane domain." Molecular and Cellular Endocrinology **229**(1-2): 57.

<http://www.sciencedirect.com/science/article/B6T3G-4DW3CTN-1/2/30559f4b88b821ae45af0bd78aa5f09b>

Inactivating mutations in the LH receptor are the predominant cause for male pseudohermaphroditism in subjects with Leydig cell hypoplasia (LCH). The severity of the mutations, correlates with residual receptor activities. Here, we detail the clinical presentation of one subject with complete male pseudohermaphroditism and LCH. We identify within the proband and her similarly afflicted sibling a homozygous T to G transversion at nucleotide 1836 in exon 11 of the LH/CGR gene. This causes conversion of a tyrosine codon into a stop codon at codon 612 in the seventh transmembrane domain, resulting in a truncated receptor that lacks a cytoplasmic tail. In vitro, in contrast to cells expressing a normal LHR, cells transfected with the mutant cDNA exhibit neither surface binding of radiolabeled hCG nor cAMP generation. In vitro expression under the control of the LHR signal peptide of either a wild type or mutant LHR-GFP fusion protein shows no differences in receptor cellular localization. In conclusion, the in vitro studies suggest that residues in the seventh transmembrane domain and cytoplasmic tail are important for receptor binding and activation without playing a major role in receptor cellular trafficking.

Saward, L. and P. Zahradka (1996). "Insulin is required for angiotensin II-mediated hypertrophy of smooth muscle cells." Molecular and Cellular Endocrinology **122**(1): 93.

<http://www.sciencedirect.com/science/article/B6T3G-3W3NFG8-9/2/c2184a3931196634ffc2aa99e5ae0f7d>

The prevalence of hypertension and atherosclerosis among subjects with hyperinsulinemia supports the premise of a direct metabolic link between insulin and angiotensin II at the cellular level. In the present study, the effect of insulin on the angiotensin II-induced growth of A10 smooth muscle cells (SMC) was investigated. Treatment of quiescent A10 cells with angiotensin II caused an increase in RNA synthesis, proto-oncogene c-fos mRNA levels and cell size

dependent upon pretreatment with insulin. The insulin requirement was independent of its actions as a growth factor, since a pre-treatment of at least 24 h with insulin was essential for growth stimulation by angiotensin II. Using RT-PCR, insulin was shown to regulate AT2 receptor expression in both quiescent and differentiating cells. These data suggest the AT2 receptor, which mediates the growth effects of angiotensin II in A10 cells, may be the critical target for the effect of insulin.

Scaldaferri, L., K. Arora, et al. (1996). "Expression of PACAP and its type-I receptor isoforms in the rat ovary." Molecular and Cellular Endocrinology **117**(2): 227.

<http://www.sciencedirect.com/science/article/B6T3G-3VXN39T-G/2/67461a1765a47657b9f38c8930175915>

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a member of the secretin/glucagon/vasoactive intestinal peptide (VIP)/growth hormone releasing hormone (GHRH) family of neuropeptides, several of which stimulate steroidogenesis in ovarian granulosa cells. PACAP receptors are of two major subtypes; the type I receptor (PACAP-I-R) has much higher affinity for PACAP than VIP, and the type II receptor (PACAP-II-R) has similar affinity for both peptides. In the rat ovary, expression of the PACAP gene was demonstrated by amplification of ovarian RNA by the reverse transcription/polymerase chain reaction (RT-PCR). In addition, hybridization of Northern blots of rat ovarian poly(A)⁺ RNA with a 706-nt rat hypothalamic PACAP-I-R cDNA probe revealed the presence of a 7.0 kb PACAP receptor transcript, similar to that detected in brain and hypothalamus. RT-PCR using specific primers for the PACAP-I-R gene yielded products of the expected size with RNA obtained from ovarian tissue, brain, and hypothalamus. The authenticity of the PCR products was confirmed by Southern blotting and nested PCR, which revealed at least three splice variants of the PACAP-I-R in the rat ovary. These findings demonstrate that both PACAP and PACAP-I-R isoforms are expressed in the rat ovary, where they could exert autocrine or paracrine actions on granulosa cell function.

Sermon, K., S. Seneca, et al. (2001). "PGD in the lab for triplet repeat diseases -- myotonic dystrophy, Huntington's disease and Fragile-X syndrome." Molecular and Cellular Endocrinology **183**(Supplement 1): S77.

<http://www.sciencedirect.com/science/article/B6T3G-441N4PC-K/2/78f500fdb12933f9859b4b869f595ae8>

Myotonic dystrophy (DM), Huntington's disease (HD) and Fragile X syndrome (FRAXA) are three monogenic disease which are caused by so-called dynamic mutations. These mutations are caused by triplet repeats inside or in the vicinity of the gene which have the tendency to expand beyond the normal range thus disrupting the normal functioning of the gene. We describe here our experiences from 1995 to May 2000 with PGD for these three triplet repeat diseases.

Seuntjens, E., H. Vankelecom, et al. (1999). "Targeted ablation of gonadotrophs in transgenic mice affects embryonic development of lactotrophs." Molecular and Cellular Endocrinology **150**(1-2): 129.

<http://www.sciencedirect.com/science/article/B6T3G-3WM5C4D-G/2/4b4da433827bf8df70281e05366cc6f6>

Ablation of pituitary gonadotrophs was obtained in transgenic mice expressing diphtheria toxin A (DTA) under control of the -313/+48 bovine glycoprotein hormone [alpha]-subunit ([alpha]SU) promoter, previously shown to be active in mouse gonadotrophs but not in thyrotrophs. Development of hormone-producing cell types was assessed on the day of birth by computer-assisted image analysis on paraffin-embedded, immunostained pituitary sections. Six out of 50 transgenic F0 ('founder') mice (3 males and 3 females) showed a nearly complete disappearance of gonadotrophs but not of thyrotrophs. The number of lactotrophs and the relative area occupied by PRL-immunoreactivity were significantly reduced in the gonadotroph-depleted mice. The size of lactotroph clusters was smaller in the absence of gonadotrophs. The number and immunoreactive area of corticotrophs and somatotrophs, on the other hand, were not significantly affected by gonadotroph ablation. Based on the reported evidence that fetal ovaries do not produce steroid hormones as a result of lack of expression of at least three of the steroidogenic enzymes, P450scc, P450c17, and P450arom, the present observations can hardly be explained by a decline in estrogen levels due to gonadotroph ablation. Rather, the present data indicate that gonadotrophs directly stimulate the development of lactotrophs during fetal and early postnatal life, consistent with previous in vitro observations, and/or that gonadotrophs may share a cell-lineage relationship with a subpopulation of lactotrophs.

Shen, R., M. Sumitomo, et al. (2000). "Identification and characterization of two androgen response regions in the human neutral endopeptidase gene." Molecular and Cellular Endocrinology **170**(1-2): 131.

<http://www.sciencedirect.com/science/article/B6T3G-4233BNC-D/2/987a54374f43c51de959723e36a9e306>

Transcription of the human neutral endopeptidase 24.11 (NEP) gene is androgen regulated in prostate cancer cells. Homology search identified a sequence GTCACAagAGTTCT similar to the ARE consensus sequence GGTACAnnnTGTTCT within the 3'-untranslated region of the NEP mRNA. A double-stranded radiolabelled oligonucleotide containing this NEP-ARE sequence formed a DNA-protein complex with nuclear proteins from LNCaP cells or COS-7 cells co-transfected with an androgen receptor (AR) expression vector, and with full-length AR synthesized by baculovirus in mobility shift assays. Unlabeled NEP-ARE or consensus ARE but not mutated NEP-ARE replaced radiolabelled NEP-ARE. Steroid-dependent enhancement of transcription was assayed by transfecting ptkCAT reporter constructs containing the NEP-ARE into CV-1/AR cells and prostate cancer cells (PC-3/AR). Enhancement of chloramphenicol acetyltransferase (CAT) activity was increased four-fold by androgen, seven-fold by dexamethasone and three-fold by progesterone in CV-1/AR cells, and the NEP-ARE bound to glucocorticoid and progesterone receptor in mobility shift assays. We next performed DNase-I footprinting analysis of the NEP promoter and identified a 23 bp sequence GGTGCGGGTCGGAGGGATGCCCA (NEP-ARR) which was protected from DNase I cleavage by nuclear extracts from COS-7 cells expressing AR. This sequence was 62.5% homologous to an androgen responsive region (PSA-ARR) identified in the promoter of the prostate specific antigen (PSA) gene. A double-stranded radiolabelled oligonucleotide containing this NEP-ARR sequence formed DNA-protein complex with AR but not GR proteins. Unlabeled NEP-ARR, PSA-ARR and NEP-ARE replaced radiolabelled NEP-ARR. Steroid-dependent enhancement of transcription assays in PC-3/AR cells revealed that the enhancement of CAT activity was increased 2.3-fold by androgen, but not by glucocorticoid or progesterone. In a thymidine kinase promoter, the NEP-ARE and NEP-ARR together stimulated a five-fold increase in promoter activity in PC cells. These data suggest that steroid regulation of the NEP gene involves at least two elements including a typical ARE which binds androgen, progesterone and glucocorticoid receptors, and a unique ARR which only binds androgen receptor.

Shozu, M., Y. Zhao, et al. (2000). "TGF- β 1 stimulates expression of the aromatase (CYP19) gene in human osteoblast-like cells and THP-1 cells." Molecular and Cellular Endocrinology **160**(1-2): 123.

<http://www.sciencedirect.com/science/article/B6T3G-3YS34B7-G/2/c73ceae48348f8645e48ab656ada6b3c>

Recent evidence has shown that bone is not only a target of estrogen action but also a source of local estrogen production. Bone cells such as osteoblasts express aromatase (P450arom) and the expression of P450arom in osteoblasts is positively regulated in a tissue specific fashion, as in the case of other tissues which express P450arom. To clarify the physiological factors regulating expression of P450arom in bone, we tested TGF- β 1 using osteoblast-like cells obtained from human fetuses as well as THP-1 cells. TGF- β 1 increased IL-1 β +DEX-induced aromatase activity in osteoblast-like cells, while it inhibited activity in skin fibroblasts. Similar enhancement of aromatase activity by TGF- β 1 was found in DEX-stimulated THP-1 cells and this cell line was used for further experiments. In THP-1 cells, TGF- β 1 enhanced DEX-induced aromatase activity almost linearly by 12 h and thereafter. Increased levels of P450arom transcripts were also demonstrated by RT-PCR at 3 h of TGF- β 1 treatment and thereafter. Cyclohexamide abolished enhancement of activity but did not inhibit the accumulation of P450arom transcripts induced by TGF- β 1. Increase in P450arom expression by TGF- β 1 was attributable to expression driven by promoter I.4. TGF- β 1 did not change the half life of P450arom transcripts. To identify the cis-acting elements responsible for TGF- β 1 action on aromatase expression, transient transfection assays were performed using a series of deletion constructs for promoter I.4 (P450-I.4/Luc). Two constructs (-410/+14 and -340/+14) that contain a functional glucocorticoid response element (GRE) and downstream sequence showed significant increase of luciferase activity in response to TGF- β 1. Deletion and mutation of the GRE in P450-I.4/Luc (-340/+14) abolished the TGF- β 1. The luciferase activity of a (GRE)1-SV40/Luc construct was also stimulated by TGF- β 1. These results indicate that TGF- β 1 increases the expression of P450arom at the level of transcription through promoter I.4, at least in part via an enhancement of transactivation activity of the GR in THP-1 cells. TGF- β 1 is suggested to be one of the physiological up-regulatory factors of bone aromatase.

Simmen, R. C. M., L. Badinga, et al. (1993). "Chromosomal organization of the gene encoding porcine antileukoprotease and functional analysis of the promoter region in endometrial and placental cells." Molecular and Cellular Endocrinology **97**(1-2): 101.

<http://www.sciencedirect.com/science/article/B6T3G-47NWFBW-36/2/227a7e2ad7c8488900daff83269b999a>

The apparent preferential expression of the elastase/ cathepsin G protease inhibitor antileukoprotease (ALP) in endometrium of species with epitheliochorial placenta suggests mechanisms of transcriptional regulation unique to these mammalian species. To begin to define the cis-acting regulatory elements involved in the endometrial transcription of the ALP gene, the porcine ALP gene was isolated and characterized. The porcine gene spans at least 13 kb and consists of 5 exons and 4 introns. This genomic structure, except for an additional exon, is similar to that of the human gene where the first three exons encode the signal peptide, trypsin/ cathepsin G binding region, and elastase binding region, respectively. The positions of the 16 cysteine residues in exons 2 and 3 of the human gene are conserved in the porcine gene. The porcine gene contains a TATA box at -29 nucleotide (nt), and sequences with limited homology to those which might bind the transcription factors AP-1, AP-2, Sp-1 and Oct-1. The functional promoter activity of the ALP-5' flanking DNA was examined using chimeric ALP-chloramphenicol acetyl transferase (CAT) DNA constructs, after transient transfection in human (ECC-1, Ishikawa) and rabbit (HRE-H9) endometrial and human trophoblastic (JEG-3) cell lines. A 887 nt fragment of the ALP-5'-flanking region (- 887ALP-pCAT-E) was active in these cell lines, with the highest

promoter activity observed in the ECC-1. Progressive 5' deletion of the 887 nt fragment up to -243 nt had no effect on CAT gene expression in all cell lines, relative to the longest construct. Results suggest that the approximately 240 bp fragment most proximal to the transcription initiation site confers basal and limited endometrial tissue-specific promoter activity to the ALP gene 5'-flanking region. These studies also establish the ECC-1 cell line as an in vitro model system to elucidate the control of ALP gene transcription in the endometrium.

Too, C. K. L., A. Giles, et al. (1999). "Estrogen stimulates expression of adenine nucleotide translocator ANT1 messenger RNA in female rat hearts." Molecular and Cellular Endocrinology **150**(1-2): 161.

<http://www.sciencedirect.com/science/article/B6T3G-3WM5C4D-K/2/22aada3530f90294fd330c5ca1bd6bca>

The identification of estrogen-responsive genes in the heart, is necessary to understand estrogen-induced changes in cardiac function. Using Delta RNA fingerprinting, we demonstrate that a single injection of estradiol benzoate (50 [mu]g, s.c.) revealed mRNA species that were elevated, down-regulated, or were unaffected in the heart tissue of ovariectomized female rats. One of the upregulated genes was identified, by cloning and sequencing, to have 95.8% (230/240) identity with the 3' end of the rat ant1 gene encoding the mitochondrial adenine nucleotide translocator, ANT1. Using the isolated ANT1 cDNA (280 bp) as a probe in Northern analysis, estrogen was shown to upregulate the expression of cardiac ANT1, by at least 3-fold in female rats, from as early as 1 h to as long as 24 h. In contrast, estrogen treatment had no effect on ANT1 expression in heart tissue from male rats. RNA yields were low in rat atria and no transcript was detectable by Northern analysis. Using primers specific to the known rat ANT1 gene, the estrogen upregulation of the cardiac ANT1 transcript in female rat was confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR); a predicted product of 249 bp was obtained and this was stimulated by at least 3-fold upon estrogen treatment for 24 h.

Vanden Broeck, J., D. Veelaert, et al. (1996). "Molecular cloning of the precursor cDNA for schistostatins, locust allatostatin-like peptides with myoinhibiting properties." Molecular and Cellular Endocrinology **122**(2): 191.

<http://www.sciencedirect.com/science/article/B6T3G-3W2T670-8/2/f3b0b8320e25c67f2af9fef23068a1b3>

The cDNA encoding the precursor polypeptide for schistostatins, allatostatin-like peptides which have been shown to inhibit peristaltic movements of the lateral oviducts of *Schistocerca gregaria*, has been cloned and sequenced. Translation of this sequence reveals the presence of a pre-proschistostatin consisting of 283 amino acids. It contains ten different peptide sequences which are flanked by dibasic cleavage sites and C-terminal amidation signals. Eight of these peptides were identical to the schistostatins (or Scg-ASTs) that were previously purified from *Schistocerca gregaria* brain extracts. Two novel peptide sequences were discovered. One of these is the first AST-like peptide which has a C-terminal valine residue. Two peptides contain within their sequence an internal dibasic site which suggests a possible role for alternative processing and/or degradation. The schistostatin precursor differs from cockroach pre-proallatostatins in size, in sequence and in organization. It contains a lower number of peptides (10 versus 13 or 14) which are interrupted only once by an acidic spacer region (versus four in *Diploptera punctata* and *Periplaneta americana*). Northern analysis showed the presence of a 2.4 kb mRNA band in the locust central nervous system and midgut. This indicates that schistostatins, like other ASTs, are a good example of insect brain/gut peptides.

VanderHeyden, T. C., P. W. Wojtkiewicz, et al. (2000). "Mouse growth hormone transcription factor Zn-16: unique bipartite structure containing tandemly repeated zinc finger domains not reported in rat Zn-15." Molecular and Cellular Endocrinology **159**(1-2): 89.

<http://www.sciencedirect.com/science/article/B6T3G-3YDGFYR-B/2/330897c3548224195b3e394d5828b522>

Rat Zn-15 is a transcription factor activating GH gene expression by synergistic interactions with Pit-1, named for 15 DNA-binding zinc fingers, including fingers IX, X, and XI that are responsible for GH promoter binding. In this study, a mouse cDNA for Zn-15 was characterized. The predicted 2192-amino acid mouse protein is 89% identical to rat (r) Zn-15 overall, and is 97% similar in the C-terminal domain necessary for binding the GH promoter. However, the mouse cDNA encodes 16 zinc fingers, and sequences of rZn-15 pituitary cDNAs were the same as the mouse (m) Zn-16; the rat sequence in GenBank has a one nucleotide offset of a 17-bp segment in the finger V region. The mouse and corrected rat sequences contain four tandemly repeated fingers in the N-terminus, each separated by seven amino acids, typical of zinc finger proteins of the transcription factor IIIA-type. Analysis of mZn-16 expression by RT-PCR showed that the mRNA is produced at similar levels in normal and GH-deficient Ames dwarf (Prop-1) mouse pituitaries at postnatal day 1. Mouse Zn-16 mRNA also was detected by ribonuclease protection assay in the pre-somatotrophic mouse cell line GHFT1-5. The Zn-16 protein is bipartite in that the N-terminal half displays tandem spacing typical of most zinc finger proteins, while the C-terminal portion contains long linkers between fingers that cooperatively bind to a DNA response element. Expression in early postnatal pituitary and in pre-somatotrophic cells suggests that Zn-16 could play a role in pituitary development prior to somatotroph differentiation.

Voss, T. C., L. R. Goldman, et al. (2001). "GH mRNA levels are elevated by forskolin but not GH releasing hormone in GHRH receptor-expressing MtT/S somatotroph cell line." Molecular and Cellular Endocrinology **172**(1-2): 125.

<http://www.sciencedirect.com/science/article/B6T3G-427JWGY-1J/2/9d484055d765d2d8608e80386ceb5204>

The MtT/S somatotroph cell line should be a growth hormone-releasing hormone (GHRH)-responsive model system for the study of physiological control of growth hormone (GH) transcription because GH secretion from these cells is stimulated by GHRH. To examine the GH transcriptional activity of these cells, endogenous GH mRNA levels were measured using a ribonuclease protection assay following treatment under a variety of hormonal conditions. While omission of serum led to reduction of GH mRNA to 22% of control levels by 2 days and to 8% by 5 days ($P=20$ nmol/106 cells per h in both serum-containing and serum-free media, and that GHRH had no effect on cAMP levels, suggesting constitutive activation. To rule out the possibility of autocrine stimulation by GHRH produced endogenously, GHRH mRNA was not detectable in MtT/S cells using RT-PCR amplification. The stimulatory G-protein [α] subunit, mutations of which are known to activate adenylate cyclase constitutively in acromegaly, was sequenced but found not to differ from normal pituitary in the regions most commonly mutated. Finally, treatment with 10 [μ]M forskolin, to directly activate adenylate cyclase, increased GH mRNA to 140% of controls in TDM, and to 163% in serum-free medium after 2 days, and to 166% in TDM-treated cells and 174% in serum-free culture after 5 days (all Pgps mutations).

Wang, L., L.-P. Xie, et al. (2001). "Presence of gonadotropin-releasing hormone (GnRH) and its mRNA in

rat pancreas." Molecular and Cellular Endocrinology **172**(1-2): 185.

<http://www.sciencedirect.com/science/article/B6T3G-427JWGY-1S/2/519d326fb21fe9999bd1c0b76558bf34>

Gonadotropin-releasing hormone (GnRH) has been reported to exist in many non-hypothalamic tissues, such as the placenta, gonads, and mammary glands, while there still have been no reports concerning the existence and expression of GnRH in the mammalian digestive system. Immunocytochemistry and in situ hybridization results show that GnRH molecule and GnRH mRNA are both exclusively distributed in exocrine pancreas, and RT-PCR result further proves that GnRH transcription units do exist in the pancreas, which possess the same sequence as the hypothalamus GnRH mRNA. Quantitative analysis indicates that mRNA levels in rat pancreas remain at a low level (less than 10% of that in hypothalamus) without sexual or developmental difference. This is the first report suggesting the existence and gene expression of GnRH in rat pancreas.

Williams, C. P., C. Postic, et al. (1999). "Isolation and characterization of the mouse cytosolic phosphoenolpyruvate carboxykinase (GTP) gene: evidence for tissue-specific hypersensitive sites." Molecular and Cellular Endocrinology **148**(1-2): 67.

<http://www.sciencedirect.com/science/article/B6T3G-3VTYSMT-8/2/2ff88846b64c32c7b4ea8f1b33f27131>

A 72 kilobase pair DNA fragment that contains the mouse phosphoenolpyruvate carboxykinase (PEPCK) gene locus, pck1, was isolated from a genomic bacterial artificial chromosome library. The region from ~-5.5 to +6.6 kilobase pairs relative to the pck1 transcription start site was sequenced and exhibits a high degree of homology to the rat and human genes. Additionally, the chromatin structure of the PEPCK gene in mouse liver resembles that seen in rat. Backcross panel analysis of a microsatellite sequence confirms that the gene is located on chromosome 2. Hypersensitive site analysis was performed on nuclei isolated from the adipocyte cell line 3T3-F442A in the preadipose and adipose states. Several hypersensitive sites are present in the undifferentiated 3T3-F442A cells, before PEPCK mRNA is detected. The same sites are present after differentiation, however, the sensitivity of mHS 3 increases relative to the others. We conclude that the chromatin is open in 3T3-F442A cells and that factors are able to bind in the undifferentiated state but that something else is required for transcription.

Yarney, T. A., M. R. Sairam, et al. (1993). "Molecular cloning and expression of the ovine testicular follicle stimulating hormone receptor." Molecular and Cellular Endocrinology **93**(2): 219.

<http://www.sciencedirect.com/science/article/B6T3G-47N6MJR-JG/2/832005b95edd318dd058231bf964f9f1>

A sheep testicular cDNA library constructed in pcDNA1 vector was screened with a probe generated by polymerase chain reaction (PCR) and corresponding to a 1.6 kb fragment of the rat luteinizing hormone receptor cDNA. Several clones hybridizing to the rat probe at low stringency were sequenced to obtain 95% of the putative full-length ovine follicle-stimulating hormone receptor (oFSH-R) cDNA. The missing 5' region was obtained by PCR amplification of the cDNA library. Sequencing revealed a 2085 nucleotide open reading frame encoding a mature protein of 678 amino acids (74,580 daltons). The oFSH-R is remarkably similar (> 90%) to the human and rat FSH receptors, has a structural motif like the G protein-coupled family of receptors and contains 3 potential sites for N-linked glycosylation. RNA blot analysis revealed two major

transcripts of 2.6 kb and 6.7 kb in size and a smaller transcript of about 1 kb in the sheep testis. A 53 residue segment in the extracellular domain unique to the receptor contains more than 50% of residues bearing functional side chains that could participate in ligand (FSH) interaction and/or signal transduction. Transfection of human fetal kidney cell line (293) with the cloned oFSH receptor cDNA based in pcDNA1/Neo vector revealed functional expression. Labeled oFSH bound to receptor expressed on the membrane with high affinity and specificity. In stably transfected 293 cells, purified oFSH and hFSH but not oLH stimulated cyclic AMP accumulation. Chemically deglycosylated oFSH (DG-oFSH) was inactive in these cells but it effectively blocked the action of native hormone. Thus, the functional characteristics of the cloned receptor are similar to the natural receptor in testis.

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Gurney, M. E. and H. Yamamoto (1991). "Expression of recombinant rat ciliary neurotrophic factor in *Escherichia coli* and its synergy with basic fibroblast growth factor." Molecular and Cellular Neuroscience **2**(5): 369.

<http://www.sciencedirect.com/science/article/B6WNB-4F0PTB1-W/2/e5b7a9e88d3fd9ce8bc79b9fc48ffbf>

Rat CNTF was expressed in *Escherichia coli* using a T7 RNA polymerase vector system. rCNTF was obtained as an insoluble aggregate at levels approaching 70% of total *E. coli* protein. After extraction, renaturation, and purification by anion-exchange chromatography and gel filtration the yield of biologically active rCNTF was nearly 0.5 mg/ml of *E. coli* culture broth. The effects of rCNTF and bFGF on cultured chick ciliary neurons were compared. bFGF was primarily neuritogenic and did not support ciliary neuron survival beyond 2 days in culture. rCNTF induced neuritogenesis more slowly and supported ciliary neuron survival in culture for a longer period. bFGF strongly potentiated the effect of rCNTF. Without bFGF, the ED50 for rCNTF was 102 +/- 25 pg/ml. With bFGF, the potency of rCNTF increased to an ED50 of 42 +/- 6 pg/ml. Chick nodose neurons also responded to rCNTF and their response was potentiated by bFGF. Thus, bFGF modulates the response of ciliary and nodose neurons to CNTF in vitro and may have a similar effect in vivo.

Juryne, M. J., C. P. Riley, et al. (2003). "TIGR is upregulated in the chronic glial scar in response to central nervous system injury and inhibits neurite outgrowth." Molecular and Cellular Neuroscience **23**(1): 69.

<http://www.sciencedirect.com/science/article/B6WNB-48FK4SW-7/2/39ba92863fa0ad23b241cef2152eaf6b>

Reactive astrocytes respond to central nervous system (CNS) injury and disease by elaborating a glial scar that is inhibitory to axonal regeneration. To identify genes that may be involved in the astrocytic response to injury, we used differential display polymerase chain reaction and an in vivo model of the CNS glial scar. Expression of the trabecular meshwork inducible glucocorticoid response (TIGR) gene was increased in gliotic tissue compared with the uninjured cerebral cortex. Increased TIGR expression by reactive astrocytes was confirmed by in situ hybridization, quantitative reverse transcriptase-polymerase chain reaction, immunoblot analysis, and

immunohistochemistry. Although mutations of the TIGR gene have been implicated in glaucoma, a function for TIGR has not been reported. Since TIGR is secreted, we assessed a possible role in inhibition of neuronal regeneration with an in vitro bioassay and found that this protein is a potent inhibitor of neurite outgrowth. Thus, TIGR is a newly identified component of the CNS glial scar that is likely to contribute to neuronal regenerative failure characteristic of the mammalian CNS.

Kury, P., H. Koller, et al. (2003). "Cyclic AMP and tumor necrosis factor-[alpha] regulate CXCR4 gene expression in Schwann cells." Molecular and Cellular Neuroscience **24**(1): 1.

<http://www.sciencedirect.com/science/article/B6WNB-48PVDG8-N/2/6234126ecff62831690858a7dc289d96>

Rat peripheral nerve Schwann cells have been shown to express the [alpha]-chemokine receptor CXCR4 as well as the corresponding ligand stromal cell-derived factor-1 (SDF-1). We have investigated gene regulatory mechanisms acting on the expression of CXCR4 in cultured rat Schwann cells and found that receptor expression at transcript- and protein levels is directly dependent on intracellular cyclic AMP. Such increased levels of CXCR4 expression were found to be efficiently reversed by the action of tumor necrosis factor-[alpha] (TNF[alpha]). We also provide evidence that the POU box transcription factor Oct-6/SCIP is involved in the control of CXCR4 transcription. Finally, we could demonstrate that CXCR4 activation by SDF-1[alpha] increases the number of dying Schwann cells, indicating that this receptor/ligand interaction is modulating cell survival. Our data, therefore, suggest that in the Schwann cell lineage signal transduction cascades controlled by the activation of TNF- and CXCR4 receptors are functionally coupled.

Kusy, S., L. Funkelstein, et al. (2003). "Redundant functions but temporal and regional regulation of two alternatively spliced isoforms of Semaphorin 3F in the nervous system." Molecular and Cellular Neuroscience **24**(2): 409.

<http://www.sciencedirect.com/science/article/B6WNB-496NKDJ-6/2/cb8e3b6dcf762646fc5db398bf100479>

SEMA3F is a secreted semaphorin that affects axon and cell guidance in the developing nervous system, and is also thought to have anti-tumor activity. Two spliced forms of SEMA3F have been identified that differ by the insertion of 31 amino acids in the sema domain. Here, we investigated the bioactivity of these isoforms and show, using coculture and binding assays, that they share common axonal chemorepulsive properties and binding to neuropilin receptors. SEMA3F isoforms were also found to regulate endothelial cell morphology by remodeling lamellipodial protrusions. Although Sema3F expression globally decreased during mouse development, we noted an enrichment of the longest isoform at postnatal stages in some territories such as the brainstem and spinal cord. These results indicate that although functionally redundant in cell culture assays, Sema3F spliced forms are characterized in vivo by a temporal and regional specific regulation during maturation of the nervous system.

Sikela, J. M., N. J. Leidenheimer, et al. (1991). "Localization and functional expression of alternatively spliced forms of the GABAA receptor [gamma]2 subunit." Molecular and Cellular Neuroscience **2**(4): 338.

<http://www.sciencedirect.com/science/article/B6WNB-4DYM8XC-9/2/bbad97d573982c1a78e06b591989b2ca>

Recent studies have identified two alternatively spliced forms of the GABAA receptor [gamma]2 subunit that differ by the presence ([gamma]2L) or absence ([gamma]2S) of an eight-amino acid segment. This insert in the [gamma]2L isoform exists in the proposed cytoplasmic loop region, between M3 and M4, and contains a consensus sequence for phosphorylation by protein kinase C. To examine the regional distribution of this novel receptor subunit in the brain, [gamma]2L subunit mRNA was detected using both in situ hybridization histochemistry and PCR amplification methods. Hybridization histochemistry with a [gamma]2L, subunit-specific oligonucleotide probe revealed that the [gamma]2L, subunit mRNA is widely distributed throughout the mouse brain. The highest levels of expression are found in the cerebral cortex, hippocampus, olfactory lobe, and cerebellum. The presence of the [gamma]2L, subunit in these regions was confirmed using PCR. Additionally, PCR experiments detected yes subunit mRNA in the cerebral cortex and hippocampus but not in the cerebellum. To examine the functional properties of the [gamma]2 subunit isoforms, [gamma]2S and [gamma]2L, subunit mRNAs were coexpressed with [alpha]1[beta]1 subunit mRNAs in *Xenopus* oocytes. These experiments indicate that the [gamma]2L and [gamma]2S subunit variants exhibit similar pharmacological properties, including the ability of both isoforms to confer diazepam sensitivity to the receptor complex. In addition, potentiation of GABA responses by pentobarbital in oocytes expressing either subunit isoform is similar. These data indicate that the presence or absence of the additional eight amino acids in the [gamma]2 subunit isoforms does not appear to alter the response of the GABAA receptor complex to either benzodiazepines and barbiturates at the level of protein phosphorylation present in the oocyte.

Tortarolo, M., P. Veglianesi, et al. (2003). "Persistent activation of p38 mitogen-activated protein kinase in a mouse model of familial amyotrophic lateral sclerosis correlates with disease progression." Molecular and Cellular Neuroscience **23**(2): 180.

<http://www.sciencedirect.com/science/article/B6WNB-48FK4SW-1/2/e9eed170a04bd49b549f60c56a56a301>

The p38 mitogen-activated protein kinase (p38MAPK) is activated via phosphorylation in neurones and glial cells by a variety of stimuli including oxidative stress, excitotoxicity, and inflammatory cytokines. Activated p38MAPK can in turn induce phosphorylation of cytoskeletal proteins and activation of cytokines and nitric oxide, thus contributing to neurodegeneration. We investigated the expression and distribution of p38MAPK in the spinal cord of transgenic mice expressing a superoxide dismutase 1 mutation (SOD1G93A), a model of familial amyotrophic lateral sclerosis (ALS). Accumulation of p38MAPK was found by immunoblotting in the spinal cord of G93A mice during the progression of disease, but no changes were detected in its mRNA levels. Immunostaining for phosphorylated p38MAPK in lumbar spinal cord sections of SOD1G93A mice at the presymptomatic and early stages of disease showed an increased labeling in motor neurones that colocalized with phosphorylated neurofilaments in vacuolized perikarya and neurites, as detected by confocal microscopy. As the disease progressed, activated p38MAPK also accumulated in hypertrophic astrocytes and reactive microglia, as demonstrated by colocalization with GFAP and CD11b immunostaining, respectively. These data suggest that activation of p38MAPK in motor neurones and then in reactive glial cells may contribute, respectively, to the development and progression of motor neuron pathology in SOD1G93A mice.

Zaratin, P. F., A. Quattrini, et al. (2005). "Schwann cell overexpression of the GPR7 receptor in inflammatory and painful neuropathies." Molecular and Cellular Neuroscience **28**(1): 55.

<http://www.sciencedirect.com/science/article/B6WNB-4DXB9YN-1/2/3462dade0b819d5de0299a9b73992ba2>

The human 7-transmembrane receptor GPR7 has sequence similarity to opioid and somatostatin receptors, and can be activated by the recently discovered neuropeptides NPB and NPW. This receptor is highly expressed in the nervous system, with suggested roles in neuroendocrine events and pain signaling. In this study, we investigated whether the GPR7 receptor is expressed in the peripheral nervous system under normal and pathological conditions. A low level of GPR7 receptor was observed in myelin-forming Schwann cells in both normal human and rat nerve, and in primary rat Schwann cell cultures. Peripheral nerve samples taken from patients exhibiting inflammatory/immune-mediated neuropathies showed a dramatic increase of GPR7 receptor expression restricted to myelin-forming Schwann cells. Complementary animal models of immune-inflammatory and ligation-induced nerve injury and neuropathic pain similarly exhibited an increased myelin-associated expression of GPR7 receptor. These results suggest a relationship between the pathogenesis of inflammatory/immune-mediated neuropathies, GPR7 receptor expression, and pain transmission.

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Ahmad, S., E. Mokaddas, et al. (2004). "Rapid detection of ethambutol-resistant Mycobacterium tuberculosis strains by PCR-RFLP targeting embB codons 306 and 497 and iniA codon 501 mutations." Molecular and Cellular Probes **18**(5): 299.

<http://www.sciencedirect.com/science/article/B6WNC-4CHRGDY-1/2/0fc1a263987e61e7c08ada24452448da>

Mutations at embB gene codons 306 and 497 and iniA gene codon 501 occur frequently in ethambutol (EMB)-resistant Mycobacterium tuberculosis strains worldwide. The identification of these mutations in resistant strains has been achieved by labor-intensive DNA sequencing or by tedious amplification protocols followed by restriction endonuclease digestion. In this report, we describe PCR-restriction fragment length polymorphism (RFLP)-based methods for determining substitutions at embB codons 306 and 497 and iniA codon 501 directly in BACTEC cultures of M. tuberculosis isolates. The wild-type and mutant alleles are revealed by easily interpretable and different RFLP patterns. The methods optimized initially on reference strains were tested directly on BACTEC cultures of 25 randomly selected clinical M. tuberculosis isolates, seven of which were determined to contain EMB-resistant strains by phenotypic drug susceptibility testing. The PCR-RFLP methods identified mutations in four of seven EMB-resistant strains with three isolates containing mutated embB codon 306 and one isolate containing mutated embB codon 497. The results of PCR-RFLP were confirmed by DNA sequencing. The worldwide prevalence figures for mutations at embB codons 306 and 497 and iniA codon 501 suggest that nearly half of EMB-resistant M. tuberculosis strains could be identified within one working day even in developing countries equipped with simple PCR technology instead of weeks required for phenotypic drug susceptibility testing. Further, since EMB resistance is also associated with multiple-drug resistance from some geographical locations, detection of EMB resistance may also lead to rapid identification of multidrug-resistant strains of M. tuberculosis.

An, S. F., D. Franklin, et al. (1992). "Generation of digoxigenin-labelled double-stranded and single-

stranded probes using the polymerase chain reaction." Molecular and Cellular Probes **6**(3): 193.

<http://www.sciencedirect.com/science/article/B6WNC-4DYN471-30/2/5e63dbdc394136ce873a50ed261eedb>

As the polymerase chain reaction (PCR) can be used for the generation of vector-free probes, the optimum conditions for incorporation of digoxigenin-11-dUTP into hepatitis B virus (HBV) probes have been investigated. High yields of double-stranded or single-stranded probes can be obtained by utilizing a pair of primers or one primer alone. The probes were tested by dot-blot hybridization on HBV plasmid DNA, slot-blot hybridization on total cellular RNA of Alexander cells and Southern blot hybridization on cellular DNA of Alexander cells and HBV plasmid DNA. They were also tested by in situ hybridization (ISH) on HBV-positive biopsy liver tissue. A ratio of dig-dUTP:dTTP of 1:3 gave highest sensitivity in DNA hybridization. No loss of amplification efficiency and sensitivity was observed when the final concentration of dig-11-dUTP and dTTP was reduced to 20 [mu]M and 60 [mu]m respectively, compared to 200 [mu]m each of dATP, dCTP, dGTP. Several different sizes of double-strand probes were compared by dot-blot hybridization. Longer probes were more sensitive. Strong signal could also be obtained by combination of two or three small probes, which have overlapping sequences. Single-stranded DNA probes had advantages of simplicity of use, high sensitivity and strand specificity.

Bobba, A., E. Marra, et al. (2003). "Non-radioactive detection of five common microsatellite markers for ATP7B gene in Wilson disease patients." Molecular and Cellular Probes **17**(6): 271.

<http://www.sciencedirect.com/science/article/B6WNC-49HSTV6-1/2/feb91b218644ec50acae01df1aa9a62e>

Haplotype analysis using microsatellite markers is a useful indicator of specific mutations and is often exploited as the first large-scale screening technique to carry out the molecular characterization of the disease gene in probands from a specific population. However, the methodologies available are still cumbersome and require the use of either radioactive compounds or specialized equipment suitable to follow fluorescent dyes. Both these techniques may not be available for newly developing clinical laboratories. We have set up a sensitive and easy-to-use protocol to characterize five closely spaced, highly polymorphic microsatellite polymorphisms (CA repeats) that span the Wilson disease (WD) region, i.e. D13S316, D13S133, D13S301, D13S314, D13S315. The technique described here for the analysis of the WD gene microsatellite system relies on the quick detection method of silver staining, avoiding the use of toxic or sophisticated equipment. This approach could be the method of choice to implement molecular genetic testing in clinical laboratories, even those not especially equipped for DNA analysis and in particular in newly developed molecular genetics centers in countries whose population has not yet been characterized for WD-causing ATP7B gene mutations.

Bottero, M. T., A. Dalmaso, et al. (2004). "Development of a multiplex PCR assay for the identification of pathogenic genes of Escherichia coli in milk and milk products." Molecular and Cellular Probes **18**(4): 283.

<http://www.sciencedirect.com/science/article/B6WNC-4CB0HNX-1/2/47788539570c45d133bf7f2ac2ed128a>

A multiplex PCR for the simultaneous detection of some pathogenic genes of enteropathogenic, enterotoxigenic and verocytotoxin-producing Escherichia coli was developed. In this study primers found in literature as well as primers to the purpose designed were used. In this way, it was

possible to generate specific fragments of 96, 170, 229, 285, 348, 414 and 510 bp for Hly_A, St, EaeA, Lt, Vt1, UidA and Vt2 genes, respectively. When applied to bacterial strains experimentally inoculated in milk and milk products, the proposed PCR showed a detection limit of 5 x 10⁴ CFU/ml for Hly_A, St, EaeA, Vt1 primers, while for Lt and Vt2 primers the limit resulted of 10⁶ CFU/ml.

Brogan, J. M., J. Acciai, et al. (1992). "Development of a DNA probe for *Ureaplasma urealyticum*." Molecular and Cellular Probes **6**(5): 411.

<http://www.sciencedirect.com/science/article/B6WNC-4DYN471-2F/2/331343dc3f5c815d3f106a7aec237c7>

Ureaplasma urealyticum has been associated with a variety of disease conditions in humans. However, its exact etiologic role has not been well established because of the difficulties encountered in cultural diagnosis and the time needed for positive identifications. A DNA probe which is specific for a target DNA sequence unique to this suspected pathogen offers a rapid, sensitive and specific means of diagnosis. This study details the development of a polymerase chain reaction system for *U. urealyticum*. Using conventional hybridization techniques, a cloned genomic fragment was found to be specific for this organism. Sequencing of part of this probe DNA permitted the assignment of oligonucleotide primers which amplified a 186 bp target segment. This PCR system is specific for *U. urealyticum* but not for other closely related species of mycoplasma. This highly sensitive diagnostic technique will aid in determining the etiologic role, tissue tropism and dynamics of pathogenesis of this organism, and thereby result in better patient care.

Carlson, S. A. and M. T. Wu (2003). "Avoidance of false PCR results with the integron-retron junction in multiple antibiotic resistant *Salmonella enterica* serotype Typhimurium." Molecular and Cellular Probes **17**(4): 183.

<http://www.sciencedirect.com/science/article/B6WNC-4938M0C-1/2/923b86c6e539907e236960ab9970126e>

Salmonella infections continue to cause gastrointestinal and systemic disease throughout the world. Another concern with this pathogen is the ability to acquire integrons that confer resistance to multiple antibiotics. For multiresistant *Salmonella enterica* serotype Typhimurium, the most common multiresistant *Salmonella* serotype, an integron structure can be found between *thdF* and a retron. Our objective was to investigate the utility of a 450 bp *thdF*-retron amplicon as an indicator of an insertless *thdF*-retron junction thus indicating an integron-free strain. Surprisingly, we found that the 450 bp *thdF*-retron amplicon was present, and thus incorrectly suggesting an integron-free status, in some multiresistant *S. enterica* serotype Typhimurium isolates. However, this phenomenon was not observed if the isolate was enriched in the presence of two antibiotics. This demonstrates that, within some individual clinical isolates of multiresistant *S. enterica* serotype Typhimurium, there exists a small subpopulation of integron-free bacteria. Consequently, it appears that the *thdF*-retron amplicon is an inaccurate predictor of integron status in *S. enterica* serotype Typhimurium unless multiresistance is used as a selection tool during enrichment.

Caterina, K. M., J. S. Frasca, et al. (2004). "Development of a multiplex PCR for detection of avian adenovirus, avian reovirus, infectious bursal disease virus, and chicken anemia virus." Molecular

and Cellular Probes **18**(5): 293.

<http://www.sciencedirect.com/science/article/B6WNC-4CHRGDY-2/2/5f78e57c627066509a23c40245c79db5>

A multiplex polymerase chain reaction (mPCR) was developed and optimized for the simultaneous detection and differentiation of avian reovirus (ARV), avian adenovirus group I (AAV-I), infectious bursal disease virus (IBDV), and chicken anemia virus (CAV). Four sets of specific oligonucleotide primers were used in this test for ARV, AAV-I, IBDV, and CAV. The mPCR DNA products were visualized by gel electrophoresis and consisted of fragments of 365 bp for IBDV, 421 bp for AAV-I, 532 bp for ARV, and 676 bp for CAV. The mPCR assay developed in this study was found to be sensitive and specific. Detection of PCR-amplified DNA products was 100 pg for both CAV and IBDV, and 10 pg for both ARV and AAV-I and this mPCR did not amplify nucleic acids from the other avian pathogens tested. The mPCR demonstrated similar sensitivity in tests using experimental fecal cloacal swab specimens that were spiked with ARV, AAV-I, IBDV, and CAV, and taken from specific pathogen free (SPF) chickens. This mPCR detected and differentiated various combinations of RNA/DNA templates from ARV, AAV-I, CAV, and IBDV without reduction of amplification from feces.

Cuppens, H., I. Buyse, et al. (1992). "Simultaneous screening for 11 mutations in the cystic fibrosis transmembrane conductance regulator gene by multiplex amplification and reverse dot-blot." Molecular and Cellular Probes **6**(1): 33.

<http://www.sciencedirect.com/science/article/B6WNC-4DYVPKN-2P/2/b5d61e28b1c93341d2dbe43abc6cc142>

An assay is described in which 11 mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene can be screened simultaneously. Six different exons of the CFTR gene are amplified in a single multiplex amplification. Biotinylated dUTP is incorporated into the different fragments during the amplification process. A sample of this mixture is then hybridized to 21 different poly-dT tailed oligonucleotide probes which are bound to a nylon membrane. In order to screen the different mutations in a single step hybridization, the length of the different oligonucleotides and the amount used in the assay were optimized. The detection is performed by binding avidin-alkaline phosphatase to the biotin, followed by a chemiluminescent reaction. By means of this fast and sensitive assay, about 85% of all the cystic fibrosis mutations in the Belgian population can be detected.

Exner, M. M. and M. A. Lewinski (2002). "Sensitivity of multiplex real-time PCR reactions, using the LightCycler and the ABI PRISM 7700 Sequence Detection System, is dependent on the concentration of the DNA polymerase." Molecular and Cellular Probes **16**(5): 351.

<http://www.sciencedirect.com/science/article/B6WNC-47CJ5D5-5/2/1ab695694b42544a7b3da1e86006035b>

The introduction of multiplex PCR techniques to clinical laboratories has provided a means to streamline assays and to produce multiple results with minimal effort. While this methodology is very beneficial, care must be taken to ensure that reactions are properly optimized to allow for maximum sensitivity. This study was conducted to determine whether the sensitivity of multiplex-real-time PCR assays could be improved by increasing the concentration of DNA polymerase within a reaction. Multiplex reactions were designed to simultaneously detect the human HLA-DQ gene and a sequence from the UL83 region of the CMV genome. Two real-time PCR systems,

one utilizing AmpliTaq Gold DNA polymerase and the ABI 7700 Sequence Detection System, and one utilizing FastStart Taq DNA polymerase and the Roche LightCycler were tested. The results indicated that increasing the AmpliTaq Gold concentration from 0.050 to 0.10 U/[μ] and the FastStart Taq concentration from 0.1875 to 0.375 U/[μ] increased detection sensitivity from 5000 to 50 CMV copies per PCR reaction. In separate experiments, commercially prepared mastermixes were utilized for both real-time PCR platforms as per the manufacturer's suggestions or with the addition of supplemental DNA polymerase. In assays designed to detect 4 CMV genome copies per reaction, the addition of 2.5 U of AmpliTaq Gold to TaqMan Universal Mastermix increased the detection rate from 21 to 67%, and the addition of 5 U of FastStart Taq to FastStart DNA Master Hybridization Probes mastermix increased the detection rate from 17 to 56%. These results indicate that increasing the DNA polymerase concentration in multiplex real-time PCR reactions may be a simple way to optimize assay sensitivity.

Fortina, P., R. Conant, et al. (1992). "Fluorescence-based, multiplex allele-specific PCR (MASPCR) detection of the [Δ]F508 deletion in the cystic fibrosis transmembrane conductance regulator (CFTR) gene." Molecular and Cellular Probes 6(4): 353.

<http://www.sciencedirect.com/science/article/B6WNC-4DYN471-F/2/12122f842c21a790567637d58adfbce2>

Cystic fibrosis (CF) is a common genetic disorder in Caucasians, and in some populations 70% of cases are associated with a 3 base pair (bp) deletion ([Δ]F508) in the CFTR gene. We have implemented a fluorescence-based, multiplex allele-specific polymerase chain reaction (MASPCR) assay for deletion of the [Δ]F508 mutation. Different allele-specific fluorescently-tagged primers are used in the PCR reaction to distinguish between normal and [Δ]F508 alleles. Fluorescent PCR products are then visualized in a single lane on an agarose gel following electrophoresis combined with real-time multicolour fluorescence detection. The approach simplifies diagnosis of the most common mutation in the CFTR gene, and holds promise for a multiplex allele-specific, fluorescence-tagged gene amplification strategy for detection of additional CF mutations which may result in more cost-effective testing without increasing the risk of missed or erroneous diagnoses.

Gage, K. L., R. D. Gilmore, et al. (1992). "Detection of *Rickettsia rickettsii* in saliva, hemolymph and triturated tissues of infected *Dermacentor andersoni* ticks by polymerase chain reaction." Molecular and Cellular Probes 6(4): 333.

<http://www.sciencedirect.com/science/article/B6WNC-4DYN471-B/2/735a4fcf32c5bebada99ad3560beff4b>

The technique of polymerase chain reaction (PCR) is potentially superior to existing methods for detecting rickettsial infections in ticks. For this reason, we developed assays for identifying rickettsial infections in ticks by PCR. Our assays amplified a 500 bp fragment from the gene encoding the rOmp B protein of *Rickettsia rickettsii*. The selected primers amplified fragments of the predicted size from all spotted fever group rickettsiae (*R. rickettsii*, *R. parkeri*, *R. conorii*, *R. sibirica*) tested. No amplified products were detected when typhus group rickettsiae (*R. canadensis*, *R. prowazekii*, *R. typhi*) were assayed. Using techniques described in this study, we reliably amplified the predicted product from hemolymph, saliva and ground leg tissue samples from live, partially fed, infected ticks. Samples derived from infected ticks preserved in 70% ethanol also were suitable for amplification by PCR. Similar assays performed with infected ticks preserved in 5% buffered formalin seldom gave positive results.

Gharizadeh, B., A. Ohlin, et al. (2003). "Multiple group-specific sequencing primers for reliable and rapid DNA sequencing." Molecular and Cellular Probes **17**(4): 203.

<http://www.sciencedirect.com/science/article/B6WNC-497HB05-1/2/01f0c767037ce348564946e0a6f8f100>

Pyrosequencing(TM) technology is a bioluminometric DNA sequencing method that employs a cascade of four enzymes to deliver sequence signals. To date this technology has been limited to the sequencing of short stretches of DNA. As an improvement to this technique, we have introduced a bacterial group-specific, multiple sequencing primer approach that circumvents sequencing of less informative semi-conservative regions of the 16S rRNA gene. This new approach is suitable for challenging templates, improving sequence data quality, avoiding sequencing of non-specific amplification products, lessening sequencing time, and moreover, this strategy should open the way for many new applications in the future. The group-specific, multiple sequencing primers can be applied in the Sanger dideoxy sequencing method as well. In addition, we have improved the chemistry of the Pyrosequencing system enabling sequencing of longer stretches of DNA, which allows numerous new applications.

Gilbert, C., D. Winters, et al. (2003). "Development of a triplex PCR assay for the specific detection of *Campylobacter jejuni*, *Salmonella* spp., and *Escherichia coli* O157:H7." Molecular and Cellular Probes **17**(4): 135.

<http://www.sciencedirect.com/science/article/B6WNC-48WPS6K-1/2/070daa389f7f3cd9496e4e9bf1fba7b8>

A triplex PCR assay was developed and evaluated for efficacy in detecting *Campylobacter jejuni*, *Salmonella* spp., and *Escherichia coli* O157:H7 in a variety of raw and ready-to-eat food products. Following a short enrichment period, artificially contaminated food samples were subjected to a triplex PCR assay, which incorporated published primers for each food pathogen, a protocol for sample collection, and a PCR procedure designed specifically for the assay. The selected primers amplified fragment sizes of 159 bp, 252 bp, and 360 bp for *C. jejuni*, *E. coli* O157:H7, and *Salmonella* spp., respectively. This assay provides specific and reliable results and allows for the cost-effective detection of all three bacterial pathogens in one reaction tube.

Gravitt, P., A. Hakenewerth, et al. (1991). "A direct comparison of methods proposed for use in widespread screening of human papillomavirus infections." Molecular and Cellular Probes **5**(1): 65.

<http://www.sciencedirect.com/science/article/B6WNC-4DYN471-1P/2/a825a684ec55eacfb930e247364e6369>

We obtained cervical swabs from 397 women participating in a human papillomavirus (HPV) prevalence study. Samples were assayed for HPV infection using ViraPap expanded cocktail (detecting HPV types 6, 11, 16, 18, 31, 33, 35, 42, 43, 44, 45, 51, 52 and 56), ViraType and PCR amplifications. Consensus primers from the L1 region were used with generic and type-specific oligonucleotide probes. Additionally, the generic amplifications were analysed with a novel restriction digest scheme. Samples positive by these methods were confirmed by PCR amplification using primers from the E5 region specific for HPV types 6, 16 and 18. The presence of human DNA in the samples was verified with amplification of the human KM-19 haplotyping

primers. Our results confirm that the PCR reporter oligomer hybridization method is more sensitive than ViraPap/ViraType, but encompasses a narrower range of HPV types. This is particularly true of the higher number types in the expanded cocktail. The narrow range seems to occur as the result of the reporter oligomer used in hybridization, rather than the consensus amplicon pair used. Amplification of a broader range of HPVs is seen on gels or using the restriction digest as confirmation of HPV infection. Both ViraPap and PCR methods of detection gave about a 10% rate of uninterpretable results. PCR methods indicated about 1.7 times as many positives, while showing overall agreement of 77.6% with ViraPap. Agreement on types ranged from 67% to 100%. All methods indicated large fractions of untypable HPVs.

Hartman, L. J., S. R. Coyne, et al. (2005). "Development of a novel internal positive control for Taqman[trademark] based assays." Molecular and Cellular Probes **19**(1): 51.

<http://www.sciencedirect.com/science/article/B6WNC-4F0GR5B-1/2/cdbcfa718a1fdc4b2757ad9df1074bb9>

Development of rapid amplification assays for the detection and identification of biological threat agents has become a focus of diagnostic efforts in recent years. The use of real-time PCR assays as diagnostic tools depends upon two critical processes. First, nucleic acid purification must provide template that is both amplifiable and free of PCR inhibitors. Second, the assays themselves must be sensitive and specific for their nucleic acid targets. A differentiation must be made between results achieved due to the lack of target nucleic acid (true negatives) and those produced due to the inability to amplify target DNA (false negatives) so confidence in negative reactions is possible. False negatives can occur when inhibitors are present in the sample being tested, especially if clinical samples such as blood are analyzed. To address the problem of detecting inhibition in purified nucleic acids, an exogenous internal positive control (IPC) based on Taqman[trademark] chemistry was developed. A previously optimized assay was cloned and the primer and probe sites were mutated to produce novel sequences with no known homology to published sequence data. The IPC was sensitive to a variety of inhibitors, including hemoglobin, heparin, EDTA, humic acids, and fulvic acid. It was also equally sensitive to inhibition when labeled with either 6FAM or ROX dyes. In addition, the IPC was successfully multiplexed with agent specific assays without any loss in their sensitivity. The designed IPC assay has proven to be an effective tool for monitoring inhibitors of PCR and builds confidence in negative results obtained with agent specific assays.

Holmberg, M., J. Wahlberg, et al. (1992). "Colorimetric detection of Plasmodium falciparum and direct sequencing of amplified gene fragments using a solid phase method." Molecular and Cellular Probes **6**(3): 201.

<http://www.sciencedirect.com/science/article/B6WNC-4DYN471-31/2/cb7625fe7e54ee4c082212a960ac460a>

A rapid colorimetric assay for the detection of DNA from Plasmodium falciparum malaria is described, allowing direct sequencing of amplified fragments in the positive samples. The method is based on amplification by the polymerase chain reaction (PCR), with incorporation of biotin and a lac operator sequence in the amplified target DNA. The PCR product was immobilized on streptavidin-coupled magnetic beads, and detected by the specific binding of an Escherichia coli lac repressor [beta]-galactosidase fusion protein. Positive samples were subsequently treated with alkali to generate single stranded templates, which were used for solid phase genomic sequencing. As targets for amplification and sequencing we selected a region of the gene for the antigen Pf155/RESA and a region of the parasite dihydrofolate reductase gene (PfDHFR/TS). We show here that both of these gene targets can be used for specific detection of P. falciparum in

patient blood samples. Genomic sequencing of five patient isolates revealed no variation in the Pf155/RESA gene fragment. In a comparison of this sequence with conserved protein domains, a marked similarity to the src homology region 3 was detected. A point mutation was found in the PfDHFR/TS gene fragment of one of the clinical samples, replacing Ser108 with Asn. This mutation has earlier been described in pyrimethamine and cycloguanile-resistant strains of *P. falciparum*.

Ibrahim, M. S., J. J. Esposito, et al. (1997). "The potential of 5' nuclease PCR for detecting a single-base polymorphism in Orthopoxvirus." Molecular and Cellular Probes **11**(2): 143.

<http://www.sciencedirect.com/science/article/B6WNC-45M2XMF-N/2/e3c78ce4b57c0c34eb51e77f9fe1f402>

A fluorogenic 5' nuclease PCR assay was evaluated for its ability to specifically detect and differentiate DNA of two Orthopoxvirus species. A pair of consensus primers that target a DNA segment of the Orthopoxvirus haemagglutinin gene, and two oligonucleotide probes, each labelled with a different fluorescent reporter dye and the same quencher dye, were used in a single-tube assay. The assay is based on the 5'→3' nuclease activity of AmpliTaq DNA polymerase that cleaves a fluorescein-labelled hybridized probe. Probe cleavage generates specific fluorescent signals whose intensity can be quantified by fluorometry. After evaluating the effects of various annealing temperatures and probe concentrations and normalizing the emission intensities of the reporter dyes, it was possible to detect and differentiate monkeypox and vaccinia virus DNAs on the basis of a single-base polymorphism. The sensitivity of the 5' nuclease PCR assay is comparable to the sensitivity of ethidium bromide-stained gels, but the assay provides higher specificity and virtually eliminates the need for laborious post-PCR processing.

Isegawa, Y., J. Sheng, et al. (1992). "Selective amplification of cDNA sequence from total RNA by cassette-ligation mediated polymerase chain reaction (PCR): Application to sequencing 6.5 kb genome segment of hantavirus strain B-1." Molecular and Cellular Probes **6**(6): 467.

<http://www.sciencedirect.com/science/article/B6WNC-4DYVPKN-1H/2/5c212d2ea010b38baa61ee9b4580f9e6>

A method, referred to as cassette-ligation mediated polymerase chain reaction (PCR), has been developed to permit selective and specific amplification of cDNA sequence from total cellular RNA. This technique comprises (i) digestion of cDNA with multiple restriction enzymes, (ii) ligation of cleavage products to double-stranded DNA cassettes possessing a corresponding restriction site and (iii) amplification of cassette-ligated restriction fragments containing a short, known sequence (but not all the other ligation products) by PCR using the specific and cassette primers; the specific primer is designed to prime synthesis from the known sequence of the cDNA whereas the cassette primer anneals to one strand of the cassette. Sequencing from the cassette primer provides information to design a new primer for the next walking step. The amplified cDNA fragments are often larger than the maximum DNA fragments (500-600 bp) that can be sequenced without the need of synthesizing internal sequencing primer. Each of such large cDNA fragments is dissected into smaller DNA fragments by repeating cassette-ligation mediated PCR exploiting different restriction sites and different sets of cassette primers. This dissection process reduces the number of specific primers to a minimum, thereby increasing the speed of sequencing and minimizing the overall cost. We have successfully applied this cDNA walking and sequencing by the cassette-ligation mediated PCR to the sequencing of an entire 6.5 kb genome segment of hantavirus strain B-1. The complete sequencing was achieved by four successive walking steps with 13 viral specific and three cassette primers, corresponding to more than 50% reduction in the number of primers necessary to sequence the cognate gene of other strains

belonging to the same virus genus.

Johnson, M., Y. G. Abs El-Osta, et al. (2004). "An electrophoretic tool for the genetic characterisation and delineation of lungworms." Molecular and Cellular Probes **18**(3): 197.

<http://www.sciencedirect.com/science/article/B6WNC-4BYJXJ0-2/2/5a48181bf135895a9186baf26c61c3f>

In the present study, PCR-based single-strand conformation polymorphism (SSCP) analysis of the second internal transcribed spacer (ITS-2) of nuclear ribosomal DNA (rDNA) was applied to the genetic characterisation of *Dictyocaulus* from red deer from New Zealand and to its differentiation from species of lungworm from cattle and other hosts. Based on SSCP profiles, *Dictyocaulus* individuals from red deer from different geographical localities in New Zealand could be readily distinguished from those representing other lungworms examined, irrespective of low-level sequence variability in the ITS-2 (0.4-2.6%) detectable among individuals. The ITS-2 of *Dictyocaulus* from red deer differed in sequence by ~7-35% from congeners from other cervid hosts, demonstrating that this parasite is genetically distinct from other species of *Dictyocaulus* for which ITS-2 sequence data are presently available. The results emphasize the need for a large-scale molecular systematic study of *Dictyocaulus* specimens from various species of cervid and other ruminant hosts and the usefulness of mutation scanning for taxonomic, epidemiological and population genetic investigations.

Latorra, D., K. Arar, et al. (2003). "Design considerations and effects of LNA in PCR primers." Molecular and Cellular Probes **17**(5): 253.

<http://www.sciencedirect.com/science/article/B6WNC-49HMTKW-1/2/d6f7bb5d3260c777aff20f41b8bac8f0>

The effects of comprehensive LNA substitution in PCR primers for amplification of human genomic DNA targets are presented in this report. Previous research with LNA in other applications has shown interesting properties for molecular hybridization including enhanced specificity in allele-specific PCR. Here we systematically modified PCR primers and conditions for the human genomic DNA targets APOB and PAH, along with a [beta]-globin amplification control, to study whether the number and position of LNA residues improves or diminishes amplification sensitivity and specificity. It was observed that the design rules for LNA substitution in PCR primers are complex and depend upon number, position and sequence context. Technical advantages were seen when compared to DNA controls for the best LNA primer designs, which were typically one to a few centrally located LNA residues. LNA advantages include increased maximum annealing temperature (T_{max}) and increased signal with limiting primer or Taq DNA polymerase. Several well-characterized designs exhibited different efficiencies with different brands of hot-start enzymes. Many shorter LNA primers were found to be functional compared to same-length non-functional native DNA controls. These results show that LNA-substituted PCR primers have potential for use in difficult PCR techniques, such as multiplex amplification at higher T_{max}, once firm LNA primer design rules are established.

Letertre, C., S. Perelle, et al. (2003). "Evaluation of the performance of LNA and MGB probes in 5'-nuclease PCR assays." Molecular and Cellular Probes **17**(6): 307.

<http://www.sciencedirect.com/science/article/B6WNC-49V3GFV->

1/2/afd418a82f36e8ac7814c0c9b3a6ca76

The aim of this study was to evaluate the use of Locked Nucleic Acids (LNA) probes in 5'-nuclease PCR, by comparison with Minor Groove Binder (MGB) probes routinely practiced in laboratories on ABI Prism 7700. The comparison was made using a collection of *Staphylococcus aureus* strains that have already been characterized by MGB 5'-nuclease PCR assays in a previous study [Mol Cell Probes, submitted for publication]. The sensitivity and specificity of 5'-nuclease PCR assays targeting the *Staphylococcal* enterotoxin genes sea to see were compared and showed that the LNA and MGB methods were equivalent. In conclusion, the LNA 5'-nuclease PCR assays developed in this work provide a specific and sensitive alternative to the well-established MGB 5'-nuclease PCR assays used for the rapid detection of bacterial pathogens genes on ABI Prism 7700.

Letertre, C., S. Perelle, et al. (2003). "A strategy based on 5' nuclease multiplex PCR to detect enterotoxin genes sea to sej of *Staphylococcus aureus*." Molecular and Cellular Probes **17**(5): 227.

<http://www.sciencedirect.com/science/article/B6WNC-49H0RCT-1/2/479cd70c2a25bd6b2a3dbdd9bb6af9b8>

We describe the development of a strategy based on 5' nuclease multiplex PCR for the rapid detection of nine enterotoxin genes (sea, seb, sec, sed, see, seg, seh, sei, sej) of *Staphylococcus aureus*. The genotyping scheme consists in identifying these nine enterotoxin genes by three 5' nuclease Triplex-PCR assays. The strategy was evaluated using a collection of *S. aureus* reference strains previously examined with conventional PCR assays, and by testing previously characterized food *S. aureus* field strains. The 5' nuclease Triplex-PCR assays correctly detected the se genes in all the reference strains. In tests with field strains there was generally excellent agreement with the results obtained by conventional PCR, except for some strains harbouring variant se genes. The detection limits of the Triplex-PCR assays evaluated using fivefold dilution of recombinant plasmids for each se gene ranged from 16 to 2000 copies of target se genes in the PCR tube. The 5' nuclease Triplex-PCR assays developed are fast and specific, and provide a useful diagnostic tool for the detection and genotyping of se genes. The development of this method is an improvement that should facilitate epidemiological investigations of staphylococcal food poisoning outbreaks.

Liguori, R., A. Argiriou, et al. (2003). "A rapid method for detecting mutations of the human LDL receptor gene by complete cDNA sequencing." Molecular and Cellular Probes **17**(1): 15.

<http://www.sciencedirect.com/science/article/B6WNC-47YY41N-5/2/98c02ea5966e11b99b8eb8ef057985e3>

We have developed and clinically tested a rapid and largely automated procedure to detect mutations in the coding region of a gene of interest. Our method relies on the automated sequencing of the complete cDNA, followed by an advanced mutation search-and-verification routine using an integrated set of computer analysis tools. We have applied our automated procedure to the diagnosis of familial hypercholesterolemia (FH) in 52 unrelated FH families, by sequencing the whole cDNA coding region of the LDLR gene. Here we report the procedures and performance of our method in the identification of the most common types of LDLR mutations: short deletions or insertions and point mutations. Our method can provide a standard procedure for the 'overnight' unequivocal identification of mutations in those genetic diseases where several different mutations, none clearly prominent, may affect a given gene.

Lucotte, G. and T. Champenois (2003). "Duplex PCR-RFLP for simultaneous detection of factor V Leiden and prothrombin G20210A." Molecular and Cellular Probes **17**(5): 267.

<http://www.sciencedirect.com/science/article/B6WNC-49M0NCG-1/2/42146fb5541af3a19ab5c5acde0c0fb3>

The authors have used a primer-engineered duplex polymerase chain reaction (PCR) -restriction fragment length polymorphism (RFLP) for the simultaneous detection of factor V Leiden and prothrombin G20210A mutations. The method involves the generation of HindIII RFLPs, and the restricted amplification products are analyzed by agarose gel electrophoresis in a single gel lane. This method is simple and inexpensive, and readily adaptable to the routine in a clinical molecular diagnostic laboratory other than our own.

Mahbubani, M. H., A. K. Bej, et al. (1990). "Detection of Legionella with polymerase chain reaction and gene probe methods." Molecular and Cellular Probes **4**(3): 175.

<http://www.sciencedirect.com/science/article/B6WNC-4DYVPKN-30/2/d9484e9c36282bf2cab9c01f90e511ac>

Methods were developed for the detection of Legionella in environmental water sources, based upon the polymerase chain reaction (PCR) and gene probes. All species of Legionella, including all 15 serogroups of L. pneumophila tested, were detected by PCR amplification of a 104 bp DNA sequence that codes for a region of 5S rRNA followed by radiolabelled oligoprobe hybridization to an internal region of the amplified DNA. Strains of L. pneumophila (all serogroups) were specifically detected based upon amplification of a portion of the coding region of the macrophage infectivity potentiator (mip) gene. Pseudomonas spp, that exhibit antigenic cross-reactivity in serological detection methods did not produce positive signals in the PCR-gene probe method using Southern blot analyses. Single cell, single gene Legionella detection was achieved with the PCR--gene probe methods.

McIntosh, I., J. R. W. Govan, et al. (1992). "Detection of Pseudomonas aeruginosa in sputum from cystic fibrosis patients by the polymerase chain reaction." Molecular and Cellular Probes **6**(4): 299.

<http://www.sciencedirect.com/science/article/B6WNC-4DYN471-5/2/cceecc03b890edeaba042e6e9f0a8da65>

A DNA amplification procedure using heat stable Taq polymerase and the polymerase chain reaction is described for the detection of Pseudomonas aeruginosa in specimens from cystic fibrosis patients. A set of primers was selected on the basis of the nucleotide sequence of the algD gene encoding GDP mannose dehydrogenase, a major enzyme in the biosynthesis of alginate by P. aeruginosa. Using this set of primers in conjunction with the polymerase chain reaction, P. aeruginosa could be specifically detected, with a sensitivity approximating 10 bacteria, in sputum harbouring large numbers of other respiratory pathogens, including Staphylococcus aureus and Haemophilus influenzae. These results suggest that amplification of specific sequences within the algD gene by the polymerase chain reaction may provide a highly sensitive and specific tool for the detection of P. aeruginosa in the early stages of pulmonary colonization.

Rahn, K., S. A. De Grandis, et al. (1992). "Amplification of an *invA* gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella*." Molecular and Cellular Probes 6(4): 271.

<http://www.sciencedirect.com/science/article/B6WNC-4DYN471-2/2/97da22fe5149c7283a408d9e24000e0c>

Amplification of nucleotide sequences within the *invA* gene of *Salmonella typhimurium* was evaluated as a means of detecting *Salmonella*. A collection of 630 strains of *Salmonella* comprising over 100 serovars, including the 20 most prevalent serovars isolated from animals and humans in Canada, was examined. Controls consisted of 142 non-*Salmonella* strains comprising 21 genera of bacteria. Cultures were screened by inoculating a single colony of bacteria directly into a polymerase chain reaction (PCR) mixture which contained a pair of primers specific for the *invA* gene. The specific PCR product was a 284 bp DNA fragment which was visualized in 2% agarose gels. With the exception of two *S. litchfield* and two *S. senftenberg* strains, all *Salmonella* strains were detected. In contrast, none of the non-*Salmonella* strains yielded the specific amplification product. Non-specific amplification of a few non-*Salmonella* strains resulted in a product that was distinctly different in size from the specific 284 bp product. Specificity of amplification was further confirmed by demonstration of hybridization of a ³²P-labelled *invA* gene fragment only to the specific 284 bp product. The detection of 99.4% of *Salmonella* strains tested and the failure to specifically amplify DNA from non-*Salmonella* strains confirm that the *invA* gene contains sequences unique to *Salmonella* and demonstrate that this gene is a suitable PCR target, with potential diagnostic applications.

Rand, K. H. and H. Houck (1990). "Taq polymerase contains bacterial DNA of unknown origin." Molecular and Cellular Probes 4(6): 445.

<http://www.sciencedirect.com/science/article/B6WNC-4DYN471-4Y/2/93b2d51a768c56b297236c561b8c74b9>

The polymerase chain reaction (PCR) was carried out with the highly conserved *E. coli* ribosomal RNA gene sequences 1376-1395 and 1521-1540. Using these primers and reaction conditions specified by the manufacturer(s), a 165 bp fragment was synthesized using Taq polymerase from three different sources in the absence of any added template. Restriction enzyme analysis suggests the source of this bacterial DNA is neither *E. coli* nor *Thermus aquaticus*. A variety of different methods to eliminate it such as treatment with DNase, restriction enzyme digestion, and CsCl₂ density gradient centrifugation were unsuccessful. Since the bacteria in which the Taq polymerase is produced are not the source of the DNA, some step(s) in the purification or reagents added to the enzyme must be involved. Thus it is likely other biological products are similarly contaminated. Although the problem is easily dealt with by running a no-template control and choosing other primers if a problem exists, it is important to recognize the potential for a false-positive result.

Read, S. C., R. C. Clarke, et al. (1992). "Polymerase chain reaction for detection of verocytotoxigenic *Escherichia coli* isolated from animal and food sources." Molecular and Cellular Probes 6(2): 153.

<http://www.sciencedirect.com/science/article/B6WNC-4DYN471-5H/2/9d8f427eb96295c62e6c55d37dbf5017>

Animals and their by-products have been implicated as important sources of verocytotoxigenic *Escherichia coli* (VTEC) associated with disease in humans. VTEC comprise a wide range of

serotypes and produce a variety of closely related verocytotoxins (VT). A pair of oligonucleotide primers, targeting conserved sequences found in VT1, VT2 and VTE genes, was used to develop a polymerase chain reaction (PCR) procedure to detect all types of VTEC. Supernatants of boiled broth cultures of VTEC (223 strains) isolated from ground beef, ground pork, raw milk, bovine faeces and porcine faeces; non-VTEC *E. coli* (72 strains); and other enteric and food bacteria (76 strains) were tested by PCR. The verocytotoxigenicity of these strains was verified by the Vero cell assay. All 223 VTEC isolates, comprising over 50 different serotypes, were detected by the PCR procedure. *Shigella dysenteriae* type 1 was the only other bacterium that was positive in this assay. As little as 1 pg of VTEC DNA and as few as 17 cfu of VTEC could be detected with this method. The results indicate that these primers detect VTEC over a wide range of serotypes. This method may be applicable as a screening procedure for the detection of VTEC in samples of foods and faeces.

Rodriguez-Lazaro, D., J. Lloyd, et al. (2004). "Unexpected detection of DNA by nucleic acid sequence-based amplification technique." Molecular and Cellular Probes **18**(4): 251.

<http://www.sciencedirect.com/science/article/B6WNC-4BV4VGG-1/2/7ce3341590f1c11cfea8a6834b707551>

Nucleic acid sequence-based amplification (NASBA) is a technique that has been previously shown to selectively mediate the detection of RNA in microbial cells. In a series of tests, nucleic acids were extracted from *Salmonella enterica* serotype Typhimurium and *Mycobacterium avium* subsp. paratuberculosis, and subjected to four enzymatic treatments prior to NASBA. These enzymatic treatments were DNase, RNase, S1 nuclease, and RNase/S1 nuclease. The results obtained were different for the two bacteria. With *S. enterica* serotype Typhimurium, RNase and RNase/S1 nuclease abolished the NASBA signal, as expected. But with *M. avium* subsp. paratuberculosis RNase, S1 nuclease, and RNase/S1 nuclease had no effect on the NASBA signal, whereas DNase treatment abolished it. This indicates that in the latter bacterium, NASBA can detect DNA, and demonstrates the necessity of verifying the nucleic acid origin of a NASBA signal if detection of RNA is objective.

Sacramento, D., H. Bourhy, et al. (1991). "PCR technique as an alternative method for diagnosis and molecular epidemiology of rabies virus." Molecular and Cellular Probes **5**(3): 229.

<http://www.sciencedirect.com/science/article/B6WNC-4DYVPKN-1Y/2/5f259cecdfb8fb9375f9f963fdf08609>

We have investigated the PCR amplification technique of viral nucleic acids as an alternative protocol for diagnosis and epidemiological studies of rabies virus. A primer set mapping in the nucleoprotein cistron allowed a specific and sensitive amplification of infected brain material, fulfilling the diagnosis requirements. One hundred samples checked by Southern or dot-blot analysis using both radioactive and non-radioactive probes showed identical results in parallel with routine techniques. For molecular epidemiological studies we selected another set of conserved primers flanking the highly evolutive pseudogene ([Psi] gene) region. This set was found to be efficient for all tested fixed rabies virus strains or wild rabies virus isolates as well as the rabies-related Mokola virus. We describe a progressive characterization of the strain that could be extended from rapid typing by a limited panel of restriction enzymes, to the ultimate identification of the nucleotide sequence by an original direct sequencing technique of amplified segments.

Stanier, P., A. D. Kitchen, et al. (1992). "Detection of human cytomegalovirus in peripheral mononuclear cells and urine samples using PCR." Molecular and Cellular Probes **6**(1): 51.

<http://www.sciencedirect.com/science/article/B6WNC-4DYVPKN-2S/2/f33be0efa66bb597043a165b0d7b6480>

Samples of peripheral blood lymphocytes from 105 different blood donors were investigated for the presence of human cytomegalovirus (HCMV) DNA using the polymerase chain reaction (PCR) with primers specific for the Pst / w fragment (IE region). Viral DNA sequences were detected in 53 samples, a fifth of which had been previously serotyped as HCMV negative. In the latter cases, Western blot analysis re-determined two out of three individuals that were resampled as seropositive. PCR could therefore be used to extend existing methods employed for the identification of HCMV infected blood samples prior to transfusion to individuals in high risk groups. In addition, the value of PCR as a diagnostic test was evaluated in a small pilot study by comparing the results obtained with urine samples from babies suffering congenital infection and from other high risk patients, with data obtained by isolation of infectious virus or through the detection of immediate early antigens in infected cultures. Data from this study indicated that PCR is at least as sensitive as the other methods used in HCMV diagnosis.

Tondeur, S., O. Agbulut, et al. (2004). "Overcoming bacterial DNA contamination in real-time PCR and RT-PCR reactions for LacZ detection in cell therapy monitoring." Molecular and Cellular Probes **18**(6): 437.

<http://www.sciencedirect.com/science/article/B6WNC-4DBSV8W-1/2/9c12b833a03a8e8eb03c6843315499f7>

Since the introduction of the polymerase chain reaction the presence of contaminating bacterial DNA in the Taq polymerase preparations has hampered the use of this technique in microbiology. Lately, this inconvenience has equally impeded gene quantification in the field of cell or gene therapy, where bacterial genes such as LacZ are often used as tags to detect vectors or cells after their injection in the recipient organism. Several means to overcome the DNA contamination of Taq Polymerase have been reported with variable degrees of decontamination efficiency and alteration of the PCR reaction. Here we propose two protocols to efficiently quantify DNA or RNA from the LacZ gene by real-time PCR using either decontamination by low concentrations of DNase I prior to PCR amplification or a highly purified Taq Polymerase which is devoid of detectable contamination.

Vaccari, G., M. Conte, et al. (2004). "Primer extension assay for prion protein genotype determination in sheep." Molecular and Cellular Probes **18**(1): 33.

<http://www.sciencedirect.com/science/article/B6WNC-49RCFKJ-1/2/7adcbb930543adceeb15906ec4f589f5>

Scrapie is a transmissible spongiform encephalopathy (TSE) which affects sheep and goats. TSEs are characterised by the conversion of the cellular prion protein (PrP^C) into the pathological form PrP^{Sc}. The occurrence of scrapie in sheep is influenced by polymorphisms in the PrP gene; in particular, three codons (136, 154 and 171) are important in conditioning the susceptibility/resistance of sheep to the disease, with the Val/Val136 Arg/Arg154 Gln/Gln171 genotype being the most susceptible and the Ala/Ala136 Arg/Arg154 Arg/Arg171, the most resistant one. The latter genotype seems to confer, in sheep, resistance to the oral infection with bovine spongiform encephalopathy, as well. The selection of genetically resistant sheep

populations represents the basis of the recent strategies against ovine TSE in the European Union (EU). Herein, we describe a rapid and simple method, based on the primer extension technique, for PrP genotype determination at codons 136, 154 and 171. Intra-laboratory validation of the method showed accuracy levels comparable to those of sequencing analysis. Such method could be used for both the application of the EU policies requiring PrP genotype analysis in all ovine TSE cases, and the large-scale genotyping claimed by the implementation of breeding programmes for genetic resistance to TSE in sheep.

Vallo, L., E. Bonifazi, et al. (2005). "Characterization of a single nucleotide polymorphism in the ZNF9 gene and analysis of association with myotonic dystrophy type II (DM2) in the Italian population." Molecular and Cellular Probes **19**(1): 71.

<http://www.sciencedirect.com/science/article/B6WNC-4DSM9Y0-5/2/90b941f20455150b556f1fd3ace8c091>

Myotonic dystrophy type 2 (DM2) is a dominant inherited disorder clinically similar to myotonic dystrophy type 1 (DM1) with a peculiar pattern of multisystemic phenotypic features. The mutation responsible for DM1 is a CTG repeat in the 3' UTR of the dystrophin protein kinase gene (DMPK) on chromosome 19q13.3, while DM2 is caused by an unstable CCTG expansion in intron 1 of the zinc finger protein 9 gene (ZNF9) on chromosome 3q21.3. Southern blotting analysis is the conventional test used to determine the size of the repeats in the molecular diagnosis of DM2. However, the large number of CCTG repeats and their somatic instability complicates this diagnostic protocol. In order to improve the DM2 test, we have recently characterised a single nucleotide polymorphism located in the first intron of the ZNF9 gene. This SNP consists in a C to A nucleotide change, which creates or disrupts an Apal enzyme restriction site, easily detectable by PCR amplification followed by restriction analysis. We genotyped this SNP in 30 unrelated DM2 patients and 70 unrelated Italian healthy individuals. Our results show that this polymorphism is in linkage disequilibrium with the DM2 mutation.

Vandenbergh, D. J., M. D. Grant, et al. (2003). "A simple tandem repeat polymorphism is present in the eighth intron of FLJ12960, a possible queuine salvage enzyme gene." Molecular and Cellular Probes **17**(6): 319.

<http://www.sciencedirect.com/science/article/B6WNC-49HMTKW-2/2/4d1d7a37d132540e2dc4690425669469>

The putative gene FLJ12960 was identified from sequence analysis of the human genome and predicted to be a member of the family of tRNA-guanine-transglycosylases for queuine biosynthesis by protein sequence similarity at the Zn-binding site (Locus Link #79691 at <http://www.ncbi.nlm.gov/LocusLink/>). FLJ12960 is immediately downstream of the Dopamine Receptor D3 (DRD3) gene. A Simple Tandem Repeat (STR) polymorphism was identified in intron 8 of the FLJ12960 gene. Primers designed to amplify the CA repeat detect 16 alleles from 121 to 151 base pairs, with a heterozygosity of 0.74.

Yolken, R. H., A. M. Sierra-Honigmann, et al. (1991). "Solid phase capture method for the specific amplification of microbial nucleic acids--avoidance of false-positive and false-negative reactions." Molecular and Cellular Probes **5**(2): 151.

<http://www.sciencedirect.com/science/article/B6WNC-4DYN471->

23/2/da37722fa139a8452c19708354977b63

DNA amplification assays such as the polymerase chain reaction are being developed for the amplification of small quantities of microbial nucleic acids. These assays offer the potential for a great deal of sensitivity. However, the high level of sensitivity increases the likelihood of cross-contamination of amplified products and the generation of false-positive reactions. In addition, substances in body fluids can inhibit the efficient performance of the amplification reactions. We have developed an assay format in which microbial nucleic acids are specifically bound to a solid phase surface. Contaminating DNA and enzyme inhibitors present in the sample are removed by washing prior to the performance of the amplification reaction. We could use this system to amplify and detect small amounts of HIV DNA diluted in whole blood. The assay system could distinguish target DNA from contaminating DNA fragments generated by prior amplification reactions.

Yoshitomi, K. J., K. C. Jinneman, et al. (2003). "Optimization of a 3'-minor groove binder-DNA probe targeting the uidA gene for rapid identification of Escherichia coli O157:H7 using real-time PCR." Molecular and Cellular Probes **17**(6): 275.

<http://www.sciencedirect.com/science/article/B6WNC-49HMTKW-3/2/b8acd04ed072ee4b691f52a73d753c57>

Enterohemorrhagic Escherichia coli are harmful human pathogens capable of causing bloody diarrhea and vomiting. An important serotype commonly associated with human illness is the E. coli O157:H7 serotype. Unlike other real-time polymerase chain reaction (PCR) methods for identifying E. coli O157:H7, this study describes the development and optimization of a real-time PCR method targeting a conserved point mutation at +93 in the uidA (gusA) gene that is unique to O157:H7, distinguishing it from non-O157:H7 serotypes. A TET-labeled Minor Groove Binder (MGB) DNA probe was designed for use in a 5' nuclease PCR assay. Using a panel of two E. coli O157:H7 strains, three E. coli non-O157:H7 strains, and one non-E. coli species, the assay was optimized for the specific detection of the E. coli O157:H7 strains. Optimal conditions were identified at high anneal/extend temperatures, low magnesium concentrations, and low probe concentrations, resulting in correct identification of E. coli O157:H7 and non-O157:H7 strains. The improved specificity of MGB probes for single base pair mismatches such as the +93 uidA mutation provides a novel approach towards rapid identification of E. coli O157:H7.

Molecular Aspects of Medicine (1)

Hochmeister, M. N. (1995). "DNA technology in forensic applications." Molecular Aspects of Medicine **16**(4): 315.

<http://www.sciencedirect.com/science/article/B6T9P-3Y5FP3Y-2/2/3311dceaa2bbf5de1f5de54ef766e8c6>

Allan, S. M., D. C. Harrison, et al. (2001). "Selective increases in cytokine expression in the rat brain in response to striatal injection of [alpha]-amino-3-hydroxy-5-methyl-4-isoxazolepropionate and interleukin-1." Molecular Brain Research **93**(2): 180.

<http://www.sciencedirect.com/science/article/B6T07-443K68G-D/2/88eaf548edc608aad9b83955b407fa6>

A number of cytokines contribute to acute experimental neurodegeneration. The cytokine response can have detrimental or beneficial effects depending on the temporal profile and balance between pro- and anti-inflammatory molecules. Our recent data suggest that the pro-inflammatory cytokine interleukin-1[beta] (IL-1[beta]) acts at specific sites (e.g., the striatum) in the rat brain to cause distant cortical injury, when co-administered with the potent excitotoxin [alpha]-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (S-AMPA). The objective of the present study was to investigate changes in the expression of several cytokines simultaneously in the rat striatum and cortex after intrastriatal administration of vehicle, S-AMPA or human recombinant (hr) IL-1[beta] alone or S-AMPA co-injected with hrIL-1[beta] using reverse transcription-polymerase chain reaction (RT-PCR; Taqman(TM) fluorogenic probes) and enzyme-linked immunosorbent assay (ELISA). Injection of S-AMPA alone increased IL-6 mRNA expression in the ipsilateral striatum after 8 h, whilst striatal injection of IL-1[beta] alone increased local IL-1[beta] and IL-1ra mRNAs. The levels of mRNA encoding IL-1[alpha], IL-1[beta], IL-1ra, IL-6, IL-10 and TNF[alpha] were markedly elevated in the ipsilateral cortex 8 h after co-injection of S-AMPA and hrIL-1[beta]. Cortical mRNA levels for IL-4, IL-18, TGF[beta] and IFN[gamma] were not significantly different between treatment groups after 2 h or 8 h. A similar pattern of change in the levels of IL-1[alpha] and IL-6 protein was observed 8 h after treatment. These data demonstrate selective increases in the expression of cytokines in areas of remote cell death in response to administration of hrIL-1[beta] and S-AMPA. Such cytokines may be involved in the ensuing damage, and further clarification of their actions could aid future therapeutic strategies for several acute neurodegenerative disorders.

Barritt, L. C., B. Fritsch, et al. (1999). "Characterization of G-protein [beta][gamma] expression in the inner ear." Molecular Brain Research **68**(1-2): 42.

<http://www.sciencedirect.com/science/article/B6T07-3WF80M0-5/2/6eb6e5a0f62e14c89d80ea86a77262b6>

Heterotrimeric guanine nucleotide binding proteins (G-proteins) are composed of a diverse set of [alpha], [beta], and [gamma] subunits, which couple cell surface receptors to intracellular effectors, such as adenylyl cyclase, phospholipase C[beta], and ion channels. Both the G[alpha] and the G[beta][gamma] dimers mediate effector activity and are believed to contribute to the complexity of the signaling pathway. Molecular and immunocytochemical techniques were employed to determine diversity of G[beta] and G[gamma] subunit expression in the murine inner ear. PCR-based assessment of [lambda]ZAP unidirectional cDNA libraries, representing the cochlea and inner ear hair cells, indicated all five known G[beta] subunits were present in the cochlea, while only a subset of G[gamma] isoforms were found. New or novel G-protein [beta] and [gamma] subunits were not detected. cDNAs representing G[beta]1-4 and G[gamma]2, G[gamma]3, G[gamma]5, G[gamma]8olf subunit transcripts were isolated. In addition, cDNAs corresponding to the G[beta]5 and G[gamma]11 isoforms exhibited restricted expression to inner and outer hair cells, respectively. Antisera specific for G[beta]3, G[beta]4, G[gamma]3, G[gamma]5 and G[gamma]11 stained spiral ganglion and neurosensory hair cells. A unique finding was the variable topological distribution of G[gamma]3 in the spiral ganglion cells along

the cochlear axis. Collectively, our results demonstrate a complementary as well as differential distribution pattern for G[beta] and G[gamma] isoforms exists in the inner ear. The co-localization of various G-protein isoforms within the same cell type suggests specific combinatorial G[beta] and G[gamma] subunit associations may preferentially be formed. Thus, the detection of multiple subunits presumably reflects the extent of the functional diversity of inner ear signaling pathways and should provide specificity of G-protein mediated pathways.

Bates, S., S. J. Read, et al. (2001). "Characterisation of gene expression changes following permanent MCAO in the rat using subtractive hybridisation." Molecular Brain Research **93**(1): 70.

<http://www.sciencedirect.com/science/article/B6T07-43V9YTJ-F/2/0e685cf73e3b9f3ce2b370d652df66b5>

Failure of several putative neuroprotectants in large multicentred clinical trials has re-focussed attention on the predictability of pre-clinical animal models of stroke. Model characterisation and relationship to heterogeneous patient sub-groups remains of paramount importance. Information gained from magnetic resonance imaging (MRI) signatures indicates that the Zea Longa model of rat middle cerebral artery occlusion may be more representative of slowly evolving infarcts. Understanding the molecular changes over several hours following cerebral ischaemia will allow detailed characterisation of the adaptive response to brain injury. Using a fully characterised model of Zea Longa middle cerebral artery occlusion we have used the representational difference analysis (RDA) subtractive hybridisation method to identify transcripts that accumulate in the ischaemic cortex. Along with a number of established ischaemia-induced gene products (including MCP-1, TIMP-1, hsp 70) we were also able to identify nine genes which have not previously been shown to accumulate following focal ischaemia (including SOCS-3, GADD45[gamma], Xin).

Bellingham, J., D. Whitmore, et al. (2002). "Zebrafish melanopsin: isolation, tissue localisation and phylogenetic position." Molecular Brain Research **107**(2): 128.

<http://www.sciencedirect.com/science/article/B6T07-474FXT3-4/2/8e05d23db514d58b6a63a2c647a3d309>

Photoreception is best understood in retinal rods and cones, but it is not confined to these cells. In non-mammals, intrinsically photosensitive cells have been identified within several structures including the pineal, hypothalamus and skin. More recently novel light sensitive cells have been identified in the inner/basal retina of both teleosts and rodents. Melanopsin has been proposed as the photopigment mediating many of these non-rod, non-cone responses to light. However, much about the melanopsin gene family remains to be clarified including their potential role as photopigments, and taxonomic distribution. We have isolated the first orthologue of melanopsin from a teleost fish and show expression of this gene in a sub-set of retinal horizontal cells (type B). Zebrafish melanopsin, and orthologues of this gene, differ markedly from the vertebrate photopigment opsins. The putative counterion is not a glutamate but a tyrosine, the putative G-protein binding domain in the third cytoplasmic loop is not conserved, and they show low levels of amino acid identity (~27%) to both the known photopigment opsins and to other members of the melanopsin family. Mouse melanopsin is only 58% identical to *Xenopus*, and 68% identical to zebrafish. By contrast, the photosensory opsin families show ~75% conservation. On the basis of their structure, genomic organisation, discrete evolutionary lineage, and their co-expression with other opsins, the melanopsins are not obvious photosensory opsins. They might represent a separate branch of photopigment evolution in the vertebrates or they may have a non-direct photosensory function, perhaps as a photoisomerase, in non-rod, non-cone light detection.

Beltramo, M., M. Campanella, et al. (2003). "Gene expression profiling of melanocortin system in neuropathic rats supports a role in nociception." Molecular Brain Research **118**(1-2): 111.

<http://www.sciencedirect.com/science/article/B6T07-49FRBP6-2/2/418586ccfd813f10342be96f072ac1bf>

The melanocortin (MC) system is involved in several biological functions. Its possible role in nociception has recently attracted attention in the field. Published data suggest that melanocortin antagonists are analgesic and agonists are hyperalgesic. Gene expression information about the MC system components (receptor, agonist and antagonist) in pain relevant areas is at present limited. To deepen our knowledge, we studied the expression of MC system components in naive, sham and neuropathic rat spinal cord and dorsal root ganglia (DRG) by PCR and quantitative real-time PCR. MC4 receptor, proopiomelanocortin (POMC) and agouti-related protein (AgRP) transcripts were detected in both spinal cord and DRG, whereas MC3 receptor was detected only in the spinal cord. To study the relationship between the MC system and chronic pain, we used the chronic constriction injury model and gene expression analysis was performed in rats showing both tactile allodynia and thermal hyperalgesia. MC4 and POMC transcript were upregulated in the spinal cord of neuropathic rats, whereas MC3 and AgRP expression were unaffected. Thus, this study demonstrates for the first time the presence of AgRP in the spinal cord and DRG, suggesting that it could play a role in the regulation of MC system activity. In addition, the upregulation of POMC and MC4, in parallel with the presence of tactile allodynia and thermal hyperalgesia, further supports the idea of MC system involvement in nociception.

Bhogal, R. K., A. L. Mitchell, et al. (2004). "Identification of putative rat ribonuclease III by differential display: a novel rat mRNA expressed in a circadian manner in the rat suprachiasmatic nucleus." Molecular Brain Research **131**(1-2): 51.

<http://www.sciencedirect.com/science/article/B6T07-4DR13JP-B/2/f90d9062b19e5ef202731f2461fac106>

The suprachiasmatic nucleus (SCN) of the hypothalamus constitutes the principal site responsible for the generation and entrainment of circadian rhythms in mammals. The mechanisms of the circadian clock involve periodic gene expression. Here we report the use of differential display reverse transcriptase polymerase chain reaction to identify a novel rat mRNA sequence which is highly homologous to human ribonuclease III. Analysis of its expression in the rat brain by in situ hybridization histochemistry showed this transcript to be expressed at differing intensities at various sites. Temporal variation in expression was observed in the SCN, with a peak at circadian time (CT) 2 and a nadir at CT14. No significant changes in its expression were detected across the cycle within the supraoptic nucleus, cingulate cortex or caudate putamen.

Bigl, M., M. Beck, et al. (2000). "Altered phosphofructokinase mRNA levels but unchanged isoenzyme pattern in brains from patients with Alzheimer's disease." Molecular Brain Research **76**(2): 411.

<http://www.sciencedirect.com/science/article/B6T07-400WXRS-X/2/1ae384a937ca3b65746974b08f169e36>

In order to find out whether the increased phosphofructokinase (PFK) activities observed in brains from Alzheimer's disease (AD) patients are associated with alterations in PFK mRNA levels, we

determined total PFK mRNA and the three different PFK isoenzyme mRNAs in AD and control patients by ribonuclease protection assay (RPA) and quantitative RT-PCR. PFK mRNA levels were found increased in some brain areas in AD patients. While all three PFK isoenzyme mRNAs were detectable in every studied brain sample, no changes of the PFK isoenzyme pattern were observed in patients with AD.

Bloom, D. C., N. T. Maidment, et al. (1995). "Long-term expression of a reporter gene from latent herpes simplex virus in the rat hippocampus." Molecular Brain Research **31**(1-2): 48.

<http://www.sciencedirect.com/science/article/B6T07-3YKMC2T-5/2/3cb2472e83b91909303540dc73f6c6f4>

A problem in utilizing herpes simplex virus (HSV) as a vector for expression of foreign genes in CNS neurons has been the inability to facilitate long-term expression of the engineered genes. Previously, we showed that the murine moloney leukemia virus LTR would drive [beta]-galactosidase ([beta]-gal) transcription for extended periods from the latent viral genome in sensory, but not motor neurons. In this communication we further evaluate the utility of the LTR promoter for use in long-term expression vectors. Following stereotactic injection of 8117/43 (an ICP4 minus, non-replicating virus with the LTR driving the [beta]-gal gene, or KD6 (an ICP4 minus non-replicating virus not expressing [beta]-gal) into the hippocampus of rats, polymerase chain reaction (PCR) analysis of viral DNA after 2 months indicated that latent infections were established. Assaying by both -gal staining and reverse transcriptase PCR we demonstrate that (1) [beta]-gal can be detected for at least 6 months in hippocampal neurons, and (2) although the number of [beta]-gal transcripts in these cells drops considerably by 2 weeks, they can be detected during the period studied. These studies indicate that the LTR promoter is active and affords long-term expression in the CNS, albeit at comparatively low levels compared to those observed at acute times.

Brandle, U., E. Guenther, et al. (1998). "Gene expression of the P2X receptors in the rat retina." Molecular Brain Research **59**(2): 269.

<http://www.sciencedirect.com/science/article/B6T07-3TMX1Y9-H/2/c6b79f7b017bb9a18669974221d4c62d>

Molecular-biological methods were used to demonstrate the expression of six P2X receptor subunits (P2X1-P2X6) in retina and choroid. Despite the considerable evidence for signalling by extracellular nucleotides in other sensory systems, few studies have been undertaken in the eye. RT-PCR for the detection of P2X subunit mRNA in the rat of different postnatal developmental stages (P23-P210) revealed the presence of P2X2 and P2X4 mRNA in the retina and choroid; P2X3, and P2X5 were detected only in the retina. There was no evidence for P2X1 and P2X6 mRNA in the ocular tissue under investigation. Our data suggest that extracellular ATP may have influences on visual processing.

Brandle, U., K. Kohler, et al. (1998). "Expression of the P2X7-receptor subunit in neurons of the rat retina." Molecular Brain Research **62**(1): 106.

<http://www.sciencedirect.com/science/article/B6T07-3V3X5DR-G/2/8d54e456793a80af454b980a54f53be7>

Despite the considerable evidence of signaling by extracellular nucleotides in other sensory systems, few studies have been undertaken in the eye. Molecular and immunohistochemical methods were used to demonstrate the expression and cellular localization of the P2X7 receptor subunit in the retina and choroid. RT-PCR was used for the detection of P2X7 subunit mRNA in the rat of different postnatal developmental stages (P23-P210) and revealed the presence of P2X7-mRNA in the retina, but not in the choroid. In the adult rat retina, immunolabelling for P2X7 was detected in a number of cells in the inner nuclear layer (INL) and ganglion cell layer (GCL), suggesting different types of amacrine cells and ganglion cells. These results demonstrate for the first time the expression of the P2X7 receptor in the mammalian retina and furthermore in distinct neuronal cell populations. Our data suggest that extracellular ATP may provide both neuromodulatory and trophic influences on visual processing.

Burcelin, R., A. G. Rodriguez-Gabin, et al. (1997). "Molecular analysis of the monomeric GTP-binding proteins of oligodendrocytes." Molecular Brain Research **50**(1-2): 9.

<http://www.sciencedirect.com/science/article/B6T07-3S13DNH-2/2/24ab43a84952fe6d7557cf04449caa86>

Vesicle transport plays an important role in the formation of myelin. Transport of proteins, including proteolipid protein and myelin associated glycoprotein, from their site of synthesis in the endoplasmic reticulum in the perikaryon of the oligodendrocytes, to myelin, takes place via carrier vesicles. The mechanisms that regulate vesicle transport in oligodendrocytes are largely unknown. The presence of monomeric GTP-binding proteins in myelin and oligodendrocytes suggested the hypothesis that these proteins participate in the regulation of vesicle transport. In an attempt to identify the Rab and Rho GTP-binding proteins present in oligodendrocytes, a cDNA library specific for these proteins was generated using a reverse transcriptase-polymerase chain reaction (RT-PCR) approach. Twelve different clones containing sequences that coded for members of the Rab and Rho families of GTP-binding proteins were isolated. This group includes Rab1, -1b, -2, -5b, -5c, -7, -8, -12, -14, -23 and Rho A. One additional clone revealed a novel cDNA sequence. Analysis of the effector loop motif indicated that this sequence encodes for a member of the Rab family. We refer to this new sequence as Rab0. Comparison of Rab0 with the most similar rat Rab sequences, Rab 14 and Rab 22, and with a recently cloned human Rab22b, showed a 71%, 72% and 94% identity, respectively. By RT-PCR analysis the Rab0 mRNA was found to be mainly expressed in oligodendrocytes and to a lesser extent in oligodendrocyte precursors, astrocytes and microglia. Moreover, the highest levels of Rab0 mRNA were observed in areas of the brain that are heavily myelinated. Rab0 mRNA was also detected in other tissues such as kidney, liver, skeletal muscle. These data provide initial evidence regarding signal transduction pathways that regulate intracellular transport in oligodendrocytes.

Cadet, P., W. Zhu, et al. (2002). "Cold stress alters *Mytilus edulis* pedal ganglia expression of [mu] opiate receptor transcripts determined by real-time RT-PCR and morphine levels." Molecular Brain Research **99**(1): 26.

<http://www.sciencedirect.com/science/article/B6T07-44VX5V7-4/2/ce24b8ec47ac8231f1defa1f6b2fe96f>

Previous pharmacological, biochemical and molecular evidence prove that [mu]-subtype opiate receptors and opiate alkaloids, i.e. morphine, are present in the ganglionic nervous system of the mollusk *Mytilus edulis* (bivalve). We now present molecular evidence on the effect of rapid temperature changes on [mu] opiate receptor expression and morphine levels. Using primers, a labeled Taq-Man probe derived from the human neuronal [mu]1 opiate receptor, and real-time RT-PCR to measure the expression of [mu] transcripts from *Mytilus* pedal ganglia, we observe, in

animals placed in cold water from room temperature, an enhanced morphine and morphine 6 glucuronide level in addition to a decrease in $[\mu]$ opiate receptor gene expression. This study provides further evidence that $[\mu]$ -type opiate receptors and morphine are expressed in mollusk ganglia and appear to be involved in physiological processes responding to thermal stress.

Cai, F., W. M. Campana, et al. (1999). "Transforming growth factor- $[\beta]$ 1 and glial growth factor 2 reduce neurotrophin-3 mRNA expression in cultured Schwann cells via a cAMP-dependent pathway." Molecular Brain Research **71**(2): 256.

<http://www.sciencedirect.com/science/article/B6T07-3XNK0RN-F/2/81fa1fbda4a1afb3bb1327e90361648a7>

The aim of the study was to determine which factors regulated the expression of neurotrophin-3 (NT-3) mRNA in cultured primary Schwann cells derived from sciatic nerve of neonatal rats. Treatment of primary Schwann cells with the adenylate cyclase activator, forskolin, or the cAMP agonist, 8-Br-cAMP, induced a significant reduction in NT-3 transcript levels. Transforming growth factor- $[\beta]$ 1 (TGF- $[\beta]$ 1) and glial growth factor 2 (GGF2) also reduced the levels of NT-3 mRNA in a dose and time-dependent manner. Treatment with nerve growth factor, brain-derived neurotrophic factor, NT-3, ciliary neurotrophic factor or interleukin-1 $[\beta]$ was without effect. The TGF- $[\beta]$ 1, GGF2 and forskolin dependent reduction in NT-3 mRNA levels involved a destabilization of transcripts which was antagonised by co-treatment with cycloheximide. The cAMP-dependent protein kinase A (PKA) inhibitor, H-89, blocked the reduction in levels of NT-3 mRNA induced by TGF- $[\beta]$ 1, GGF2 and forskolin. The data show that the effects of TGF- $[\beta]$ 1, GGF2 and forskolin on the downregulation of NT-3 mRNA, at least in part, were due to a post-transcriptional event involving a labile protein intermediate under the control of PKA. The results suggest that the down-regulation of NT-3 mRNA in Schwann cells at a site of peripheral nerve damage may be mediated via a cAMP-dependent pathway and possibly involve neuroma-related elevations in TGF- $[\beta]$ 1 and GGF2.

Calver, A. R., D. Michalovich, et al. (2003). "Molecular cloning and characterisation of a novel GABAB-related G-protein coupled receptor." Molecular Brain Research **110**(2): 305.

<http://www.sciencedirect.com/science/article/B6T07-47X149W-B/2/834598b7e23d23e019ae9211c295936f>

Using a homology-based bioinformatics approach we have analysed human genomic sequence and identified the human and rodent orthologues of a novel putative seven transmembrane G protein coupled receptor, termed GABABL. The amino acid sequence homology of these cDNAs compared to GABAB1 and GABAB2 led us to postulate that GABABL was a putative novel GABAB receptor subunit. The C-terminal sequence of GABABL contained a putative coiled-coil domain, di-leucine and several RXR(R) ER retention motifs, all of which have been shown to be critical in GABAB receptor subunit function. In addition, the distribution of GABABL in the central nervous system was reminiscent of that of the other known GABAB subunits. However, we were unable to detect receptor function in response to any GABAB ligands when GABABL was expressed in isolation or in the presence of either GABAB1 or GABAB2. Therefore, if GABABL is indeed a GABAB receptor subunit, its partner is a potentially novel receptor subunit or chaperone protein which has yet to be identified.

Cappellini, C., P. Malatesta, et al. (1999). "Characterization of a cloned *Xenopus laevis* Serotonin 5-HT_{1A}

receptor expressed in the NIH-3T3 cell line." Molecular Brain Research **63**(2): 380.

<http://www.sciencedirect.com/science/article/B6T07-3VH80K8-R/2/f7bd4d16589cef9231e68ab7cef0d3f4>

In a previous work we isolated a *Xenopus* 5-HT_{1A} receptor gene and now report the characterization of this receptor. The HindIII-XbaI fragment of this gene was cloned into the pcDNA I NEO vector and stably transfected into eukaryotic cells (NIH-3T3). To determine the specific 5-HT_{1A} receptor binding, [³H]8-OH-DPAT was used as radioligand. The selective 5-HT_{1A} receptor agonist bound only a single class of saturable high-affinity binding sites with pharmacological characteristics similar to those of the mammalian 5-HT_{1A} receptor. The effects of 5-HT_{1A} receptor activation on cell growth were also investigated in stably transfected NIH-3T3 cells. The 5-HT_{1A} agonist 8-OH-DPAT was found to increase DNA synthesis and accelerated cell growth.

Cha, J.-H. J., S. L. Kinsman, et al. (1994). "RNA editing of a human glutamate receptor subunit." Molecular Brain Research **22**(1-4): 323.

<http://www.sciencedirect.com/science/article/B6T07-485P8V9-19/2/c0500dcda820d504a2271e0d73453a48>

AMPA receptors are comprised of individual subunits, and the divalent cation permeability of assembled AMPA receptors is determined by a single amino acid residue in the second transmembrane region of the GluR-B subunit. At this site, GluR-B subunits contain an arginine while other AMPA receptor subunits contain glutamine. Interestingly, the murine gene for GluR-B actually specifies a glutamine at the divalent cation permeability site. The appearance of arginine and not glutamine in the mature GluR-B protein is thought to be a result of RNA editing of the GluR-B messenger RNA. In that AMPA receptors are thought to mediate the bulk of fast excitatory signalling within the mammalian central nervous system, this process of RNA editing may play a pivotal role in normal neural function by mediating divalent cation permeability of AMPA receptors. Disruptions of RNA editing could lead to phenotypically altered AMPA receptors, with implications for pathogenic brain processes. We report that the human GluR-B gene sequence is also edited such that there is a difference between the human GluR-B gene and the complementary DNA (cDNA), as demonstrated both with allele-specific polymerase chain reaction (PCR) and restriction enzyme digestion of PCR products. Thus, as in the rodent brain, RNA editing of an AMPA receptor subunit appears to be an important process in the human brain. Disruptions of RNA editing may have neuropathological consequences.

Chan, S. O., W. D. Lyman, et al. (1997). "Temporal and spatial expression of glutamic acid decarboxylases in human fetal brain." Molecular Brain Research **46**(1-2): 318.

<http://www.sciencedirect.com/science/article/B6T07-3R37W4K-19/2/816a9e62d44b56bfc9d7ad63874b4af8>

The expression of two isoforms of glutamic acid decarboxylase, GAD67 and GAD65, was analyzed in central nervous system (CNS) tissues obtained from normal second trimester human fetuses after elective termination of pregnancy. After RT-PCR amplification of sequences contained in total RNA extracts, Southern blotting indicated that GAD67 and GAD65 mRNAs can be detected in frontal pole tissue as early as the 12th week of gestation (12 GW). GAD67 message is strongly expressed during early second trimester and decreases slightly thereafter but remains abundant. In contrast, GAD65 message decreases rapidly and becomes

undetectable by the 19 GW. However, GAD67 and GAD65 are similar in their spatial expression in the CNS at 22 GW. GAD67 and GAD65 messages are highly expressed in the cerebellum but expressed in low levels, if at all, in the spinal cord during this gestational period. These results suggest that GAD67 may have a greater role in neuron differentiation than GAD65 during human brain development.

Chang, A., P. P. Li, et al. (2003). "cAMP-Dependent protein kinase (PKA) subunit mRNA levels in postmortem brain from patients with bipolar affective disorder (BD)." Molecular Brain Research **116**(1-2): 27.

<http://www.sciencedirect.com/science/article/B6T07-493HSMV-1/2/6a6be20939e0fcd0bfb66ce0620b8f5a>

Earlier findings of elevated basal and stimulated PKA activities, and increased immunoreactive levels of PKA regulatory and catalytic subunits in discrete postmortem brain regions from bipolar disorder (BD) patients suggest that disturbances in PKA are involved in the pathophysiology of BD. PKA subunit mRNA levels were measured using SYBR Green real-time RT-PCR to determine if previously observed differences in immunoreactive levels of PKA RII[beta] and C[alpha] subunits were associated with corresponding changes in mRNA levels in temporal and frontal cortices from the same BD patients and matched controls. In distinct contrast to the higher immunolabeling levels of the PKA subunits previously reported in the BD brain, there were no significant differences in RII[beta] and C[alpha] subunit mRNA levels in the temporal and frontal cortices of BD patients compared with controls. These findings infer that the elevated PKA immunolabeling and activity found in the selected cerebral cortical regions of BD postmortem brain were due to a posttranscriptional mechanism, rather than changes in regulation of gene transcription and/or mRNA stability of the PKA subunits.

Chiang, L. W., F. E. Schweizer, et al. (1994). "Nitric oxide synthase expression in single hippocampal neurons." Molecular Brain Research **27**(1): 183.

<http://www.sciencedirect.com/science/article/B6T07-485P5VK-X/2/3f06c0f9a0b2d0a419a36db01df6edc0>

The presence of nitric oxide synthase (NOS) in CA1 pyramidal cells of the rat hippocampus was demonstrated by single-cell PCR. NOS-specific primers were used to amplify mRNA isolated from single hippocampal neurons. The sequence of the major amplification-product obtained was identical to that of the constitutively expressed brain-isoform of NOS. These results confirm immunocytochemical data that NOS is present in CA1, and, therefore, nitric oxide could function as a retrograde messenger in long-term potentiation.

Chinta, S. J., H. V. Pai, et al. (2002). "Constitutive expression and localization of the major drug metabolizing enzyme, cytochrome P4502D in human brain." Molecular Brain Research **103**(1-2): 49.

<http://www.sciencedirect.com/science/article/B6T07-45W39G4-1/2/2a8f796fe179fe2bf855d05b37e4c913>

Cytochrome P4502D6, an important isoform of cytochrome P450, mediates the metabolism of several psychoactive drugs in liver. Quantitatively, liver is the major drug metabolizing organ,

however metabolism of drugs in brain could modulate pharmacological and pharmacodynamic effects of psychoactive drugs at their site of action and explain some of the variation typically seen in patient population. We have measured cytochrome P450 content and examined constitutive expression of CYP2D mRNA and protein in human brain regions by reverse transcription polymerase chain reaction, Northern and immunoblotting and localized it by in situ hybridization and immunohistochemistry. CYP2D mRNA was expressed constitutively in neurons of cerebral cortex, Purkinje and granule cell layers of cerebellum, reticular neurons of midbrain and pyramidal neurons of CA1, CA2 and CA3 subfields of hippocampus. Immunoblot studies demonstrated the presence of cytochrome P4502D protein in cortex, cerebellum, midbrain, striatum and thalamus of human brain. Immunohistochemical localization showed the predominant presence of cytochrome P4502D not only in neuronal soma but also in dendrites of Purkinje and cortical neurons. These studies demonstrate constitutive expression of cytochrome P4502D in neuronal cell population in human brain, indicating its possible role in metabolism of psychoactive drugs directly at or near their site of action, in neurons, in human brain.

Cho, T. T. and A. I. Farbman (1999). "Neurotrophin receptors in the geniculate ganglion." Molecular Brain Research **68**(1-2): 1.

<http://www.sciencedirect.com/science/article/B6T07-3WF80M0-1/2/fa75e295e8ecdcb5918eeee4f9465388>

We examined the distribution of the high affinity neurotrophin receptors (trkA, trkB, and trkC) in the rat geniculate ganglion. Previous work had shown that during early (prenatal) development, trkB and its two ligands, BDNF and NT-4/5, were most important for survival of almost all neurons. Using nested polymerase chain reaction (PCR), we showed that trkA, trkB, and trkC transcripts were expressed, and the mRNAs for trkB and trkC were more abundant than that for trkA. We modified and improved the method for direct reverse transcription in situ PCR and localized trkB mRNA in approximately one third of the neurons in the ganglion. Immunohistochemical data confirmed that approximately the same fraction of neurons was immunoreactive with antibody vs. trkB, and an approximately equal fraction was immunoreactive with trkC antibody. These results are consistent with the notion that both BDNF/trkB and NT-3/trkC play important roles in maintenance of the geniculate ganglion neurons and possibly the peripheral taste system in the young postnatal rat.

Cho, Y., T.-W. L. Gong, et al. (2004). "Noise overstimulation induces immediate early genes in the rat cochlea." Molecular Brain Research **130**(1-2): 134.

<http://www.sciencedirect.com/science/article/B6T07-4D5JY3F-2/2/b456e67680c101b0eeec53b3b1a33764>

In mammals, exposure to intense noise produces a permanent hearing loss called permanent threshold shift (PTS), whereas a moderate noise produces only a temporary threshold shift (TTS). Little is known about the molecular responses to such high intensity noise exposures. In this study we used gene arrays to examine the early response to acoustic overstimulation in the rat cochlea. We compared cochlear RNA from noise-exposed rats with RNA from unexposed controls. The intense PTS noise induced several immediate early genes encoding both transcription factors (c-FOS, EGR1, NUR77/TR3) and cytokines (PC3/BTG2, LIF and IP10). In contrast, the TTS noise down-regulated the gene for growth hormone. The response of these genes to different noise intensities was examined by quantitative RT-PCR 2.5 h after the 90-min noise exposure. For most genes, the extent of induction correlates with the intensity of the noise exposure. Three proteins (EGR1, NUR77/TR3, and IP10) were detected in many regions of the unexposed cochlea. After exposure to 120 dB noise, these proteins were present at higher levels

or showed extended expression in additional regions of the cochlea. LIF was undetectable in the cochlea of unexposed rats, but could be seen in the organ of Corti and spiral ganglion neurons following noise. NUR77/TR3 was a nuclear protein before noise, but following noise translocated to the cytoplasm. These studies provide new insights into the molecular response to noise overstimulation in the mammalian cochlea.

Citron, B. A., J. E. Zoloty, et al. "Tissue transglutaminase during mouse central nervous system development: Lack of alternative RNA processing and implications for its role(s) in murine models of neurotrauma and neurodegeneration." **Molecular Brain Research In Press, Corrected Proof**
<http://www.sciencedirect.com/science/article/B6T07-4FDJ7C8-2/2/5831e75b6eee3a5e4f6b30375e5b4714>

Tissue transglutaminase (tTG) is a member of a multigene family principally involved in catalyzing the formation of protein cross-links. Unlike other members of the transglutaminase family, tTG is multifunctional since it also serves as a guanosine triphosphate (GTP) binding protein (G[alpha]h) and participates in cell adhesion. Different isoforms of tTG can be produced by proteolysis or alternative splicing. We find that tTG mRNA is expressed at low levels in the mouse CNS relative to other tissues, and at lower levels in the CNS of mouse in comparison to that of human or rat. tTG mRNA levels are higher in the heart compared to the CNS, for example, and much higher in the liver. Within the CNS, tTG message is lowest in the adult cerebellum and thalamus and highest in the frontal cortex and striatum. In the hippocampus, tTG expression is highest during embryonic development and falls off dramatically after 1 week of life. We did not find alternative splicing of the mouse tTG. At the protein level, the predominant isoform is [not, vert, similar]62 kDa. In summary, tTG, an important factor in neuronal survival, is expressed at low levels in the mouse CNS and, unlike rat and human tTG, does not appear to be regulated by alternative splicing. These findings have implications for analyses of rodent tTG expression in human neurodegenerative and neurotrauma models where alternative processing may be an attractive pathogenetic mechanism. They further impact on drug discovery paradigms, where modulation of activity may have therapeutic value.

Confaloni, A., A. Crestini, et al. "Rat nicastrin gene: cDNA isolation, mRNA variants and expression pattern analysis." **Molecular Brain Research In Press, Corrected Proof**
<http://www.sciencedirect.com/science/article/B6T07-4FPX2GC-1/2/8deec95a53ff24b033675b5ae2cf212a>

Nicastrin is a type 1 transmembrane glycoprotein that interacts with presenilin, Aph-1, and Pen-2 proteins to form a high molecular complex with gamma secretase activity. Then, nicastrin has a central role in presenilin-mediated processing of beta-amyloid precursor protein and in some aspects of Notch/glp-1 signaling in vivo. Here, we isolated a rat nicastrin cDNA and investigated gene expression in embryonic and adult rat tissues. The predicted amino acid sequence is comprised of 708 residues and showed a high degree of identity with other vertebrate orthologs. Besides full-length nicastrin mRNA, we identified an alternative spliced variant lacking the whole exon 3 and predicted to encode a 62-residue-long truncated protein. Full-length nicastrin mRNA was observed to be ubiquitously expressed, while the spliced variant was preferentially transcribed in the nervous system, whether in embryonic or adult neural tissues. Studies performed on primary cell cultures demonstrated that the short isoform was expressed in neurons, but not in astrocyte and microglial cells. Further experiments performed to verify the presence of the variant in neuroblastoma culture failed to show any truncated protein. Treatments by cyclohexamide showed the involvement of a quality control-based surveillance mechanism, which selectively degrades the exon 3-skipped isoform. In summary, this is the first report describing a novel skipped isoform of nicastrin which may suggest a new possible control mechanism based on the alternative splicing and nonsense-mediated mRNA decay to regulate

brain protein expression and provide newer insights into potential implication in Alzheimer's disease.

Connell, J. W., T. Rodriguez-Martin, et al. "Quantitative analysis of tau isoform transcripts in sporadic tauopathies." Molecular Brain Research **In Press, Corrected Proof**
<http://www.sciencedirect.com/science/article/B6T07-4FTS2RH-1/2/7f309513c5ecec1d7144f877a22cd2ed>

A number of neurodegenerative diseases, including Alzheimer's disease (AD), are characterized by intraneuronal accumulation of the tau protein. Some forms of FTDP-17 are caused by mutations in the tau gene affecting exon 10 splicing. Therefore, dysregulation of tau pre-mRNA splicing may be a contributing factor to sporadic tauopathies. To address this question, we devised a real-time RT-PCR strategy based on the use of a single fluorogenic probe to evaluate the ratio between tau isoforms containing or lacking exon 10 (4R/3R ratio) in post-mortem brain samples. We found a two- to six-fold increase in the 4R/3R ratio in cases of FTDP-17 linked to a splice site mutation, hence confirming the validity of the strategy. The difference in the 4R/3R ratio in the superior temporal and superior frontal gyri between AD and control brains was not statistically significant. Similarly, there was no significant difference in the 4R/3R ratio between Pick's disease cases and controls, indicating that the predominance of tau3R protein in PiD reflects post-translational modifications of specific isoforms. This study indicates that post-translational events are likely to be the main factors controlling tau isoform composition in sporadic tauopathies and highlights the benefit of quantitative RT-PCR in the assessment of splicing abnormalities in tauopathies.

Crispino, M., C. P. Capano, et al. (2001). "Messenger RNAs in synaptosomal fractions from rat brain." Molecular Brain Research **97**(2): 171.

<http://www.sciencedirect.com/science/article/B6T07-44MGNFC-1/2/94cbbb85282bc585a0f5919085e6cc41>

Synaptosomal fractions from rat brain have been analyzed with semi-quantitative RT-PCR methods to determine their content of mRNAs coding for presynaptic, postsynaptic, glial, and neuronal proteins. Each mRNA was determined with reference to the standard HPRT mRNA. In our analyses, mRNAs were considered to be associated with synaptosomes only if their relative amounts were higher than in microsomes prepared in a polysome stabilizing medium, rich in Mg⁺⁺ and K⁺ ions, or in the homogenate. According to this stringent criterion, the following synaptosomal mRNAs could not be attributed to microsomal contamination and were assumed to derive from the subcellular structures known to harbor their translation products, i.e. GAT-1 mRNAs from presynaptic terminals and glial processes, MAP2 mRNA from dendrites, GFAP mRNA from glial processes, and TAU mRNA from neuronal fragments. This interpretation is in agreement with the involvement of extrasomatic mRNAs in local translation processes.

D'Arcangelo, G., G. G. Miao, et al. (1996). "Detection of the reelin breakpoint in reeler mice." Molecular Brain Research **39**(1-2): 234.

<http://www.sciencedirect.com/science/article/B6T07-3W496SN-VI/2/a9ddfebea06ae9cf7529cc41dfa24116>

Disruption of the reelin gene by partial deletion causes the neurological phenotype known as

reeler. Here we report the cloning and sequencing of the reelin breakpoint region from the Jackson reeler strain (rl). Based on this sequence, we developed a polymerase chain reaction screen that allows the identification of mutant mice prior to the appearance of the phenotype. The assay also permits discrimination of heterozygous from wild-type mice. These findings provide a strategy for the characterization of the early anatomical and physiological consequences of the reeler mutation.

Duzhy, D. E., Y. Sakai, et al. (2004). "Cloning and developmental expression of Shaker potassium channels in the cochlea of the chicken." Molecular Brain Research **121**(1-2): 70.

<http://www.sciencedirect.com/science/article/B6T07-4BCXM1S-3/2/c39719b36d4e0fa7f9ba290eafa25ef1>

Signal coding by the receptor and neuronal cells of the auditory system involves various ion channels that modulate a sound stimulus. The genes that encode a number of these ion channels and their accessory subunits are presently unknown for channels found in the sensory epithelium and cochlear nerve. Among these genes are those that encode delayed rectifier and transient type potassium channels found in both the sensory cells and the ganglion. Here, we report the cloning and developmental expression of Shaker family members that include cKv1.2, cKv1.3, cKv1.5, and the Shaker-related cGMP-gated potassium channel cKCNA10. Clones were obtained by screening a chicken embryonic cochlea cDNA library using, as a probe, a mixture of two DNA fragments of cKv1.2 and cKv1.3 obtained by the reverse transcription polymerase chain reaction (RT-PCR). Sequence analysis revealed chicken homologues of Kv1.2, Kv1.3, Kv1.5 and cGMP-gated potassium channels with a deduced amino acid homology of 96-98%, 82-84%, 67-71% and 67-79% to correspondent mammalian homologues. During development of chicken inner ear, RT-PCR studies show expression of cKv1.2, cKv1.3 and cKv1.5 as early as Embryonic Day (ED) 3, while cKCNA10 was detected at low levels beginning on ED6 and was highly expressed by ED9. Additionally, analysis of expression in different parts of the cochlea showed that these genes were co-expressed in different regions of the cochlea, including the cochlear ganglion, sensory epithelium, lagena, and tegmentum. This expression pattern suggests the potential for the formation of heteromeric channels from the corresponding [alpha]-subunits in these various tissues.

Edwards, M. A., R. A. Loxley, et al. (2004). "Unique levels of expression of N-methyl--aspartate receptor subunits and neuronal nitric oxide synthase in the rostral ventrolateral medulla of the spontaneously hypertensive rat." Molecular Brain Research **129**(1-2): 33.

<http://www.sciencedirect.com/science/article/B6T07-4CYWTB0-1/2/05fcbd08b048b0f14502ee991abf8887>

The rostral ventrolateral medulla (RVLM) is the major brainstem region contributing to sympathetic control of blood pressure. We have compared the expression of N-methyl--aspartate (NMDA) receptor subunits (NR1, NR2A-D), NR1 splice variants (NR1-1a/1b, -2a/2b, -3a/3b, -4a/4b), and the neuronal and inducible isoforms of NO synthase (nNOS and iNOS) in the RVLM of Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR), based on the hypothesis that altered NMDA receptor make-up or altered expression of endogenous NO may be associated with the increase in sympathetic output described from this site in hypertension. Total RNA was extracted and reverse transcribed from the RVLM of mature male WKY and SHR (16-23 weeks). Conventional polymerase chain reaction (PCR) indicated that only the NR1 splice variants NR1-2a, NR1-2b, NR1-4a and NR1-4b were expressed in the RVLM of either species. Quantitative real-time PCR indicated that for both strains of rat, mRNA for the NR1 subunit (all splice variants) was the most abundant (16.5-fold greater, $P \leq 0.05$). This was confirmed

immunohistochemically for nNOS expression. These results demonstrate differential expression levels of NMDA receptor subunits and NOS isoforms in the RVLM region of SHR when compared to WKY rats.

Eva, C., M. Fusco, et al. (1992). "Nerve growth factor modulates the expression of muscarinic cholinergic receptor messenger RNA in telencephalic neuronal cultures from newborn rat brain." Molecular Brain Research **14**(4): 344.

<http://www.sciencedirect.com/science/article/B6T07-484NCGY-8B/2/43984521b2b5503a4195ed91973bdc48>

The effect of nerve growth factor (NGF) on muscarinic receptor subtypes was investigated in a primary culture of telencephalic neurons prepared from neonatal rats. The treatment with 100 ng/ml of NGF significantly enhanced choline acetyltransferase (ChAT) activity and intracellular acetylcholine (ACh) content during cultivation. The same treatment induced an early transient increase of the number of muscarinic cholinergic receptors (mAChR), as measured by [3H]quinuclidinyl benzilate binding to cell homogenate, that was followed by a dramatic decrease of the receptor density from the 9th day of culture. Atropine completely prevented the decrease of the maximal number of muscarinic recognition sites induced by NGF. Prolonged exposure of telencephalic neurons to NGF also induced a significant reduction of the relative content of the messenger RNA (mRNA) encoding m1 and m3 receptors, while the m4 transcript was increased by the treatment. We suggest that the prolonged stimulation of cholinergic neurons by NGF induces a downregulation of m1 and m3 mAChR and their mRNAs on the postsynaptic site, while it increases the synthesis of the functionally distinct m4 receptor subtype, which might be presynaptically localized on cholinergic neurons. The transient increase of the receptor number that occurs at the first days of culture was not paralleled by changes in the relative content of mAChR mRNAs and might be associated with the trophic activity of NGF on cholinergic synapses during early development.

Fathi, Z., P. M. Battaglini, et al. (1998). "Molecular characterization, pharmacological properties and chromosomal localization of the human GALR2 galanin receptor." Molecular Brain Research **58**(1-2): 156.

<http://www.sciencedirect.com/science/article/B6T07-3T8P2J7-J/2/f1a247a9f5c544cf6c313c20f964e2ed>

The neuropeptide galanin mediates a diverse spectrum of biological activities by interacting with specific G protein-coupled receptors. We have used homology genomic library screening and polymerase chain reaction (PCR) techniques to isolate both genomic and cDNA clones encoding the human homolog of the recently cloned rat GALR2 galanin receptor. By fluorescence in situ hybridization, the gene encoding human GALR2 (GALNR2) has been localized to chromosome 17q25.3. The two coding exons of the human GALNR2 gene, interrupted by an intron positioned at the end of transmembrane domain III, encode a 387 amino acid G protein-coupled receptor with 87% overall amino acid identity with rat GALR2. In HEK-293 cells stably expressing human GALR2, binding of porcine galanin is saturable and can be displaced by galanin, amino-terminal galanin fragments and chimeric galanin peptides but not by carboxy-terminal galanin fragments. In HEK-293 cells, human GALR2 couples both to G[alpha]q/11 to stimulate phospholipase C and increase intracellular calcium levels and to G[alpha]i/o to inhibit forskolin-stimulated intracellular cAMP accumulation. A wide tissue distribution is observed by reverse transcriptase (RT)-PCR analysis, with human GALR2 mRNA being detected in many areas of the human central nervous system as well as in peripheral tissues.

Fathi, Z., A. M. Cunningham, et al. (1997). "Cloning, pharmacological characterization and distribution of a novel galanin receptor." Molecular Brain Research **51**(1-2): 49.

<http://www.sciencedirect.com/science/article/B6T07-3S1PFHR-7/2/fbc6dfc28bd830046d2ad2f941cb3d01>

The neuropeptide galanin mediates a diverse spectrum of biological activities by interacting with specific G-protein-coupled receptors. Through expression cloning, human and rat GALR1 receptor cDNA clones have previously been isolated and characterized. In this study, we have used homology screening to isolate a rat brain cDNA clone encoding a second galanin receptor subtype, the GALR2 receptor. The isolated cDNA encodes a 372-amino-acid G-protein-coupled receptor that shares 38% overall amino-acid identity with the rat GALR1 receptor. The pharmacological profile of the rat GALR2 receptor is similar to that of the rat GALR1 receptor. The rat GALR2 receptor binds galanin, N-terminal galanin fragments, and the putative galanin receptor antagonists galantide, C7, M35 and M40 with high affinity but it does not bind C-terminal galanin fragments. Galanin increases intracellular inositol phosphate levels in HEK 293 cells expressing the rat GALR2 receptor via a pertussis toxin-insensitive G-protein. The rat GALR2 receptor mRNA is highly expressed in several brain regions, including hypothalamus and hippocampus as well as the anterior pituitary, with lower levels of expression detected in amygdala, and regions of cortex. It is also highly expressed in the GH3 pituitary cell line and in gut tissues, and to a lower extent in spleen, lung, skeletal muscle, heart, kidney, liver and testis. These results suggest that GALR2 receptor mediates galanin's regulation of pituitary hormone secretion and possibly food intake.

Follesa, P. and M. K. Ticku (1995). "Chronic ethanol treatment differentially regulates NMDA receptor subunit mRNA expression in rat brain." Molecular Brain Research **29**(1): 99.

<http://www.sciencedirect.com/science/article/B6T07-3YGV58W-15/2/fe86e448ee542cd93457126f984ee1db>

Previous studies have implicated the N-methyl--aspartate receptor (NMDAR) complex in the physical dependence and withdrawal effects of chronic ethanol administration. In this study, we examined the effect of chronic ethanol administration and ethanol withdrawal on the NMDAR subunit R1, R2A, R2B, and R2C mRNA levels in rat hippocampus, cerebral cortex, and cerebellum. Using the RNase protection assay, we compared the levels of the NMDAR subunits mRNAs in ethanol-treated and control rats. Our results indicate that chronic ethanol administration and ethanol withdrawal do not change the NMDAR R1 subunit mRNA levels in cerebral cortex, hippocampus, or cerebellum at any time point. In contrast, 9 h after the last ethanol administration the R2A and R2B mRNA subunits were elevated by [ap] 40% in cerebral cortex, and [ap] 30% in hippocampus with respect to the levels in control animals. At 48 h the mRNA levels returned to the control levels. The chronic ethanol treatment did not alter R1, R2A, and R2C subunit mRNA levels in cerebellum. Our results demonstrate that chronic ethanol administration produces a differential regulation of the genes encoding the various subunits of the NMDAR.

Frostholm, A., J. E. Evans, et al. (2000). "Harmaline-induced changes in gamma aminobutyric acidA receptor subunit mRNA expression in murine olivocerebellar nuclei." Molecular Brain Research **85**(1-2): 200.

<http://www.sciencedirect.com/science/article/B6T07-423JKMG-S/2/2bd304c420a855db2090501da7f53522>

Increased CNS activity in the form of electrically or chemically induced seizures is known to alter the properties of GABAA receptors. The tremorgen, harmaline, causes a bursting pattern of activity in inferior olivary neurons, the effects of which are transmitted throughout the olivocerebellar circuit to other regions of the CNS. In situ hybridization was used to determine the effect of this increased activity on gamma aminobutyric acidA (GABAA) receptor subunit gene expression in the cerebellar Purkinje cell layer, deep cerebellar nuclei and inferior olivary complex of adult mice. In Purkinje cells, the expression of [alpha]1, [beta]2, and [gamma]2 mRNAs was increased only slightly (2 transcript levels were initially elevated (26%), but dropped to control levels immediately thereafter. The expression of [alpha]2, [alpha]4, [beta]3 and [gamma]1 mRNAs in olivary neurons was affected differentially by harmaline administration. The [alpha]4 transcript was increased, reaching >60% above control levels at 6 h post-injection. A smaller increase was observed for [alpha]2 mRNA, while [beta]3 and [gamma]1 transcripts dropped below control levels during the same period. The expression of corticotropin-releasing factor mRNA was also elevated in the olivary complex. These data indicate that while Purkinje cells and deep cerebellar neurons are only minimally affected, harmaline induced changes in cellular properties may result in increased numbers of [alpha]4-containing, diazepam-insensitive, GABAA receptors in olivary neurons.

Frostholm, A., D. Zdilar, et al. (1992). "Ontogeny of GABAA/benzodiazepine receptor subunit mRNAs in the murine inferior olive: transient appearance of [beta]3 subunit mRNA and [3H]muscimol binding sites." Molecular Brain Research **16**(3-4): 246.

<http://www.sciencedirect.com/science/article/B6T07-485H1Y9-B/2/47c8f2c54d45c5ac5ceb0f58b1e86541>

The GABAA/benzodiazepine receptor consists of at least four subunits, [alpha], [beta], [gamma] and [delta], each comprised of several variants. The developmental expression of the [alpha]1, [beta]1-3, [gamma]2 and [delta] subunits was studied in the murine inferior olivary nucleus by in situ hybridization with antisense cRNA probes. The postnatal appearance and distribution of [3H]flunitrazepam and [3H]muscimol binding sites, [alpha] and [beta] subunit-specific ligands respectively, were also studied autoradiographically. The [beta]3 subunit was transiently expressed in each of the subnuclei of the inferior olive: The signal was strong at birth, increased throughout postnatal week 1 and rapidly declined thereafter to low adult levels. A similar pattern of labeling was observed with [3H]muscimol. Detectable levels of [alpha]1 subunit mRNA hybridization signal and [3H]flunitrazepam binding sites were also present in the inferior olive at birth, decreasing thereafter. Low to moderate levels of [beta]1, [beta]2, and [gamma]2 subunit mRNAs were present in olivary neurons throughout postnatal development, while [delta] mRNAs were largely absent. It has been reported previously that, during the 2nd postnatal week, the ratio of climbing fiber terminals to Purkinje cells is reduced from 3: 1, as observed in neonates, to the 1: 1 relationship observed in the adult cerebellar cortex. Our results raise the possibility that the subunit composition of the GABAA/benzodiazepine receptor in inferior olivary neurons undergoes changes during development, and that this process may be related to the elimination of multiple climbing fiber innervation of cerebellar Purkinje cells.

Gerhart, D. Z., R. L. Leino, et al. (1994). "GLUT1 and GLUT3 gene expression in gerbil brain following brief ischemia: an in situ hybridization study." Molecular Brain Research **25**(3-4): 313.

<http://www.sciencedirect.com/science/article/B6T07-485H291-5M/2/846ced34a13e585f5e11b131d288d1dd>

GLUT1 and GLUT3 mRNAs in normal and post-ischemic gerbil brains were examined qualitatively and semi-quantitatively using in situ hybridization in conjunction with image analysis. Coronal brain sections at the level of the anterior hippocampus were prepared three hours, one day, and three days after animals were subjected to six min of ischemia. The sections were hybridized with vector- and PCR-generated RNA probes labeled with 35S. Microscopic evaluation of hybridized brain sections coated with autoradiographic emulsion indicated that GLUT1 mRNA was associated with brain microvessels, choroid plexus, and some ependymal cells. GLUT1 mRNA was not observed in neurons, except that one day following ischemia, this mRNA was induced in neurons of the dentate gyrus. GLUT3 mRNA was detected only in neurons. Image analysis of film autoradiograms revealed that both the GLUT1 and GLUT3 messages increased following ischemia but returned nearly to control levels by day three. In the CA1 region of the hippocampus the increase in GLUT3 mRNA was not statistically significant, and by day three the level had fallen significantly below the control, coinciding with the degeneration of the CA1 neurons. Our results suggest that the brain possesses mechanisms for induction and up-regulation of glucose transporter gene expression.

Grafstein-Dunn, E., K. H. Young, et al. (2001). "Regional distribution of regulators of G-protein signaling (RGS) 1, 2, 13, 14, 16, and GAIP messenger ribonucleic acids by in situ hybridization in rat brain." Molecular Brain Research **88**(1-2): 113.

<http://www.sciencedirect.com/science/article/B6T07-42R0S6R-K/2/fa475951770b91b954868b009faea808>

Regulators of G-protein signaling (RGS) proteins are a novel family of GTPase-activating proteins that interact with G[alpha] subunits of the Gi/o, Gz, Gq and G12/13 subfamilies to dampen G-protein-coupled receptor (GPCR)-mediated signaling by accelerating intrinsic G[alpha]-GTPase activity. In the present study, we report on messenger ribonucleic acid (mRNA) localization in rat brain of six RGS genes by in situ hybridization. The distribution patterns of RGS2, RGS13, RGS14 and GAIP (G[alpha] interacting protein) overlapped in most brain regions examined. Highest regional expression was observed for RGS2 in the cerebral cortical layers, striatum, hippocampal formation, several thalamic and hypothalamic nuclei and hindbrain regions such as the pontine, interpeduncular and dorsal raphe nuclei. Levels of RGS14 mRNA closely paralleled those of RGS2 expression levels throughout most brain regions. RGS13 mRNA was enriched in the hippocampal formation, amygdala, mammillary nuclei as well as the pontine and interpeduncular nuclei. GAIP expression levels were highest in the hippocampal formation with moderate to low levels present in all other regions studied. Of the six RGS genes probed, RGS16 mRNA displayed a discrete localization predominantly in the thalamic midline/intralaminar and principal relay nuclei, and the hypothalamic suprachiasmatic nucleus. RGS1 mRNA signal was not detected in brain. In conclusion, the in situ hybridization studies for RGS2, RGS13, RGS14, RGS16 and GAIP mRNAs extend our knowledge of the distribution of RGS genes expressed in the rat central nervous system, and indicate overlapping RGS-enriched regions that may be indicative of functional diversification in GPCR signaling pathway modulation.

Gray, C. W. and A. J. Patel (1993). "Regulation of [beta]-amyloid precursor protein isoform mRNAs by transforming growth factor-[beta]1 and interleukin-1[beta] in astrocytes." Molecular Brain Research **19**(3): 251.

<http://www.sciencedirect.com/science/article/B6T07-4859N5F-7C/2/d7cbd85eea9f1bfff303fb2762fa09bd>

In cultured astrocytes, all three major transcripts of [beta]-amyloid precursor protein (APP) were expressed with the ratio for APP695, APP751 and APP770 isoform mRNAs being 1:4:2. In

comparison with controls, treatment of astrocytes with transforming growth factor-[beta]1 (TGF-[beta]) produced about 6 fold increase in total APP mRNA, while elevation in the interleukin-1[beta] (IL-1[beta]) treated group was small and may relate to the mitogenic effect of IL-1[beta] on astrocytes. Treatment of astrocytes with cytokines also produced marked changes in the upregulation in expression of different APP isoforms. The net increase in mRNAs of KPI-containing isoforms APP751 and APP770 was relatively more than for the APP695 isoform. This phenomenon was mainly related to the differences in the expression of KPI-containing APP isoforms and APP695 isoform in the controls. The present findings provide further evidence for the involvement of astrocytes in a cascade of events leading to the development of senile plaques in Alzheimer's disease and Down's syndrome.

Gubba, E. M., J. W. Fawcett, et al. (2004). "The effects of corticosterone and dehydroepiandrosterone on neurotrophic factor mRNA expression in primary hippocampal and astrocyte cultures." Molecular Brain Research **127**(1-2): 48.

<http://www.sciencedirect.com/science/article/B6T07-4CS4RRD-1/2/2cc31664fb6870b1ec91ec36dd8a773b>

The effects of corticosterone (CORT) and dehydroepiandrosterone (DHEA) on the expression of growth factor mRNA in either primary hippocampal cultures or astrocyte-enriched cultures from E18 CD rats was studied. In mixed primary cultures, 1 [mu]M CORT up-regulated basic fibroblast growth factor (bFGF; FGF2) after 6 h of exposure, but down-regulated nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) were unchanged. A 100 [mu]M NMDA alone decreased NT-3, increased BDNF, but had no effect on NGF or FGF2. Concurrently administered CORT had no additional effect on either NGF, BDNF or NT-3, but up-regulated FGF2. In astrocytic cultures, 1 [mu]M CORT increased FGF2 and NT-3, but decreased BDNF and NGF. A dose-response study confirmed these results. DHEA (100 nM) up-regulated NGF after 3 h, but not at other time points (6, 12, 24, 48 h). It had no effect on the other growth factors in mixed primary cultures. In astrocytic cultures, there was no effect of DHEA. Adding DHEA or its sulphate (up to 1 [mu]M) to CORT did not alter the latter's action on growth factor mRNA expression. These results show that CORT has a selective action on growth factor expression, which was greater in astrocytic than in mixed cultures, that CORT amplifies or moderates activity-induced expression following NMDA, but that DHEA does not influence the effects of CORT on growth factor mRNA expression under these conditions.

H. Wheeler-Schilling, T., K. Marquardt, et al. (2000). "Expression of purinergic receptors in bipolar cells of the rat retina." Molecular Brain Research **76**(2): 415.

<http://www.sciencedirect.com/science/article/B6T07-400WXRS-Y/2/5210503c23a7c0e2ea8f2ed6e5337471>

P2X receptors are ligand-gated ion channels which are activated by excitatory neurotransmitter ATP. Despite considerable evidence of signaling by extracellular nucleotides in other sensory systems, P2X receptors in the visual system have only rarely been studied, and almost nothing is known about their functional significance in the retina. To determine whether ATP plays a role in the modulation of vertical retinal signal pathways, we examined the expression of P2X receptor mRNA in freshly isolated bipolar cells of the rat retina (Brown Norway, P25) using the single-cell RT-PCR technique. Positive amplification signals were found in about 33% of the bipolar cells for P2X3, P2X4 and P2X5 but not for P2X7 mRNA. We conclude that at least a subpopulation of bipolar cells in the rat retina expresses ionotropic P2 receptors of the P2X type and that these possibly exert a neuromodulatory influence on information processing in the retina.

Han, S.-H., D. Murchison, et al. "Low voltage-activated calcium and fast tetrodotoxin-resistant sodium currents define subtypes of cholinergic and noncholinergic neurons in rat basal forebrain."

Molecular Brain Research In Press, Corrected Proof

<http://www.sciencedirect.com/science/article/B6T07-4F9F8GX-1/2/725ce0716e4627dbc0b0745f3ca77980>

Neurons of the basal forebrain (BF) possess unique combinations of voltage-gated membrane currents. Here, we describe subtypes of rat basal forebrain neurons based on patch-clamp analysis of low-voltage activated (LVA) calcium and tetrodotoxin-resistant (TTX-R) sodium currents combined with single-cell RT-PCR analysis. Neurons were identified by mRNA expression of choline acetyltransferase (ChAT+, cholinergic) and glutamate decarboxylase (GAD67, GABAergic). Four cell types were encountered: ChAT+, GAD+, ChAT+/GAD+ and ChAT-/GAD- cells. Both ChAT+ and ChAT+/GAD+ cells (71/75) displayed LVA currents and most (34/39) expressed mRNA for LVA Ca²⁺ channel subunits. Cav3.2 was detected in 31/34 cholinergic neurons and Cav3.1 was expressed in 6/34 cells. Three cells expressed both subunits. No single neurons showed Cav3.3 mRNA expression, although BF tissue expression was observed. In young rats (2-4 mo), ChAT+/GAD+ cells displayed larger LVA current densities compared to ChAT+ neurons, while these latter neurons displayed an age-related increase in current densities. Most (29/38) noncholinergic neurons (GAD+ and ChAT-/GAD-) possessed fast TTX-R sodium currents resembling those mediated by Na⁺ channel subunit Nav1.5. This subunit was expressed predominately in noncholinergic neurons. No cholinergic cells (0/75) displayed fast TTX-R currents. The TTX-R currents were faster and larger in GAD+ neurons compared to ChAT-/GAD- neurons. The properties of ChAT+/GAD+ neurons resemble those of ChAT+ neurons, rather than of GAD+ neurons. These results suggest novel features of subtypes of cholinergic and noncholinergic neurons within the BF that may provide new insights for understanding normal BF function.

Harada, N., K. Yamada, et al. (1992). "Regulation of aromatase cytochrome P-450 (estrogen synthetase) transcripts in the quail brain by testosterone." Molecular Brain Research **15**(1-2): 19.

<http://www.sciencedirect.com/science/article/B6T07-4859NCP-9Y/2/65b60ddedd7c10783274c0652e6526da>

The aromatase cytochrome P-450 (P-450AROM) cDNA, which was identified by homologies in the DNA and in the deduced amino acid sequences with human P-450AROM cDNA, was isolated from a brain cDNA library of Japanese quail, demonstrating the presence of RNA transcripts of P-450AROM in the quail brain. To determine trace amounts of P-450AROM mRNA in the brain and to examine the effects of testosterone on its expression, a quantitative PCR method of RNA transcripts was developed. Brain total RNA was subjected to reverse transcription reaction and then quantitated by PCR from cDNA with a fluorescent dye-labeled primer. The quantity of P-450AROM mRNA was calculated by using an internal standard of modified P-450AROM (m-P-450AROM) RNA. The brain P-450AROM was primarily transcribed in the hypothalamus area (1.15±0.14 amol/[μg of RNA) and traces of transcripts only were detected in the cerebellum (0.038±0.005 amol/[μg of RNA). The P-450AROM mRNA in the hypothalamus of castrated quail was low (0.270±0.078 amol/[μg of RNA) and increased 4- to 5-fold following treatment with testosterone. These results demonstrate, for the first time, that the increase in P-450AROM activity that is observed in the brain following treatment with testosterone results from a pretranslational regulation of the P-450AROM by androgens.

Hardin-Pouzet, H., P. Giraudon, et al. (1996). "Glucocorticoid upregulation of glutamate dehydrogenase

gene expression in vitro in astrocytes." Molecular Brain Research **37**(1-2): 324.

<http://www.sciencedirect.com/science/article/B6T07-3WXX2SG-1C/2/7063cc863f74b415313c635944b66a16>

Glutamate, the major excitatory neurotransmitter, is preferentially catabolized in astrocytes by glutamate dehydrogenase (GDH). Treatment of an astrocytic cell line with hydrocortisone (10⁻⁵ M) resulted in increased expression of GDH mRNA. Transfection of the cells with truncated parts of the GDH promoter showed that genomic responsive elements activated by hydrocortisone are localized in the -557/+1 region of the promoter. This control of GDH expression by glucocorticoids may be involved in their protective effect against glutamate excitotoxicity.

Harrison, D. C., R. P. Davis, et al. (2001). "Caspase mRNA expression in a rat model of focal cerebral ischemia." Molecular Brain Research **89**(1-2): 133.

<http://www.sciencedirect.com/science/article/B6T07-42T4FSW-N/2/f9b07d41be65769de0b4f7f2940f8ff3>

Proteins of the caspase family are involved in the signalling pathway that ultimately leads to programmed cell death (apoptosis), which has been reported to occur in some experimental models of stroke. In a previous paper we used quantitative reverse transcription and polymerase chain reaction (RT-PCR) to characterise changes in the mRNA expression of one member of this family, caspase-3, in a rat model of permanent focal ischemia. Here we have used this technique to study the expression of a further three caspases which are involved in different aspects of caspase signalling. Caspase-8, involved in Fas-mediated apoptosis, was upregulated in the cortex of ischemic rats. Caspase-11, which leads to the synthesis of the functional form of the cytokine interleukin-1[beta], also showed increased expression, but with a different temporal profile from caspase-8. In contrast, caspase-9, which forms part of the pathway signalling through the mitochondria, showed a decrease in expression. The expression of a further four caspases (1, 2, 6 and 7) has also been characterised in a simpler experiment. These caspases all showed distinctive patterns of expression following the induction of ischemia. These data lead us to conclude that caspase expression as a whole is under very strict transcriptional control in this model. Certain elements of caspase signalling, such as the Fas-induced pathway and the events upstream of IL-1[beta] processing, are upregulated, while others are not. This may be due to some form of genetic program activated in response to ischemia in the brain and may highlight which biological pathways are modulated.

Harrison, D. C., A. D. Medhurst, et al. (2000). "The use of quantitative RT-PCR to measure mRNA expression in a rat model of focal ischemia -- caspase-3 as a case study." Molecular Brain Research **75**(1): 143.

<http://www.sciencedirect.com/science/article/B6T07-3YDG207-K/2/0a52cbf3332d36fc9d1f0df70e1be77b>

Quantitative reverse transcription and polymerisation chain reaction (RT-PCR) using Taqman(TM) fluorogenic probes has been used to measure changes in gene expression in the cerebral cortex of rats in the permanent middle cerebral artery occlusion (pMCAO) model of focal ischemia. The mRNA levels of three housekeeping genes have been analysed in this model to determine which gene showed least change following experimental insult. In the lesioned cortex, [beta]-actin mRNA increased at 24 h, while the levels of cyclophilin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) did not change. We have also used this methodology to

examine modulations in the level of caspase-3 mRNA during focal ischemia in the rat. Caspase-3 mRNA showed a 41% increase at 6 h post-MCAO, which was specific to the lesioned cortex. This change became more pronounced with time, showing an increase of 220% at 24 h. This methodology enables changes in mRNA expression to be analysed more sensitively and quantitatively than other available techniques and highlights the need for careful choice of control or housekeeping genes used for RNA comparisons.

Hashimoto, N., H. Yamanaka, et al. (2001). "Expression of hepatocyte growth factor in primary sensory neurons of adult rats." Molecular Brain Research **97**(1): 83.

<http://www.sciencedirect.com/science/article/B6T07-44CNR5J-3/2/622c0f6b0b367e3054eae46cc283f07>

We examined the expression of hepatocyte growth factor (HGF) mRNA and its receptor, c-met mRNA, in the dorsal root ganglia (DRG) and spinal cord of naive adult rats using in situ hybridization histochemistry (ISHH) and the reverse transcription-polymerase chain reaction (RT-PCR) technique. HGF mRNA was expressed in 25.0% of DRG neurons and 80.5% of HGF mRNA-positive neurons expressed trkA mRNA. In the lumbar spinal cord, c-met mRNA signals were observed in the superficial layer of dorsal horn. These results suggest that the HGF/cMet system may be involved in sensory transmission.

Herrington, R. J., S. A. Nanda, et al. (2004). "The effects of acute stress on the regulation of central and basolateral amygdala CRF-binding protein gene expression." Molecular Brain Research **131**(1-2): 17.

<http://www.sciencedirect.com/science/article/B6T07-4DBX7P9-1/2/5d068ef9d8df23989286a2d60d03874a>

Corticotropin-releasing factor (CRF) is a key mediator of the behavioral, autonomic, and endocrine responses to stress. CRF binds two receptors and a CRF-binding protein (CRF-BP), which may inactivate or modulate the actions of CRF at its receptors. The amygdala is an important anatomical substrate for CRF and contains CRF, its receptors, and CRF-BP. Few studies have examined the effects of acute stress on the regulation of amygdala CRF-BP with other CRF system genes. Therefore, we examined the time course of the effects of acute restraint stress on central (CeA) and basolateral (BLA) amygdala CRF system genes. Consistent with our previous study, acute stress increased BLA CRF-BP mRNA shortly after stress offset. Surprisingly, BLA CRF-BP mRNA remained elevated up to 21 h after the stressor. This effect was selective in the BLA as stress did not alter CeA CRF-BP mRNA, and there were no changes in CRF or CRF receptor mRNAs in either amygdala nucleus. These results suggest that alterations in BLA CRF-BP gene expression are a primary response of the BLA/CeA CRF system to acute stress. Because CRF-BP can modulate CRF action, changes in amygdala CRF-BP levels after stress exposure may affect the ability of an organism to adapt to future stressors.

Hirst, W. D., N. Y. Cheung, et al. (1998). "Cultured astrocytes express messenger RNA for multiple serotonin receptor subtypes, without functional coupling of 5-HT₁ receptor subtypes to adenylyl cyclase." Molecular Brain Research **61**(1-2): 90.

<http://www.sciencedirect.com/science/article/B6T07-3TYNN0P-9/2/1085298406828f13fed212546d126127>

The literature describing the expression of 5-HT receptor subtypes by astrocytes is controversial and incomplete. It is clear that primary cultures of astrocytes express receptors of the 5-HT₂ family coupled to phospholipase C and of the 5-HT₇ receptor family positively coupled to adenylyl cyclase. Cultured astrocytes have also been reported to express receptors of the 5-HT₁ family, although the exact subtypes present are unknown. In the present study we have investigated which of the known rat G-protein coupled 5-HT receptor mRNAs are expressed by cultured astrocytes. Reverse transcriptase-polymerase chain reaction (RT-PCR) revealed expression of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_{5B}, 5-HT₆ and 5-HT₇ receptor mRNAs in astrocytes derived from 2-day old rats and cultured for 10-12 days. Messenger RNAs for 5-HT₄ and 5-HT_{5A} receptors were not detected. The functional expression of 5-HT₁ receptor subtypes was investigated by measuring the ability of 5-HT₁ receptor agonists: 8-OH-DPAT (5-HT_{1A} receptors), RU24969 (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, and 5-HT_{1F} receptors) or sumatriptan (5-HT_{1B}, 5-HT_{1D}, and 5-HT_{1F} receptors) to modulate forskolin or isoproterenol stimulated cAMP production. These compounds, at concentrations up to 10 [μ]M, did not significantly attenuate cAMP production. These results indicate that although astrocytes express mRNA for each of the five 5-HT₁ receptor subtypes which have been isolated from the rat, these receptors are not coupled to the inhibition of adenylyl cyclase.

Honkaniemi, J., S. M. Massa, et al. (1996). "Global ischemia induces apoptosis-associated genes in hippocampus." Molecular Brain Research **42**(1): 79.

<http://www.sciencedirect.com/science/article/B6T07-3P8WWD9-B/2/d7a5e5c31045b97fe4222464bb9c4d80>

Using in situ hybridization, Northern blotting and RT-PCR we studied the post-ischemic expression of bcl-2, bcl-x, bax and ICE. One day following 5 min or 10 min of global ischemia bcl-2 and bcl-x mRNAs were induced in CA1 hippocampal pyramidal neurons while bax was unchanged. By 72 h after ischemia the expression of bcl-2, and bcl-x and bax mRNAs decreased in CA1. The large isoform of bcl-x (bcl-xL), detected using RT-PCR, decreased in whole hippocampus by 24-72 h after ischemia relative to the putative short (bcl-xS) and transmembrane deleted (bcl-x[Delta]TM) forms. Oligonucleotides to interleukin-1[beta] convertase (ICE), which detected the expected 2-kb transcript and two lesser 1.5- and 3-kb hybridizing species, demonstrated slight mRNA induction in the CA1 at 72 h following ischemia. DNA nick end-labeling at 3 days following ischemia showed DNA fragmentation in neurons limited to the CA1 region of hippocampus following 5 min ischemia, while DNA fragmentation was detected in CA1, CA3, dentate gyrus and cortical neurons following 10 min ischemia. The data support the view that hippocampal neurons might undergo an apoptosis-like death after global ischemia. Since global ischemia decreases total protein synthesis especially in the CA1 region, the increases in bcl-2 mRNA levels may not necessarily lead to increased Bcl-2 protein levels. This may explain why the CA1 neurons die despite the prominent induction of the protective bcl-2 gene. The observed decrease by 24 h in the bcl-xL/bcl-xS ratio which preceded DNA fragmentation may participate in the cell death produced by ischemia. However, because of the ischemia-induced decrease in total protein synthesis, the decreased bcl-xL/bcl-xS ratio does not necessarily lead to a changed ratio in the amount of the appropriate proteins. Since ICE like mRNA was induced at 72 h when the CA1 neurons were dead, the significance of this ICE-like mRNA induction remains unclear.

Huang, X. C., U. V. Shenoy, et al. (1997). "Modulation of angiotensin II type 2 receptor mRNA in rat hypothalamus and brainstem neuronal cultures by growth factors." Molecular Brain Research **47**(1-2): 229.

<http://www.sciencedirect.com/science/article/B6T07-3S0MHR6->

X/2/ff184324f0223fd780b477872e2bac60

This study investigates the regulatory effects of growth factors upon angiotensin II type 2 (AT2) mRNA levels in neurons co-cultured from newborn rat hypothalamus and brainstem. Incubation of cultured neurons with nerve growth factor (NGF; 5-50 ng/ml) caused time-dependent changes in the steady-state levels of AT2 receptor mRNA. Short-term (0.5-1.0 h) incubations with NGF resulted in significant increases in AT2 receptor mRNA, whereas longer-term incubations (4-24 h) caused significant decreases. Activation of NGF receptors is known to stimulate phospholipase C-[gamma] and subsequently activate protein kinase C (PKC). Incubation of cultures with the PKC activator, phorbol-12-myristate-13-acetate (PMA; 100 nM), caused temporal changes in AT2 receptor mRNA levels similar to those observed with NGF. By contrast, insulin (0.1-10 [mu]g/ml) elicited only significant decreases in AT2 receptor mRNA levels. The observed abilities of NGF and insulin to regulate the expression of AT2 receptor mRNA are consistent with the fact that the AT2 receptor gene promoter region contains several cis DNA regulatory elements that respond to growth factor-stimulated transcription factors. These novel observations which show that NGF and insulin can regulate AT2 receptor mRNA in neurons derived from neonatal rat CNS lend support to the idea that AT2 receptors have a role in development and differentiation.

Ikeda, T., S. Kitayama, et al. (2001). "Nerve growth factor down-regulates the expression of norepinephrine transporter in rat pheochromocytoma (PC12) cells." Molecular Brain Research **86**(1-2): 90.

<http://www.sciencedirect.com/science/article/B6T07-4299Y92-K/2/87062a8394c416703691fbc6736c8823>

Functional expression of norepinephrine transporter (NET) and its regulation were examined in rat pheochromocytoma cell line, PC12. Nerve growth factor (NGF) decreased [3H]-norepinephrine (NE) uptake in association with a decrease in NET mRNA levels. On the other hand, levels of tyrosine hydroxylase mRNA increased in PC12 cells treated with NGF for 4-24 h, while Oct-2 mRNA levels decreased at 4 h with NGF then recovered for 8-24 h in the presence of NGF. Both bFGF and EGF reduced [3H]NE uptake, although they failed to affect NET mRNA levels. To examine the NET transcriptional regulation, we identified the 5'-noncoding region of rat NET mRNA by the rapid amplification of cDNA end (RACE) method and cloned the 5'-flanking region of NET gene. The newly identified exon encodes the untranslated region of rat NET mRNA upstream of the known 5'-region including ATG start codon. Constructs having green fluorescent protein (GFP) as reporter were made with the cloned NET gene, and promoter activity was examined in CHO and SK-N-SH cells transiently transfected and in PC12 cells stably transfected with NET-GFP constructs. The results indicate that the 2.1 kb NET flanking region displays promoter activity and is responsible for the NGF-induced down-regulation of NET expression.

Imaizumi, K., M. Tsuda, et al. (1994). "Differential expression of sgk mRNA, a member of the Ser/Thr protein kinase gene family, in rat brain after CNS injury." Molecular Brain Research **26**(1-2): 189.

<http://www.sciencedirect.com/science/article/B6T07-485RK1F-W/2/4bff8057c15e112190bbf4e74be51300>

We cloned genes the expression of which were induced 3 days after cortical injury of rat brain by a differential display technique, and four novel and known sequences were isolated. Among these sequences, the sgk gene which was recently identified as a novel member of the serine/threonine protein kinase gene family, was selected for analysis of its expression patterns in rat brain by northern blotting and in situ hybridization, because hybridization signals were strong at the lesion

sites. Expression of *sgk* mRNA was induced within 3 days after injury, and was maintained at a high level for at least 14 days. The cells which strongly expressed the *sgk* gene were in the deep layers of the cortex and in the corpus callosum. In situ hybridization analysis for *sgk* and myelin proteolipid protein mRNA using serial sections showed that the distribution of both signals was very similar at the damaged regions. Therefore, it is likely that the *sgk* transcript is expressed by oligodendrocytes after brain injury. Investigation of the developmental expression of the *sgk* gene showed that neurons in layers I and II of the cortex, lateroposterior and laterodorsal thalamic nucleus, and ventral posterolateral and posteromedial thalamic nucleus strongly expressed *sgk* mRNA at postnatal day 1 and day 7, but these neurons showed no expression in fetal or adult brain. These results suggest that the induction of *sgk* gene may be associated with a series of axonal regenerations after brain injury, and in addition, the *sgk* gene may also play important roles in the development of particular groups of neurons in the postnatal brain.

Inoue, K., M. Sakaitani, et al. (1995). "Cloning and expression of a bovine glutamate transporter." Molecular Brain Research **28**(2): 343.

<http://www.sciencedirect.com/science/article/B6T07-3YGV2GR-K/2/b9cbe584de1534d591cb6bd1231207b3>

We have isolated a 3845 base-pair cDNA (BNGLUAS) encoding a bovine glutamate transporter (bovine GLAST) by screening a bovine retina cDNA library with an oligonucleotide probe corresponding to conserved regions of known glutamate transporters. The cDNA sequence predicted a protein of 542 amino acids and displayed 96% and 97% amino acid identity with the rat GLAST/GluT-1 and human GLAST, respectively. Expression of the bovine GLAST in *Xenopus* oocytes revealed Na⁺-dependent [¹⁴C]-glutamate uptake and electrogenic glutamate uptake.

Iwata, N., M. Virkkunen, et al. (1998). "Identification of a naturally occurring Pro15-Ser15 substitution in the serotonin5A receptor gene in alcoholics and healthy volunteers." Molecular Brain Research **58**(1-2): 217.

<http://www.sciencedirect.com/science/article/B6T07-3T8P2J7-S/2/eef45acc7f9019eac16d780ad514343>

We screened the serotonin5A receptor gene coding region in 186 unrelated alcoholic patients and 187 controls. A relatively abundant amino acid substitution and two synonymous DNA substitutions were detected. Two synonymous variants, A12T and C789T, had rarer-allele frequencies of 23% and 1%, respectively. The Pro15Ser substitution is located in the amino terminal, extracellular domain of the receptor adjacent to a putative phosphorylation site. Pro15Ser had rarer-allele frequencies of 8.1% and 5.9% in Finnish alcoholic patients and controls, respectively (p=n.s.).

Jabs, R., E. Guenther, et al. (2000). "Evidence for P2X3, P2X4, P2X5 but not for P2X7 containing purinergic receptors in Muller cells of the rat retina." Molecular Brain Research **76**(2): 205.

<http://www.sciencedirect.com/science/article/B6T07-400WXRS-2/2/64f680b029c163f72e6904434ba3ab0f>

P2X receptors are ligand-gated ion channels activated by ATP. They are expressed in a broad variety of tissues. To date, eight P2X receptor subunits (P2X1-P2X7, P2XM) have been cloned.

In spite of the considerable evidence of signaling by extracellular nucleotides in other sensory systems, only few studies have been undertaken in the retina. In earlier studies, we have demonstrated that there is mRNA expression of the P2X2-5 and P2X7 subunits in the rat retina. In the present study, molecular biological methods were used to investigate expression of P2X receptor mRNA in freshly isolated Muller cells (MCs) of the adult rat retina (Brown Norway). A total of 36 MCs was analyzed, employing the single-cell RT-PCR. A positive amplification signal of 11/14 for P2X3-mRNA, 5/10 for P2X4-mRNA, 3/10 for P2X5-mRNA and 0/8 for P2X7-mRNA was revealed. Additionally, the astroglial identity of the cells under studied was confirmed in 10 cases by simultaneous amplification of RT-PCR products of glutamine synthetase (GS)- and P2X-mRNA. We conclude that MCs of rat retina express ionotropic P2 receptors, which, in addition to other functions, may play a key role within the recently described long range calcium signaling and the fast direct glia-neuron interactions in the rat retina.

Jeohn, G. H., R. C. C. Chang, et al. (2000). "Post-transcriptional inhibition of lipopolysaccharide-induced expression of inducible nitric oxide synthase by Go6976 in murine microglia." Molecular Brain Research **79**(1-2): 18.

<http://www.sciencedirect.com/science/article/B6T07-40VT3B1-2/2/7f2c5e432fcb2f540b47e674192abf78>

Glia in the brain respond to various toxins with an increased expression of inducible nitric oxide synthase (iNOS) and an increased production of nitric oxide (NO). Here, we report that lipopolysaccharide (LPS)-induced expression of iNOS was down-regulated post-transcriptionally through the destabilization of iNOS mRNA by the indolocarbazole compound, Go6976, in murine microglia. This Go6976 effect is specific for iNOS since tumor necrosis factor [alpha] was unaffected by the compound. Interestingly, the post-transcriptional effects ascribed to Go6976 were not observed with other inhibitors of protein kinase A, C (PKC), G, or protein tyrosine kinases. Instead, these kinases appear to affect the iNOS/NO system at the transcriptional level. In the past, Go6976 has been reported to be a rather specific inhibitor of PKC in vitro. Results from our experiments, through prolonged treatment with phorbol esters and with the various PKC inhibitors including phorbol ester-insensitive PKC isotype inhibitor, suggest that the Go6976-mediated post-transcriptional regulation of iNOS gene expression and NO production in microglia is not mediated through its reputed effects on PKC activity. Since the effects of various neurotoxins and certain neurodegenerative diseases may be manifested through alterations in the iNOS/NO system, post-transcriptional control of this system may represent a novel strategy for therapeutic intervention.

Jeohn, G. H., B. Wilson, et al. (2000). "The indolocarbazole Go6976 protects neurons from lipopolysaccharide/interferon-[gamma]-induced cytotoxicity in murine neuron/glia co-cultures." Molecular Brain Research **79**(1-2): 32.

<http://www.sciencedirect.com/science/article/B6T07-40VT3B1-3/2/e625bfacacf792061a8483aa5ec556f9>

The expression of inducible nitric oxide synthase (iNOS) and the production of nitric oxide (NO) after exposure to endotoxins has been implicated in immune-mediated neurotoxicity. The indolocarbazole compound Go6976, which has been described as a selective protein kinase C (PKC) inhibitor in vitro, rescued neurons from lipopolysaccharide/interferon-[gamma] (LPS/IFN[gamma])- or interleukin-1[alpha]/tumor necrosis [alpha]/IFN[gamma] (IL-1[alpha]/TNF[alpha]/IFN[gamma])-induced cytotoxicity in murine primary neuron-glia co-cultures. Other compounds known to inhibit PKC, Ro31-8220, GF109203X, Go7874, H7, staurosporine and H89, failed to rescue neurons from the LPS/IFN[gamma]-induced cytotoxicity. These results

suggest that the neuroprotection by Go6976 from the LPS/IFN[gamma]-induced neuronal cell death is not mediated through its reputed effects on PKC activity. The neuroprotection paralleled the inhibition of iNOS gene expression and NO production. However, further analyses correlating NO production with the extent of neurotoxicity suggested that additional mechanism(s) besides the inhibition of the iNOS/NO system may be responsible for the neuroprotective effects of Go6976. An understanding of the mechanism underlying the neuroprotective effect of Go6976 may provide key insights into potential interventions for immune-mediated neurodegenerative diseases.

Kasai, K., T. Yamashita, et al. (2003). "Induction of mRNAs and proteins for Na/K ATPase [alpha]1 and [beta]1 subunits following hypoxia/reoxygenation in astrocytes." Molecular Brain Research **110**(1): 38.

<http://www.sciencedirect.com/science/article/B6T07-47FDMXT-1/2/e48bcce151c0a68c6073ad1a19a87883>

Characteristics of the cellular response to oxygen deprivation and subsequent reoxygenation (hypoxia/reoxygenation) include redirection of energy metabolism, increased glucose utilization and expression of oxygen-regulated proteins. Inhibition of protein synthesis during early reoxygenation period prevented effective astrocyte adaptation to hypoxia/reoxygenation, resulting in eventual cell death. To elucidate the role of astrocytes in the central nervous system in response to hypoxia/reoxygenation, we analyzed the cDNA library derived from the cultured rat astrocytes subjected to 24 h of hypoxia followed by reoxygenation by differential display, and isolated a cDNA corresponding to Na/K ATPase [alpha]1 subunit. The expression of Na/K ATPase [alpha]1 subunit mRNA as well as [beta]1 subunit mRNA was transiently increased after reoxygenation, whereas hypoxia itself did not induce any gene expression change. Na/K ATPase [alpha]1 subunit protein was transiently increased, whereas the protein expression for Na/K ATPase [beta]1 subunit showed sustained induction after reoxygenation. Overexpression of [beta]1 subunit in HEK 293 cells subjected to hypoxia/reoxygenation promoted survival of the cells. These findings suggest that Na/K ATPases may contribute to maintain the cellular environment of astrocytes subjected to hypoxia/reoxygenation.

Kashiwagi, K., Y. Iizuka, et al. (2003). "Differences in nitric oxide production: a comparison of retinal ganglion cells and retinal glial cells cultured under hypoxic conditions." Molecular Brain Research **112**(1-2): 126.

<http://www.sciencedirect.com/science/article/B6T07-4841424-5/2/395d975b6fa25ad56807fb7265183fe7>

The aim of this study was to compare the effects of hypoxia on nitric oxide synthase (NOS) expression and the production of NO between isolated retinal ganglion cells (RGCs) and retinal glial cells. Reverse transcription-polymerase chain reaction (RT-PCR) was employed to examine the presence of neuronal NOS mRNA, inducible NOS mRNA, and endothelial NOS mRNAs in the two cell types. RGCs and retinal glial cells were separately cultured under hypoxic (10% O₂) or control (20% O₂) conditions. Changes in NOS-mRNA expression were quantified by real-time PCR, and nitrite in the medium was measured up to 96 h of culture. The effects of non-NOS- and iNOS-selective inhibitors on hypoxia-induced release of nitrite in the culture medium were evaluated. RT-PCR revealed the presence of three types of NOSs in the two types of cultured cells. Hypoxic culture conditions significantly changed the expression of all NOS mRNAs in retinal glial cells but not in RGCs. NO production showed significant changes corresponding to those of NOS mRNAs in retinal glial cells but not in RGCs, and both NOS inhibitors significantly reduced hypoxia-induced nitrite release in retinal glial cells. Retinal glial cells but not RGCs may be the

major source of NO under hypoxic conditions.

Kawano, H., T. Nakatani, et al. (2004). "Identification and characterization of novel developmentally regulated neural-specific proteins, BRINP family." Molecular Brain Research **125**(1-2): 60.

<http://www.sciencedirect.com/science/article/B6T07-4CC80CC-2/2/0e37a8b0314f7eb84b0437b1596c0c07>

Processes of neuronal differentiation involve activation of a set of neuronal specific genes and cessation of cell proliferation in postmitotic neurons. Previous studies revealed that bone morphogenetic protein (BMP) and retinoic acid (RA) play important roles in the differentiation of peripheral sympathetic neurons such as the synergistic induction of responsiveness to specific neurotrophic factors. In the present study, while trying to clarify the mechanism of the BMP/RA-actions, we identified a novel neural-specific protein, BMP/RA-inducible neural-specific protein-1 (BRINP1) which shows no similarity to other known proteins. Subsequently, two homologous proteins, BRINP2 and BRINP3, making up the BRINP family, are identified. Individual BRINP genes have distinct regulatory mechanisms of expression within the nervous system. In rodent brain, BRINP1 is expressed from earlier developmental stage, i.e. E9.5, and widely expressed in various neuronal layers and nuclei of the adult animal, while BRINP2 and BRINP3 were detectable from E11.5 and expressed in rather limited regions in a complementary manner. During the course of perinatal development of sympathetic neurons, BRINP1 is induced from earlier embryonic stage and further increased toward adult stage, while BRINP3 expressed from earlier stage is replaced by BRINP2 expression which increases postnatally in accordance with the action of BMP2 and RA. Furthermore, when expressed in nonneuronal cells, all three BRINP family proteins suppressed the cell cycle progression. Possible physiological functions of BRINP family members in the development of the nervous system are discussed.

Kobayashi, M., M. Fujii, et al. (1998). "Bone morphogenetic protein-2 and retinoic acid induce neurotrophin-3 responsiveness in developing rat sympathetic neurons." Molecular Brain Research **53**(1-2): 206.

<http://www.sciencedirect.com/science/article/B6T07-3S3DNY9-R/2/6dbb22ea638c4019490b7042e078a44f>

Expression of the receptor tyrosine kinase, Trk, determines the specificity of neurotrophin responsiveness of different neuronal populations during development. Recently it has become apparent that sympathetic neurons of rat superior cervical ganglia (SCG) acquire sensitivity to neurotrophin-3 (NT3) before they become dependent on the target-derived nerve growth factor (NGF) for their survival by sequential induction of TrkC and TrkA. The mechanism controlling the expression of TrkC as well as the source of NT3 at their initial developmental stage has, however, not been clarified. Here we show that the treatment of the perinatal rat SCG neurons which express high levels of trkA mRNA with bone morphogenetic protein-2 (BMP2) induced the expression of trkC mRNA. Induction of the functional TrkC receptor by BMP2 was confirmed by the enhancement of the survival response of these neurons to NT3. Treatment of SCG neurons with retinoic acid (RA) promoted the effect of BMP2 on the induction of trkC mRNA levels. BMP2 treatment, on the other hand, promoted the effect of RA on the suppressions of trkA mRNA levels and the NGF-dependent survival of the SCG neurons. Furthermore, BMP2/RA treatment induced the endogenous expression of NT3. These results indicate that specific environmental signals can regulate neurotrophin responsiveness of developing sympathetic neurons by differential alteration of the trk and neurotrophin expressions.

Kobori, N., G. L. Clifton, et al. (2002). "Altered expression of novel genes in the cerebral cortex following experimental brain injury." Molecular Brain Research **104**(2): 148.

<http://www.sciencedirect.com/science/article/B6T07-46R9NFD-C/2/a629aad03adc6e0e610c3cb2b394d21a>

Damage to the cerebral cortex results in neurological impairments such as motor, attention, memory and executive dysfunctions. To examine the molecular mechanisms contributing to these deficits, mRNA expression was profiled using high-density cDNA microarray hybridization after experimental cortical impact injury in mice. The mRNA levels at 2 h, 6 h, 24 h, 3 days and 14 days after injury were compared with those of control animals. This revealed 86 annotated genes and 24 expression sequence tags (ESTs) as being differentially expressed with a 1.5-fold or greater change. Quantitative real-time PCR analysis was used to independently verify these results for selected genes. Seven functional classes of genes were found to be altered following injury, including transcription factors, signal transduction genes and inflammatory proteins. While a few of these genes have been previously reported to be differentially regulated following injury, the most of the genes have not been previously implicated in traumatic brain injury (TBI) pathophysiology. For example, consistent with previous reports, the transcription factor c-jun and the neurotrophic factor bdnf mRNA levels were altered as a result of TBI. Among the novel genes, the mRNA levels for the high mobility group protein 1 (hmg-1), the regulator of G-protein signaling 2 (rgs-2), the transforming growth factor [beta] inducible early growth response (tieg), the inhibitor of DNA binding 3 (id3), and the heterogeneous nuclear ribonucleoprotein H (hnmp h) were changed following injury. The functional significance of these genes in neurite outgrowth, neuronal regeneration, and plasticity following injury are discussed.

Kojima, N., T. Shirao, et al. (1993). "Molecular cloning of a developmentally regulated brain protein, chicken drebrin A and its expression by alternative splicing of the drebrin gene." Molecular Brain Research **19**(1-2): 101.

<http://www.sciencedirect.com/science/article/B6T07-485H22S-27/2/537ed5ba2ae1a165938ca92a3bbda491>

Drebrins are developmentally regulated proteins found in the chicken brain and are classified into three forms, E1, E2 and A. Previously we isolated two cDNAs corresponding to the embryonic drebrin mRNAs from a chick embryo cDNA library. They differed in that an internal 129-nucleotide sequence, designated ins1, was inserted in the cDNA encoding drebrin E2 and was deleted in the other cDNA encoding drebrin E1. To search for the cDNA clone encoding drebrin A, a cDNA library of 1-day-old chick brains was screened using embryonic drebrin cDNA fragments as probes. Consequently, a novel cDNA was isolated, the sequence of which was entirely identical with that of drebrin E2 except for the insertion of a 138-nucleotide sequence, designated ins2, in the 5' direction immediately upstream from ins1. Since the translation product of the entire coding region was similar to that of drebrin A, this cDNA should correspond to the mRNA for drebrin A. Sequencing analysis of three drebrin cDNAs clearly indicated that the heterogeneity of chicken drebrins was caused by the insertion or deletion of the two sequences, ins1 and ins2. The amino-terminal half region including ins2 and two short sequences in the carboxyl-terminal region of the predicted drebrin A were highly evolutionarily conserved. Cloning and sequencing of the drebrin gene revealed that ins1 and ins2 were independently encoded by separate exons and three drebrin isoforms were thought to arise by alternative splicing from a single drebrin gene. The difference in the time course of expression and tissue distribution of each drebrin suggests that the machinery of alternative splicing site selection of the drebrin gene is regulated in a developmental stage-dependent and tissue-specific manner.

Konu, O., X. Xu, et al. (2004). "Application of a customized pathway-focused microarray for gene expression profiling of cellular homeostasis upon exposure to nicotine in PC12 cells." Molecular Brain Research **121**(1-2): 102.

<http://www.sciencedirect.com/science/article/B6T07-4BCXM1S-7/2/e3754a9d3f81704aef001d5d649a46a9>

Maintenance of cellular homeostasis is integral to appropriate regulation of cellular signaling and cell growth and division. In this study, we report the development and quality assessment of a pathway-focused microarray comprising genes involved in cellular homeostasis. Since nicotine is known to have highly modulatory effects on the intracellular calcium homeostasis, we therefore tested the applicability of the homeostatic pathway-focused microarray on the gene expression in PC-12 cells treated with 1 mM nicotine for 48 h relative to the untreated control cells. We first provided a detailed description of the focused array with respect to its gene and pathway content and then assessed the array quality using a robust regression procedure that allows for the exclusion of unreliable measurements while decreasing the number of false positives. As a result, the mean correlation coefficient between duplicate measurements of the arrays used in this study (control vs. nicotine treatment, three samples each) has increased from 0.974 $\pm$ 0.017 to 0.995 $\pm$ 0.002. Furthermore, we found that nicotine affected various structural and signaling components of the AKT/PKB signaling pathway and protein synthesis and degradation processes in PC-12 cells. Since modulation of intracellular calcium concentrations ([Ca²⁺]_i) and phosphatidylinositol signaling are important in various biological processes such as neurotransmitter release and tissue pathogenesis including tumor formation, we expect that the homeostatic pathway-focused microarray potentially can be used for the identification of unique gene expression profiles in comparative studies of drugs of abuse and diverse environmental stimuli, such as starvation and oxidative stress.

Kursula, P., V.-P. Lehto, et al. (1998). "Expression of the amino acid dimorphism in the small myelin-associated glycoprotein cytoplasmic domain in rat peripheral nerves during postnatal development." Molecular Brain Research **54**(2): 252.

<http://www.sciencedirect.com/science/article/B6T07-3VCKRSV-7/2/32d37ce784348eff74cca97bbab543b0>

The myelin-associated glycoprotein (MAG) is one of the proteins expressed during the period of myelin formation and is believed to play a major role in the initiation of myelination. It exists as two differentially expressed isoforms, L- and S-MAG, that are generated by alternative mRNA splicing. A nucleotide dimorphism at the mRNA level resulting in an Arg/Pro dimorphism in the cytoplasmic tail of the S-MAG protein has previously been detected in the rat brain. In this study, we show that this dimorphism is detectable in the rat peripheral nervous system. We propose an allelic origin for the dimorphism and demonstrate the differential expression of the S-MAG alleles in the sciatic nerves of heterozygous rats during the period of active myelination. We also present data on the properties of the two S-MAG cytoplasmic domains produced as GST fusion proteins. The importance of this differentially expressed amino acid dimorphism is discussed, taking into account both its probable effect on the S-MAG cytoplasmic domain function and its significance in functional and structural studies concerning the S-MAG protein.

Li, B., X. Xi, et al. (2003). "Distribution of glucokinase, glucose transporter GLUT2, sulfonylurea receptor-1, glucagon-like peptide-1 receptor and neuropeptide Y messenger RNAs in rat brain by

quantitative real time RT-PCR." Molecular Brain Research **113**(1-2): 139.

<http://www.sciencedirect.com/science/article/B6T07-48JJD4F-J/2/9c915737ac083a2b1f006ef4ca15cef6>

Glucokinase (GK), glucose transporter GLUT2, sulfonylurea receptor-1 (SUR1), glucagon-like peptide-1 receptor (GLP-1R) and neuropeptide Y (NPY) have been proposed to be involved in central glucose sensing or regulation of food intake. In this study, we combined tissue micropunch and real time reverse transcription polymerase chain reaction (RT-PCR), and measured GK, GLUT2, SUR1, GLP-1R and NPY mRNA expression in discrete areas in the hypothalamus and the hindbrain.

Luntz-Leybman, V., A. Frostholt, et al. (1993). "GABAA/benzodiazepine receptor [gamma]2 subunit gene expression in developing normal and mutant mouse cerebellum." Molecular Brain Research **19**(1-2): 9.

<http://www.sciencedirect.com/science/article/B6T07-485H22S-1V/2/84a613805b9850a35042755cc609540d>

Recent studies have identified several subunits ([alpha], [beta], [gamma] and [delta]) of the [gamma]-aminobutyric acidA/benzodiazepine receptor; each consists of several variants. The [gamma] subunit appears to mediate the interaction of the [alpha] and [beta] subunits making the receptor capable of modulation by benzodiazepines. In the present studies, the expression of mRNA encoding the [gamma]2 subunit was examined in the cerebellum during development and in adult Purkinje cell degeneration, lurcher and reeler mutant mice. In the normal adult cerebellum, in situ hybridization with [35S]cRNA probes revealed a strong signal over the Purkinje cell layer and deep cerebellar nuclei, and a weaker signal over basket, stellate and granule cells. Labeling over Purkinje cells was detectable at birth, gradually becoming stronger and more punctate during postnatal weeks 1 and 2, as Purkinje cells formed a monolayer between the molecular and granule cell layers. Adult levels of grain density were reached by P20. The external germinal layer, which contained proliferating granule cells, was unlabeled throughout development; however, weak labeling was detected over the internal granular layer at the end of postnatal week 1, as granule cells began their migration across the molecular layer. During the second postnatal week, punctate labeling became visible over the molecular layer in a distribution indicative of basket and stellate cells. In adult Purkinje cell degeneration and lurcher mutants, in which Purkinje cells have degenerated, no punctate labeling characteristic of mature Purkinje cells was detected. In adult and developing reeler mutants, where all classes of cells are malpositioned throughout the cerebellum, the punctate hybridization signal was present and clearly associated with Purkinje cells in all cortical regions. Our results suggest that developing Purkinje cells express the [gamma]2 gene at a time prior to receiving GABAergic inhibitory input, and that the continued expression in the adult is not affected by the absence of afferents.

Ma, E., T. Xu, et al. (1999). "Gene regulation by O2 deprivation: an anoxia-regulated novel gene in *Drosophila melanogaster*." Molecular Brain Research **63**(2): 217.

<http://www.sciencedirect.com/science/article/B6T07-3VH80K8-2/2/5d5419854705442eb38474bad284f9a7>

Organisms, across the animal kingdom, vary in their tolerance or susceptibility to cell injury from O2 deprivation. In this study we have taken advantage of the genetically well studied fruit fly to dissect basic mechanisms underlying their ability to tolerate lack of O2. Using differential display

and molecular techniques, we cloned and characterized a novel gene, named *fau*, which is up-regulated considerably following anoxia in *Drosophila melanogaster*. Northern blot analysis revealed that the transcript of this gene is ~0.9 kb in length with an open reading frame encoding a small hydrophilic protein (~14.4 kDa). This protein has no homology to previously described gene products but has many potential phosphorylation sites. In situ hybridization showed that this gene is located in region 7C-D on the *Drosophila* X-chromosome and its transcript concentrated in the lamina and cortical neurons of the *Drosophila* central nervous system (CNS). Transgenic flies showed that over-expression of *fau* significantly reduced the recovery time of the flies from anoxia. We conclude that (1) this study provided a framework on which the mechanisms underlying anoxia tolerance can be dissected in the fruit fly and (2) *fau* gene plays an important role in the regulation of tissue responsiveness to O₂ deprivation.

Macdonald, R., S. Bingham, et al. (2001). "Determination of changes in mRNA expression in a rat model of neuropathic pain by Taqman(TM) quantitative RT-PCR." Molecular Brain Research **90**(1): 48.

<http://www.sciencedirect.com/science/article/B6T07-433P6FX-C/2/48413e0890aec8894a4611abcb47f77f>

The aim of this study was to develop a rapid and accurate high throughput method of screening multiple genes across a single sample set to detect changes in gene expression in the dorsal root ganglion (DRG) following partial sciatic nerve ligation in the rat. Using Taqman quantitative RT-PCR, we show that expression of a number of genes, including galanin, vasointestinal peptide and neuropeptide Y are rapidly increased 24 h post-operation in the DRGs on the ligated side only. Other genes tested, including vanilloid receptor-1, substance P, galanin receptor-2 and housekeeping genes did not alter. Analysis of the expression of ASIC4 showed a small difference in expression at 7 days post ligation. By applying a statistical method for analysis of multiple variables, partial least squares, we show that the expression change of ASIC4 was significantly altered on the ligated side even though the change was small. This method will allow us to rapidly identify changes in expression of candidate genes that may be involved in adaptive responses in the DRG due to nerve injury.

Marracci, S., D. Cini, et al. (1997). "Cloning and developmental expression of 5-HT_{1A} receptor gene in *Xenopus laevis*." Molecular Brain Research **47**(1-2): 67.

<http://www.sciencedirect.com/science/article/B6T07-3S0MHR6-8/2/211063fcea3bc5653a96c6424bc657b>

The aim of our work is to investigate the potential involvement of serotonin and its G-protein-coupled receptors in neural differentiation or other developmental processes in *Xenopus laevis*. By using a RT-PCR strategy, we isolated a cDNA fragment from *X. laevis* brain showing high amino-acid similarity with the mammalian 5-HT_{1A} receptor. We used this fragment to isolate a cDNA clone containing a single ORF of 408 amino-acids with an overall amino-acid identity of 73% with the human and rat 5-HT_{1A} receptor. This structural similarity suggests that this clone encodes the *Xenopus* homolog of the mammalian 5-HT_{1A} receptor (X5-HT_{1A}). In order to establish a possible role for this receptor in development, we analyzed the pattern of its gene expression during embryogenesis, larval stages and in adult brain by in situ hybridization. The first signal of mRNA expression appears in the rostral part of brain stem at stage 22, when the first neurons start differentiation [38, 21]. In later stages of development, the cells expressing X5-HT_{1A} transcripts appear to correspond to serotonergic neurons. By stage 41, X5-HT_{1A} mRNA is also detected in the inner nuclear layer (INL) of the developing retina. This pattern of expression is maintained until stage 46, i.e. at the beginning of metamorphosis. In adult, additional brain areas express X5-HT_{1A} mRNA, particularly in telencephalon, diencephalon and mesencephalon.

On the whole, our data show that the X5-HT1A receptor mRNA is developmentally regulated, with expression first appearing in differentiating serotonergic neurons, where this receptor may mediate, through an autocrine regulatory pathway, the trophic action of serotonin on developing serotonergic system.

McKinley, D. D., D. J. Lennon, et al. (1995). "Cloning, sequence analysis and expression of two forms of mRNA coding for the human [beta]2 subunit of the GABAA receptor." Molecular Brain Research **28**(1): 175.

<http://www.sciencedirect.com/science/article/B6T07-3YGV2HR-1H/2/5d2411e258ccb9181655a448339061cb>

Two forms of cDNA coding for the human GABAA [beta]2 subunit have been cloned and sequenced. The two sequences differ by a 114 base pair insertion. The insert contains a phosphorylation consensus sequence for calmodulin-dependent protein kinase II. Quantitative PCR studies show that h[beta]2L cDNA represents 10-15% of total h[beta]2 cDNA in the 10 brain substructures tested. Analysis of human genomic southern blots suggests that the two forms might arise by differential splicing.

Misawa, H., J. Matsuura, et al. (1997). "Human choline acetyltransferase mRNAs with different 5'-region produce a 69-kDa major translation product." Molecular Brain Research **44**(2): 323.

<http://www.sciencedirect.com/science/article/B6T07-3P6BGCP-J/2/9338ddeda8b0fefc08ada807a2c40ea3>

Choline acetyltransferase (ChAT, EC 2.3.1.6) is the biosynthetic enzyme for acetylcholine. We have previously shown that multiple ChAT mRNA species with different 5'-noncoding regions are expressed in the rat and mouse. However, the diversity of ChAT mRNA species in human has not completely been elucidated. In this work N1- and N2-type ChAT cDNAs were cloned from a human brain cDNA library and the N-exon located in the human ChAT gene. Polymerase chain reaction analysis indicates that four species of ChAT mRNAs (R-, N1-, N2- and M-types) are produced in human brain and spinal cord. In all human transcripts, the ATG initiation codon in the rat, mouse and pig was replaced by ACG, which does not serve as an initiation codon for translation. In vitro translation and mammalian expression analyses revealed that N1-, N2- and R-type mRNAs give rise to a single 69 kDa enzyme, while M-type mRNA produces both 82 and 69 kDa enzymes. The translation efficiency of M-type mRNA was lower than that of the other mRNA species. Moreover, the translation efficiency of human ChAT mRNAs was considerably lower than that of rat ChAT mRNA, suggesting that the ATG codons for human ChAT are unfavorable for translation initiation compared with the initiation codon for rat ChAT. These results provide rational explanations for the previous reports that human ChAT protein purified from the brain and placenta had 66-70 kDa molecular mass, and that ChAT activity in a single motor neuron of human was far lower than that of other vertebrates. Sequencing of monkey ChAT gene showed that the initiation ATG in rodent ChAT was also replaced by ACA in the monkey.

Moshnyakov, M., U. Arumae, et al. (1996). "mRNAs for one, two or three members of trk receptor family are expressed in single rat trigeminal ganglion neurons." Molecular Brain Research **43**(1-2): 141.

<http://www.sciencedirect.com/science/article/B6T07-3RSGFBD-H/2/f29a7dd8e0ca73e5b053f1b81314582b>

We studied the expression of mRNAs of neurotrophin (NTF) receptors trkA, trkB and trkC in single rat trigeminal ganglion neurons at embryonic days 12 and 16 to determine, whether single trigeminal ganglion neurons express one trk family member or coexpress several of them. For that purpose we elaborated a sensitive technique of reverse transcriptase-polymerase chain reaction to detect all neurotrophin receptors in a single neuron. Expression of neurofilament light chain mRNA was used as a positive marker to confirm the recovery of mRNAs from single neurons. Neurofilament-positive samples were subsequently analyzed for the expression of mRNAs for catalytic trkA, trkB and trkC, and in some cases, low-affinity neurotrophin receptor (p75). We found neurons expressing one, coexpressing two, or even all three trk receptors. In many neurons analyzed, p75 mRNA was coexpressed with trks, but we also found neurons expressing only trks without p75, and a neuron expressing p75 alone. There were also neurons containing neither trk receptors nor p75. We provide here first direct evidence that single sensory neurons can simultaneously express three or even four neurotrophin receptors.

Munch, C., B. Schwalenstocker, et al. (2000). "Differential RNA cleavage and polyadenylation of the glutamate transporter EAAT2 in the human brain." Molecular Brain Research **80**(2): 244.

<http://www.sciencedirect.com/science/article/B6T07-414NVN7-H/2/98b9d4b7c4c3e273960836c88f824d7a>

We cloned four novel transcripts of the excitatory amino acid transporter 2, named EAAT2/3UT1-4, resulting from differential cleavage and polyadenylation. Tandem poly (A) sites were found to be functional at 72, 654, 973 nucleotides and more than 2 kb downstream of the stop codon. A tissue-specific expression was identified for 3'-variants of the EAAT2 RNA, most prominently for EAAT2/3UT4 (hippocampus>cortex>>cerebellum>thalamus) as demonstrated by Northern blot analysis and quantitative PCR. We conclude, that alternative poly (A) selection may contribute to the reported differential EAAT2 protein expression under normal and diseased conditions.

Nakayama, M., Y. Gahara, et al. (1994). "Distinctive four promoters collectively direct expression of brain-derived neurotrophic factor gene." Molecular Brain Research **21**(3-4): 206.

<http://www.sciencedirect.com/science/article/B6T07-485H24C-2S/2/9e63d0fc8ea9a4c9bd0c5c1427ae0a02>

In order to get a deeper insight into comprehensive understanding of gene regulation of brain-derived neurotrophic factor (BDNF), we characterized the transcriptional apparatus of this gene on the basis of the genomic structure. The results in this study revealed that there are at least four distinctive promoters in the BDNF gene; two of them are neuron-specific and the rest are active in some non-neuronal tissues as well as neuronal ones. Although the analyses of the promoter usage pattern clarified many characteristic features in controlling these promoter activities, the most notable finding was that administration of kainic acid resulted in great activation of two out of the four promoters in hippocampal neurons in a regionally different manner and thus indicated the presence of two distinct signal transduction pathways for kainate-induced activation of BDNF gene expression in neurons. The analysis of BDNF gene expression in terms of the promoter usage pattern would provide a new and important insight into understanding a molecular control mechanism of this gene expression.

Nanda, S. A. and K. J. Mack (2000). "Seizures and sensory stimulation result in different patterns of brain derived neurotrophic factor protein expression in the barrel cortex and hippocampus." Molecular

Brain Research **78**(1-2): 1.

<http://www.sciencedirect.com/science/article/B6T07-40P8Y4K-1/2/9a74981f7be48b22df25ccd2525a3144>

Brain-derived neurotrophic factor (BDNF) is important for the development and trophic support of neurons, and may be involved in controlling axonal sprouting and synaptic plasticity. In order to investigate the activity-dependent regulation of the BDNF gene, BDNF expression was examined within the rat somatosensory cortex (SSC) and hippocampus following vibrissae stimulation, kainic acid induced seizure, and pentylenetetrazol (PTZ) induced seizure. The specific goals of this study were to determine the time course and magnitude of BDNF's activity-dependent expression, and to compare the expression patterns of three commonly used neuronal activation paradigms. Our results demonstrate three novel observations. First, the patterns of BDNF protein expression are dependent upon the neuronal stimulation model used. Both unilateral whisker stimulation (a model of experience dependent plasticity) and kainic acid induced seizure were able to increase the levels of BDNF protein within the SSC and hippocampus. In contrast, PTZ induced seizure did not increase BDNF protein levels in either tissue. Second, there is a dissociation between BDNF mRNA and protein levels following PTZ induced seizure. PTZ seizures resulted in strong increases of BDNF mRNA levels without corresponding increases of the protein. Finally, whisker stimulation resulted in an unexpected increase in BDNF mRNA and protein levels within the hippocampus. These results suggest specific types of neuronal activity can regulate gene expression differently. Furthermore, temporal and spatial differences between the expression of BDNF protein and mRNA levels suggest that the BDNF gene is regulated at the level of translation as well as transcription.

Negro, A., A. Tavella, et al. (1994). "Establishment and characterization of a CHO cell line producing a secreted form of human ciliary neurotrophic factor: neuroprotective effects of the recombinant protein." Molecular Brain Research **21**(1-2): 124.

<http://www.sciencedirect.com/science/article/B6T07-485CWX1-36/2/b4040acef9224182b54f010932d712f9>

Human ciliary neurotrophic factor (CNTF) was inserted into a mammalian expression vector linked to the prepro sequence of human nerve growth factor. A Chinese hamster ovary cell line was established by resistance to neomycin and the plasmid integrated DNA was amplified using the metallothionein gene. This cell line contained several hundred copies of the human CNTF gene and produced an NH2 terminal truncated form of human CNTF (22 kDa) which was secreted into the medium. Although the copy number of the human CNTF gene was high and its mRNA was actively transcribed, the recombinant protein secreted into the medium constituted only 35-40% of the total amount of human CNTF synthesized by these cells. Both wild-type human CNTF produced in bacterial cells and the human CNTF obtained by forced secretion were effective in protecting hippocampal pyramidal neurons from injury induced by glucose deprivation, a form of excitotoxic neurodegeneration.

Nemoto, K., M. Sekimoto, et al. (1999). "A possible mechanism of TPA-mediated downregulation of neurotrophin-3 gene expression in rat cultured vascular smooth muscle cells." Molecular Brain Research **68**(1-2): 186.

<http://www.sciencedirect.com/science/article/B6T07-3WF80M0-N/2/07f892dff46606ca7ec69e238f618a7c>

We have previously reported that in cultured rat vascular smooth muscle cells (VSMCs), neurotrophin-3 (NT-3) gene expression was suppressed by TPA (12-O-tetradecanoyl phorbol-13-acetate), which induces an AP-1 transcription factor. In the present study, to clarify the mechanism for TPA-mediated downregulation of NT-3 gene expression, effects of cycloheximide and dexamethasone (Dex) on the TPA-mediated downregulation were examined in VSMCs. Pretreatment with cycloheximide, an inhibitor of protein synthesis, or simultaneous treatment with Dex, an inhibitor of AP-1, suppressed the TPA-mediated downregulation of NT-3 gene expression. Furthermore, co-transfection of c-fos and c-jun expression vectors into VSMCs resulted in decrease in the NT-3 gene expression. The present findings suggest that TPA-induced AP-1 de novo synthesis causes the downregulation of NT-3 gene expression in VSMCs.

Niitsu, Y., O. Hori, et al. (1999). "Exposure of cultured primary rat astrocytes to hypoxia results in intracellular glucose depletion and induction of glycolytic enzymes." Molecular Brain Research **74**(1-2): 26.

<http://www.sciencedirect.com/science/article/B6T07-3YC068V-3/2/0c5296fabe2de17415bf4f8938a85a20>

Based on the neurotrophic properties of astrocytes in response to ischemia, the current work focuses on the mechanism for cultured astrocytes to adapt to a hypoxic environment. Intracellular glucose levels in primary cultured rat astrocytes exposed to hypoxia fell by 30% within 24 h, in parallel with a decrease in glycogen stores. Glycolytic metabolism was crucial for cell survival during hypoxia, as 2-deoxyglucose resulted in rapid ATP depletion and cell death. The mechanism for maintaining glucose levels under these conditions appeared to be mobilization of glycogen stores, rather than increased extracellular uptake of glucose, as gluconolactone (an inhibitor of [beta]1-4 amyloglucosidase) induced a rapid fall in cellular ATP in cultures subjected to hypoxia, whereas cytochalasin B was without affect. Addition of cycloheximide diminished the viability of astrocytes in hypoxia, suggesting an obligatory role of de-novo gene expression to respond to hypoxia. Consistently, the results of differential display suggested the induction of glycolytic enzymes, including aldolase A (EC 4.1.2.13), hexokinase II (ATP: D-hexose 6-phosphotransferase, EC 2.7.1.1), and triosephosphate isomerase (EC 5.3.1.1) in the hypoxic culture. Marked induction of these glycolytic enzymes in hypoxic astrocytes was confirmed by Northern blot analysis. These data provide a theoretical basis to understand the ability of astrocytes to tolerate ischemic condition.

Nilsson, A., K. Moller, et al. "Early changes in gene expression in the dorsal root ganglia after transection of the sciatic nerve; effects of amphiregulin and PAI-1 on regeneration." Molecular Brain Research In Press, Corrected Proof <http://www.sciencedirect.com/science/article/B6T07-4FMK8B4-4/2/ef59ee2b18cfea2266c11221ea1abfea>

To characterize the gene activity that may be required for neuronal survival and regeneration, we used the Affymetrix GeneChip Mu74A to screen 12000 genes and expressed sequence tag (EST) mRNA from L4 and L5 mouse dorsal root ganglia (DRG) 12 h and 24 h after sciatic nerve transection. At 12 h, we found 17 upregulated transcripts, and at 24 h, 49 that met our criteria of a significant 2-fold increase in expression. The alterations included a total of eight transcription factors and several genes associated with TGF-beta- and IL-6-mediated signaling. Two of the changes, amphiregulin and plasminogen activator inhibitor-1 (PAI-1), were confirmed by real-time quantitative PCR (QPCR). Addition of amphiregulin (20 ng/ml) to organ-cultured DRG stimulated axonal outgrowth while PAI-1 (20 nM) inhibited migration of Schwann cells from the ganglia.

Oh, Y., J. A. Black, et al. (1994). "The expression of rat brain voltage-sensitive Na⁺ channel mRNAs in astrocytes." Molecular Brain Research **23**(1-2): 57.

<http://www.sciencedirect.com/science/article/B6T07-485H25X-3R/2/4d26c468ef44fa449790e3ae7cb2818a>

Astrocytes from various regions of CNS have been shown to express voltage-activated Na⁺ currents. To date, three distinct subtypes (I, II and III) of Na⁺ channels have been cloned from rat brain. We have applied a combined technique of reverse transcription and polymerase chain reaction (RT-PCR) to examine the expression of rat brain Na⁺ channels in rat astrocytes in vivo and in vitro. Five PCR primer sets were used to amplify coding or 3' non-coding regions of subtype I, II, and III Na⁺ channels. We were able to amplify all three of these rat brain Na⁺ channel subtypes from rat optic nerve, which does not have neuronal cell bodies but does contain astrocytes known to express voltage-sensitive Na⁺ channels. In studies on cultured spinal cord astrocytes, we were also able to amplify all three subtypes of rat brain Na⁺ channel mRNAs. In control experiments, RT-PCR was performed on RNAs prepared from several rat tissues, including brain, skeletal muscle, and liver. Rat brain was shown to express the three Na⁺ channel subtypes as expected. In rat skeletal muscle, subtype I and III Na⁺ channel mRNAs, but not subtype II, were amplified. In rat liver, Na⁺ channel messages were not detectable. The present study provides the first direct evidence that astrocytes in vivo and in vitro express rat brain voltage-sensitive Na⁺ channel mRNAs, which have been considered as mainly neuronal-type Na⁺ channel messages.

Ozawa, K., T. Uruno, et al. (1996). "Expression of the fibroblast growth factor family and their receptor family genes during mouse brain development." Molecular Brain Research **41**(1-2): 279.

<http://www.sciencedirect.com/science/article/B6T07-3SDTRPB-16/2/d90b7636c05df75bad18ff252a32a389>

The fibroblast growth factor (FGF) family is composed of nine members and four genes encode protein tyrosine kinase receptors for them. To gain insight into the involvement of FGFs and their receptors in the development of nervous system, their expression in brains of perinatal and adult mice was examined by semi-quantitative reverse-transcription-linked polymerase chain reactions and in situ hybridization. Although all the genes, with the exception of FGF-4, were found to be expressed, FGF-3, FGF-6, FGF-7 and FGF-8 genes demonstrated higher expression in the late embryonic stages than in postnatal stages, suggesting that these members are involved in the late stages of brain development. In contrast, expression of FGF-1 and FGF-5 increased after birth. Interestingly, FGF-6 expression in perinatal mice was restricted to the central nervous system and skeletal muscles, with intense signals in the developing cerebrum in embryos but in cerebellum in 5-day-old neonates. Furthermore, FGF-receptor (FGFR)-4, a cognate receptor for FGF-6, demonstrated similar spatiotemporal expression, suggesting that FGF-6 and FGFR-4 plays significant roles in the maturation of nervous system as a ligand-receptor system. The results indicate that individual member of the fibroblast growth factor and their receptor family are expressed either sequentially or simultaneously in brain development, strongly suggesting their involvement in the regulation of a variety of developmental processes of brain, i.e., proliferation and migration of neuronal progenitor cells, neuron and glia differentiation, neurite extensions, and synapse formations.

Peinado-Ramon, P., A. Wallen, et al. (1998). "MAP kinase phosphatase-1 mRNA is expressed in embryonic sympathetic neurons and is upregulated after NGF stimulation." Molecular Brain Research **56**(1-2): 256.

<http://www.sciencedirect.com/science/article/B6T07-3SXFMR5-10/2/6fa79608c12a4d48dc96e4e642923bda>

The family of Tyr/Thr protein phosphatases, called dual-specificity phosphatases, have been implicated in the feedback regulation of the MAP kinase cascade by dephosphorylating the MAP kinases. Using low stringent cDNA screening we have isolated a chicken homologue of the CL100 phosphatase also called MAP kinase phosphatase 1 (MKP-1). The chicken MKP-1 has 84% and 85.5% identity to the rat and human amino acid sequence, respectively. Using RNase protection assay and in situ hybridization we have found that MKP-1 mRNA is expressed at low levels in most tissues during development. In embryonic dorsal root and sympathetic ganglia MKP-1 mRNA expression increases with age. The expression in large cells in dorsal root ganglia suggests that it is neurons which express MKP-1 mRNA. We also show that MKP-1 mRNA is induced in dissociated embryonic sympathetic neurons after nerve growth factor stimulation. In addition, our results show that MKP-1 mRNA is induced after NGF stimulation of fibroblasts expressing the NGF receptor TrkA, suggesting that MKP-1 is upregulated after activation of the TrkA receptor. These data show that the MKP-1 gene is regulated in a tissue and temporal specific fashion with strong expression in the developing peripheral ganglia, and suggest that the activation of MKP-1 mRNA expression by NGF is a ubiquitously induced response to TrkA activation, independent of the cellular origin or type on which the TrkA receptor is active.

Petitto, J. M., Z. Huang, et al. (1998). "Molecular cloning of the cDNA coding sequence of IL-2 receptor-[gamma] ([gamma]c) from human and murine forebrain: Expression in the hippocampus in situ and by brain cells in vitro." Molecular Brain Research **53**(1-2): 152.

<http://www.sciencedirect.com/science/article/B6T07-3S3DNY9-J/2/7e9f18bd1f201fa88f2c491b18d5bd82>

IL-2 has been implicated in various neurobiological processes of the mammalian CNS. To understand how IL-2 acts in the brain, our lab has sought to determine the molecular pharmacological characteristics of brain IL-2 receptors (IL-2R). The lymphocyte IL-2R[gamma], an essential subunit for IL-2 signaling, is also a common subunit ([gamma]c) for multiple immune cytokine receptors (e.g., IL-4R, IL-7R, IL-9R, IL-15R). Having previously cloned the [alpha] and [beta] subunits of the IL-2R heterotrimer complex from normal murine forebrain, we examined the hypothesis that the brain IL-2R[gamma] is derived from the same or a closely related gene coding sequence as that expressed by lymphocytes. In this study, we cloned and sequenced the full-length IL-2R[gamma] coding region from saline-perfused mouse forebrain and from a human hippocampal library. The cDNA sequences of IL-2R[gamma] from human and murine brain were 100% homologous to their lymphocyte sequences. Northern blot analysis showed that the mRNA transcripts in murine brain were the expected size, but the predominant transcript expressed in the brain was different than in the spleen. Compared to the spleen, very low levels of IL-2R[gamma] were expressed in the forebrain. In the murine hippocampus, a region where a number of neurobiological actions of IL-2 have been reported, IL-2R[gamma] mRNA was detected over the dentate gyrus and CA1-CA4 by in situ hybridization histochemistry. IL-2R[gamma] was found to be constitutively expressed by murine HN33.dw hippocampal neuronal cells, murine NB41A3 neuroblastoma cells, astrocyte-enriched mixed glial cell cultures, and in SCID mouse forebrain. The human cortical neuronal cell lines, HCN-1A and HCN-2, did not express the IL-2R[gamma] gene. These data suggest the possibility that, in addition to being essential in IL-2 signaling in brain, IL-2R[gamma] could be a common subunit ([gamma]c) for multiple cytokine receptors which may be operative in the mammalian CNS.

Pietruck, C., G.-X. Xie, et al. (1999). "Multiple splice patterns of cyclic AMP response element-binding protein mRNA in the central nervous system of the rat." Molecular Brain Research **69**(2): 286.

<http://www.sciencedirect.com/science/article/B6T07-3WN6HJX-G/2/6b72f63935c4afc3c661e50657184a2e>

The alternative splicing pattern of cyclic AMP response element-binding protein (CREB) in the central nervous system (CNS) of the rat has been investigated by an exon-flanking polymerase chain reaction (PCR) strategy. A series of RT-PCR studies with primer pairs flanking all possible alternative splicing sites (corresponding to a genomic region with at least one full exon and two flanking introns) has revealed multiple splice patterns in nine regions of the rat CNS. These include some novel transcripts that lack the phosphorylation site and a segment of the leucine zipper region which is crucial for dimerization and DNA binding. Some isoforms previously reported as testis-specific were also detected in the rat CNS. The findings from this study, which include differential splicing patterns among CNS regions, suggest a complex expression and functional regulation of CREB in the CNS.

Qian, Y., E. Tiffany-Castiglioni, et al. (1997). "A Menkes P-type ATPase involved in copper homeostasis in the central nervous system of the rat." Molecular Brain Research **48**(1): 60.

<http://www.sciencedirect.com/science/article/B6T07-3PKTNK0-9/2/aed1f549158ffbd62d91042de3526be8>

We previously reported that copper efflux from C6 rat glioma cells was blocked by a brief exposure to sulfhydryl reagents p-chloromercuribenzoate (PCMB) and iodoacetamide as well as dicyclohexylcarbodiimide, suggesting the possible involvement of a Cu-transporting ATPase in the efflux mechanism. In this report, we show that copper efflux from PC12 cells, a neuron-like cell line established from rat adrenal pheochromocytoma, is also inhibited by PCMB exposure. Furthermore, we show that both C6 and PC12 cells express a homolog of the Menkes gene (MNK) as detected by RT-PCR with primers designed from a mouse cDNA and confirmed by sequence analysis of the amplified product. An expected 760-bp fragment representing the transduction and phosphorylation domains and a 925-bp fragment encoding the heavy metal-binding domain of Atp7a were amplified from a RNA extract of C6 and PC12 cells. Sequence data revealed that 690 bp of the 760-bp fragment from C6 cells were an identical match to a similar fragment from PC12 cells. Both fragments encoded a 229 amino-acid polypeptide that had a 98.7% sequence homology to mouse Atp7a. In addition, 880 bp from the 925-bp fragment of the two cell lines were identical and encoded a 293 amino-acid polypeptide with 94.5% sequence homology to mouse Atp7a. These data establish that a Menkes-type Cu-transporting ATPase is expressed in rat C6 and PC12 cells and strongly support the hypothesis that both neurons and glia are involved in maintaining Cu homeostasis in the central nervous system.

Rajeevan, M. S., S. Hu, et al. (1999). "Cloning and expression of Shaker [alpha]- and [beta]-subunits during inner ear development." Molecular Brain Research **66**(1-2): 83.

<http://www.sciencedirect.com/science/article/B6T07-3W1YG48-9/2/cac80444ea29f29245147b959b2c95c5>

Sensory cells of the chicken cochlea exhibit different ion channels relative to their position along the epithelium. One of these channels conducts an A-type potassium current which is found primarily in 'short' hair cells. Here, we report the first full length cloning and developmental expression of Shaker genes from this endorgan. Clones were obtained by screening a chicken (*Gallus gallus*) cochlea cDNA library, using probes made from RHK1 (i.e., Kv[alpha]1.4) cDNA, a Shaker homologue isolated from rat heart, and hKv[beta]1.2 cDNA, a [beta] homologue isolated from human heart. Sequence analysis revealed a chick homologue of Kv[alpha]1.4, with a

deduced amino acid similarity of 76-79% to mammalian Kv[alpha]1.4, and a chick homologue of Kv[beta]1.1, with a similarity of 95% to mammalian Kv[beta]1.1. In addition, we isolated a variant of cKv[alpha]1.4 (cKv[alpha]1.4(m)) that differs in its untranslated regions and shows complete similarity in its coding region, except for the deletion of a single nucleotide. During development of the inner ear, reverse transcription-polymerase chain reaction (RT-PCR) studies show that the [beta]-subunit is expressed as early as embryonic day 3, whereas [alpha]- and [beta]-subunits are coexpressed on embryonic days 7 to 10, 14, and in adult.

Reid, R. A., D. D. Bronson, et al. (1994). "Identification and characterization of the human cell adhesion molecule contactin." Molecular Brain Research **21**(1-2): 1.

<http://www.sciencedirect.com/science/article/B6T07-485CWX1-2R/2/c58f4ce0da8d0ea417c4a142922d2ecd>

We have prepared a monoclonal antibody, Neuro-1, that recognizes the human homolog of the chicken contactin/F11 and mouse F3 cell adhesion molecules. The Neuro-1 antigen, structurally characterized as a 135 kDa glycosylphosphatidylinositol-linked glycoprotein, was immunoaffinity purified and partially sequenced. Comparison of an internal peptide sequence to that predicted from the chicken contactin/F11, mouse F3 and human contactin (reported herein) cDNA sequence identifies the Neuro-1 antigen as human contactin. Moreover, a polyclonal antisera generated against the purified Neuro-1 antigen was immunoreactive with a fragment of human contactin expressed in bacteria. The complete coding and deduced amino acid sequences of human contactin were determined and are 86% and 95% identical to the respective mouse F3 sequences. Structural features shared with contactin/F11/F3 include six immunoglobulin type C2 and four fibronectin type III-like domains, multiple sites for asn-linked glycosylation and a COOH-terminal signal peptide presumably removed during the generation of a phosphatidylinositol cell surface linkage. The potential for glycosylation and GPI-linkage is also consistent with protein chemical studies of human contactin. Contactin mRNA expression was characterized using Northern blot analyses of human tissues and cell lines. High level expression of a single contactin transcript in adult brain, and low level expression of multiple transcripts in lung, pancreas, kidney and skeletal muscle are observed. Highly expressed multiple transcripts, similar in pattern to that of pancreas, lung, kidney and skeletal muscle, are also observed in human neuroblastoma and retinoblastoma cell lines.

Ringheim, G. E., A. M. Szczepanik, et al. (1998). "Enhancement of beta-amyloid precursor protein transcription and expression by the soluble interleukin-6 receptor/interleukin-6 complex." Molecular Brain Research **55**(1): 35.

<http://www.sciencedirect.com/science/article/B6T07-3TCFW0R-5/2/b53389ac960434d63ad143464e7a5200>

We investigated a potential role for the soluble interleukin-6 receptor (sIL-6R) in modulating interleukin-6 (IL-6) function in the central nervous system by assessing IL-6 and sIL-6R effects on [beta]-amyloid precursor protein ([beta]-APP) transcription and expression in cells of human neuronal origin. Cells transfected with a luciferase reporter plasmid containing a 3.8 kb DNA fragment of the [beta]-APP promoter were shown to have inducible promoter activity when treated with phorbol ester or basic fibroblast growth factor, but not when treated with lipopolysaccharide or IL-6. PCR amplification analysis revealed the presence of mRNA encoding the signaling subunit of the IL-6 receptor complex, the gp130 subunit, at levels approximating that found in human cortical tissue. The mRNA encoding the IL-6 receptor, however, was poorly expressed and was detectable only at high amplification cycles. When purified sIL-6R protein was added together with IL-6, there was a rapid induction of promoter activity within 2 h of stimulation

followed by elevations in protein levels of both cell-associated and secreted [beta]-APP. Analysis of mRNA transcripts from human cortical brain tissue and cell cultures derived from fetal human brain demonstrated the presence of an alternatively spliced secreted form of the IL-6 receptor mRNA, suggesting that cells of the central nervous system may themselves be a source of sIL-6R protein. The capacity for sIL-6R to enhance IL-6 function and broaden the IL-6 target cell population in the brain has implications for the regulation of [beta]-APP expression in disease states such as Alzheimer's disease where elevations in brain IL-6 levels have been reported.

Rohan de Silva, H. A., A. Jen, et al. (1997). "Cell-specific expression of [beta]-amyloid precursor protein isoform mRNAs and proteins in neurons and astrocytes." Molecular Brain Research **47**(1-2): 147.

<http://www.sciencedirect.com/science/article/B6T07-3S0MHR6-K/2/3e987537cebc0d7bca80e10a1d54b2ff>

The abnormal accumulation of [beta]-amyloid (A[beta]) in senile plaques appears to be a central pathological process in Alzheimer's disease. A[beta] is formed by proteolysis of [beta]-amyloid precursor protein (APP) with several isoforms generated by alternative splicing of exons 7, 8 and 15. A semi-quantitative reverse transcription (RT)-polymerase chain reaction (PCR) analysis showed that APP695 mRNA lacking exon 7 and 8 was most abundant in primary cultures of rat neurons, while APP770 and APP751 representing, respectively, the full length and exon 8 lacking isoforms predominated in cultured astroglial cells. Antisera AP-2 and AP-4 were produced by immunizing rabbits with keyhole limpet haemocyanin coupled with synthetic peptides representing KPI region APP301-316 and A[beta] region APP670-686 of APP770, respectively. These polyclonal antisera were purified against the corresponding peptide using affinity chromatography. Western blot analysis of homogenates of relatively enriched neuronal and astroglial cultures showed that these antibodies discretely stained bands of proteins in a cell-specific manner. Dot-blot analysis using AP-2, AP-4 and 22C11 antibodies indicated that, in comparison with neurons, cultured astrocytes contained 3-fold greater KPI-containing APP isoform proteins. The amount of total APP proteins, which include both KPI-containing and KPI-lacking APP isoforms, was [ap]90% higher in astrocytes than in neurons. Consistent with these in vitro findings in cultured astrocytes, in fimbria-fornix lesioned rat hippocampus, labelling with AP-2 antibody, which specifically reacts with KPI-containing APP proteins, was mainly observed in glial fibrillary acidic protein-positive reactive astrocytes in vivo. The results showed that APP isoforms are expressed in a cell type-specific manner in the brain and, since deposition of A[beta] is closely associated with the expression of KPI-containing APP isoforms, provide further evidence for the involvement of astrocytes in plaque biogenesis.

Saragoza, P. A., J. G. Modir, et al. (2003). "Identification of an alternatively processed nicotinic receptor [alpha]7 subunit RNA in mouse brain." Molecular Brain Research **117**(1): 15.

<http://www.sciencedirect.com/science/article/B6T07-497HFM1-3/2/bb904cb5cbd485ec6205d14b4d6f55b1>

The data in this report describe the discovery and characterization of a previously unidentified alternatively processed RNA for the neuronal nicotinic receptor alpha7 subunit. The unique transcript contains an extra exon that arises from alternative splicing of intron nine of the alpha7 subunit RNA. The alpha7 subunit protein resulting from this alternatively processed RNA is truncated shortly after transmembrane domain three. The variant protein also has a predicted amino acid substitution in the large N-terminal domain as a consequence of a non-templated nucleotide substitution present in the variant alpha7 subunit RNA. The mechanism responsible for the nucleotide substitution is not known. Initial characterization of the variant alpha7 subunit suggests that it is expressed in mouse brain in a pattern similar to the standard alpha7 subunit

although at reduced levels. The variant alpha7 subunit was also found to act as a dominant-negative effector of normal alpha7 subunit function.

Schneider, A., A. Fischer, et al. (2004). "Identification of regulated genes during transient cortical ischemia in mice by restriction-mediated differential display (RMDD)." Molecular Brain Research **124**(1): 20.

<http://www.sciencedirect.com/science/article/B6T07-4C1FGS8-1/2/7af1ba0b7de7d521316d1652e7833ea3>

Cerebral ischemia induces transcriptional changes in a number of pathophysiologically important genes. Here we have systematically studied gene expression changes in the cortex after 150 min of focal cortical ischemia and 2 and 6 h reperfusion in the mouse by a fragment display technique (restriction-mediated differential display, RMDD). We identified 57 transcriptionally altered genes, of which 46 were known genes, and 11 unknown sequences. Of note, 14% of the regulated genes detected at 2 h reperfusion time were co-regulated in the contralateral cortex. Four genes were verified to be upregulated by quantitative PCR. These were Metallothionein-II (mt2), Receptor (calcitonin)-activity modifying protein 2 (ramp2), Mitochondrial phosphoprotein 65 (MIPP65), and the transcription elongation factor B2/elongin B (tceb). We could identify several genes that are known to be induced by cerebral ischemia, such as the metallothioneins and c-fos. Many of the genes identified provide hints to potential new mechanisms in ischemic pathophysiology. We discuss the identity of the regulated genes in view of their possible usefulness for pharmacological intervention in cerebral ischemia.

Shimada, J., S. Fushiki, et al. (1997). "Fibroblast growth factor-2 expression is up-regulated after denervation in rat lung tissue." Molecular Brain Research **49**(1-2): 295.

<http://www.sciencedirect.com/science/article/B6T07-3RWWXKC-17/2/39fa30f1cf7d2b06eecbf04a6f69c01>

To understand the role of fibroblast growth factor-2 (FGF-2) during the denervation-reinnervation processes which occur after lung transplantation, we studied FGF-2 gene expression in a rat lung denervation model. The temporal profile of FGF-2 mRNA in denervated rat lungs was quantitatively assessed by competitive reverse transcription polymerase chain reaction (RT-PCR) method. The level of FGF-2 mRNA was consistently higher in denervated lungs, showing a peak value on the 5th post-operative day. Immunohistochemical analysis with an anti-FGF-2 monoclonal antibody disclosed immunoreactivity in Schwann cells at the distal severed end of the nerve fascicle located at the lung hilus, 1 week post-surgery. This study indicates that FGF-2 gene expression is up-regulated following denervation and suggests possible roles of FGF-2 in the reinnervation process of lung tissue.

Shiomi, K., M. Kanemoto, et al. "Identification and differential expression of multiple isoforms of mouse Coiled-coil-DIX1 (Ccd1), a positive regulator of Wnt signaling." Molecular Brain Research In Press, Corrected Proof <http://www.sciencedirect.com/science/article/B6T07-4FBN79P-1/2/091cc7a5e2f3c083617f0b72ace44940>

The Wnt signaling plays important roles in cell growth, differentiation, polarity formation, and neural development. In the canonical pathway, two DIX domain-containing proteins, Dishevelled (Dvl) and Axin, regulate the degradation of [beta]-catenin that activates Wnt target genes through

TCF/LEF family transcription factors. Recently, we have isolated a third type of DIX domain-possessing protein, Coiled-coil-DIX1 (Ccd1). Ccd1 forms homomeric and heteromeric complexes with Dvl and Axin, and regulates the neural patterning in zebrafish embryos through Wnt pathway activation. Here, we report the isolation and characterization of mouse Ccd1. Fourteen putative mRNA isoforms are generated by different promoter usage and alternative splicing, and each isoform shows different expression patterns in various tissues. The predicted Ccd1 proteins are classified into three subtypes, and a novel form, termed Ccd1A, possesses an N-terminal calponin homology domain, suggesting an additional interaction of the isoform with actin or other proteins. When Ccd1 proteins were singularly expressed in Hela cells, they showed almost no activation of TCF-dependent reporter transcription on their own. However, when Dvl protein, at the level that did not activate Wnt pathway by itself, was co-expressed with Ccd1, the reporter transcription was greatly potentiated in Ccd1-dose-dependent manner. In addition, Ccd1- and Wnt3a-dependent activation of Wnt pathway was inhibited by Axin or a dominant negative Ccd1. These results indicate that mouse Ccd1 functions as a positive regulator of the Wnt/[beta]-catenin pathway. Furthermore, Ccd1 is highly expressed and co-localized with Wnt signaling molecules in the embryonic and adult brain, implicating the importance of Ccd1 in the Wnt-mediated neuronal development, plasticity, and remodeling.

Snell, L. D., K. R. Nunley, et al. (1996). "Regional and subunit specific changes in NMDA receptor mRNA and immunoreactivity in mouse brain following chronic ethanol ingestion." Molecular Brain Research **40**(1): 71.

<http://www.sciencedirect.com/science/article/B6T07-49HGC9G-G/2/770933731b23305e399088d172232e47>

Chronic ethanol treatment of mice has been shown to result in increased binding of dizocilpine and glutamate to hippocampal NMDA receptors. These changes were suggested to reflect an increase in NMDA receptor number that may underlie certain signs of the ethanol withdrawal syndrome. However, there was no change in binding of a competitive NMDA receptor antagonist, or of ligand binding to the glycine co-agonist site on the receptor after chronic ethanol treatment. Differential changes in the binding of particular ligands at the NMDA receptor suggested the possibility that chronic ethanol ingestion might selectively affect the expression of particular NMDA receptor subunits. Our current work demonstrates that chronic ethanol ingestion by mice, which results in the generation of physical dependence, also produces increases in the NMDA receptor NR1 subunit protein in the hippocampus and cerebellum ([ap] 50% and 95%, respectively), and produces increases in the NR2A subunit protein in the hippocampus and cortex ([ap] 25% and 40%, respectively). However, the mRNA levels for these subunits were not increased in the respective brain areas by the same ethanol treatment. The changes in NMDA receptor subunit expression in discrete areas of the brain may contribute to the previously observed changes in ligand binding and, possibly, signs of ethanol withdrawal.

Sodhi, C. P., S. Rampalli, et al. (2004). "The endocytotic pathway is required for increased A[beta]42 secretion during apoptosis." Molecular Brain Research **128**(2): 201.

<http://www.sciencedirect.com/science/article/B6T07-4CX1959-2/2/1d0b9b91a901f592985e19e3864b15ae>

Secretion and progressive cerebral accumulation of [beta]-amyloid peptides (A[beta]), which derive by endoproteolytic ('amyloidogenic') processing of [beta]-amyloid precursor protein (APP), are felt to represent collectively an early and necessary event in the pathogenesis of Alzheimer's disease. APP amyloidogenic processing can occur via secretory or endocytotic pathways, but the relative contribution of these pathways to A[beta] secretion remains to be established. The effect

of apoptosis on amyloidogenic processing and A[beta] secretion similarly is incompletely understood. We tested the hypothesis that APP processing by the endocytotic pathway represents a stress-related neural cell response, by comparing A[beta] secretion after induction of apoptosis in PC12 cells transfected either for endocytosis-competent or -deficient APP. Newly prepared adenoviral vectors encompassing targeted mutagenesis of the cytoplasmic tail YENP tetrapeptide sequence, which serves as the principal APP internalization signal, were used to express endocytosis-deficient holoprotein. We report that the endocytotic pathway is required for the generation and secretion of A[beta]42, and that secretion of this neurotoxic peptide increases significantly during apoptosis. We demonstrate additionally that more A[beta]40 apparently is generated in secretory compartments during apoptosis when APP processing by the endocytotic pathway is impaired.

Stover, T., T.-W. L. Gong, et al. (2000). "Expression of the GDNF family members and their receptors in the mature rat cochlea." Molecular Brain Research **76**(1): 25.

<http://www.sciencedirect.com/science/article/B6T07-3YSXRKR-4/2/3fadb2588e3e83a592576a00eaab4f0>

The GDNF family comprises glial cell line-derived neurotrophic factor (GDNF) and the related proteins neurturin, artemin and persephin, which form a subgroup of the TGF- β superfamily of growth factors. All four neurotrophic factors provide neuronal cell protection and cell survival. GDNF expression was found in the cochlea, and GDNF has been shown to be effective for inner ear protection from drugs and noise-induced insults. As the other members of the GDNF family also provide protective effects on neuronal cells, they may play important roles in the inner ear. We used RT-PCR to examine the expression of GDNF, neurturin, artemin, persephin and their receptors GFR α -1, GFR α -2, GFR α -3 and c-ret in whole rat cochlea as well as in functionally different subfractions (modiolus and sensorineural epithelium/lateral wall) and compared the levels of neurotrophin and receptor mRNAs in the cochlea to those in substantia nigra brain region. Our results demonstrate the expression of all GDNF family members and their receptors in cochlea and substantia nigra. However, the relative levels of mRNA were different for several genes tested in subfractions of the cochlea and/or compared to expression levels in substantia nigra. The presence of mRNA for all four members of the GDNF family and their preferred receptors in the rat cochlea suggests potential functional importance of these neurotrophic factors as protection and survival factors in the inner ear.

Suemoto, T., M. Taniguchi, et al. (1999). "cDNA cloning and expression of a novel serine protease in the mouse brain." Molecular Brain Research **70**(2): 273.

<http://www.sciencedirect.com/science/article/B6T07-3WXNYJK-B/2/63551889f37b7443051ed8f8e7ab1e47>

A cDNA for a novel serine protease, termed brain type granzyme K (B-GRK) was cloned from the mouse brain. The cDNA codes a protein similar to granzyme K (GRK) but completely different at the N-terminus. Genomic Southern and PCR analysis of the gene suggests B-GRK is the alternative transcription form of GRK. B-GRK and GRK have a different organ-specific expression pattern: B-GRK is expressed in the brain, while GRK is expressed in the spleen. The recombinant fusion protein was detected in the neuro2a cells transfected with a plasmid containing B-GRK sequence. The mRNA for B-GRK/GRK was detected in cerebral cortex, hippocampus and diencephalon of the mouse brain. In situ hybridization for B-GRK/GRK revealed that several regions in the forebrain and hypothalamus express the mRNA. Developmental analysis showed that in the prenatal stage, the mRNA was expressed also in pituitary and pineal body in addition to the brain.

Suter-Crazzolara, C., A. Lachmund, et al. (1996). "Expression of neurotrophins and their receptors in the developing and adult rat adrenal gland." Molecular Brain Research **43**(1-2): 351.

<http://www.sciencedirect.com/science/article/B6T07-3RSGFBD-1G/2/f6a54f50530640d8cae403d57c179dc9>

We have studied the postnatal expression of neurotrophins, their cognate high-affinity trk receptors and the low-affinity NGF receptor (p75) in the rat adrenal gland using RT-PCR. Neurotrophin mRNAs were detectable during the whole postnatal period. Strongest signals were obtained for BDNF and NT4/5. Expression of trkA, trkB, trkC and p75 was found at all ages studied. Signals for trkA were highest in the adult adrenal medulla, whereas signals for p75 were highest in the adult adrenal cortex. Our data suggest still largely enigmatic roles for neurotrophins in functions of the adrenal medulla and possibly also the cortex.

Suter-Crazzolara, C. and K. Unsicker (1996). "GDNF mRNA levels are induced by FGF-2 in rat C6 glioblastoma cells." Molecular Brain Research **41**(1-2): 175.

<http://www.sciencedirect.com/science/article/B6T07-3SDTRPB-T/2/032738cf5764fc80de26c6085babe129>

Glia cell line-derived neurotrophic factor (GDNF), a recently cloned member of the transforming growth factor-[beta] (TGF-[beta]) superfamily, has been implicated in the survival, morphological and functional differentiation of midbrain dopaminergic neurons and motoneurons in vitro and in vivo. The factor may thus have utility in the treatment of various human neurodegenerative disorders. Mechanisms regulating expression of GDNF in normal and diseased brain as a possible means to increase the local availability of GDNF are only beginning to be explored. We have established and employed a competitive reverse transcriptase-polymerase chain reaction (RT-PCR) to study and compare levels of expression of GDNF mRNA in several cell types and to investigate its regulation. GDNF expression was clearly evident in primary cultured astrocytes, the glioma B49 and c6 cell, but less pronounced in the Schwannoma RN22 cell lines. Little or no signal could be observed in neuroblastoma cell lines (IMR32, LAN-1) or the pheochromocytoma cell line PC12, emphasizing the glial character of this factor. Using the C6 cell line we found that fibroblast growth factor-2 (FGF-2; bFGF) can increase GDNF mRNA levels, whereas FGF-1, platelet-derived growth factor (PDGF), and vasoactive intestinal polypeptide (VIP) are apparently ineffective. Several other factors (forskolin, kainic acid, triiodothyronine, dexamethasone, GDNF, TGF-[beta]1, and interleukin-6) appear to have slightly negative effects on GDNF mRNA levels at the concentrations tested. To further explore the relationship between FGF-2 and GDNF, we also addressed the question whether GDNF, like FGF-2, may have an effect on C6 cell proliferation. We conclude that (1) glial and glial tumor cells, rather than neuronal cell lines, express GDNF, (2) that FGF-2 has a prominent inductive effect on GDNF expression and (3) that GDNF stimulates C6 cell proliferation. Finally, these data suggest that neurotrophic actions of FGF-2 in mixed glial-neuronal cell cultures might be mediated in part by GDNF.

Suzuki, M., H. Kitano, et al. (1998). "RT-PCR analysis of mRNA expression of natriuretic peptide family and their receptors in rat inner ear." Molecular Brain Research **55**(1): 165.

<http://www.sciencedirect.com/science/article/B6T07-3TCFW0R-M/2/445c354c771727428632167a1d2d6c5a>

To assess the possible physiological role of the atrial natriuretic peptide (ANP) family, we investigated the expression of mRNA of ANP, brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP), and their receptors in rat inner ear using the reverse transcription-polymerase chain reaction method. ANP and CNP message bands were detected in the inner ear, but the BNP message band was not. Amplification products of the expected sizes of ANP-A, ANP-B, and ANP-C receptors were detected in the inner ear. These results suggest that natriuretic peptide family may influence the function of the inner ear through the ANP-A, ANP-B, and ANP-C receptors.

Taguchi, A., A. Wanaka, et al. (1996). "Molecular cloning of novel leucine-rich repeat proteins and their expression in the developing mouse nervous system." Molecular Brain Research **35**(1-2): 31.

<http://www.sciencedirect.com/science/article/B6T07-3VXBRP8-4/2/13e88a7cd65199e82ea50acd6fda7422>

It is well established that leucine-rich repeat (LRR) proteins such as connectin, slit, chaoptin, and Toll have pivotal roles in neuronal development in *Drosophila* as cell adhesion molecules. However, to date, little information concerning mammalian LRR proteins has been reported. In the present study, we sought LRR proteins of the mouse brain, based on the assumption that fundamental mechanisms are conserved between different species. We screened a neonatal mouse brain cDNA library with a human partial cDNA encoding LRR protein as a probe. We obtained two independent cDNAs encoding LRR proteins, designated NLRR-1 and NLRR-2 (Neuronal Leucine-Rich Repeat proteins). We analyzed the whole sequence of NLRR-1 and partial sequence of NLRR-2. Sequence analysis showed that these two clones are about 60% homologous to each other, and that NLRR-1 protein is a transmembrane protein. Northern blot analysis and in situ hybridization histochemistry showed that both NLRR-1 and NLRR-2 mRNAs were expressed primarily in the central nervous system (CNS); NLRR-1 mRNA was also detected in the non-neuronal tissues such as cartilage, while NLRR-2 mRNA expression was confined to the CNS at all developmental stages. These results suggest that there is at least one LRR protein family in the mouse and that these molecules may play significant but distinct roles in neural development and in the adult nervous system.

Takanashi, M., H. Mori, et al. (2002). "Expression patterns of tau mRNA isoforms correlate with susceptible lesions in progressive supranuclear palsy and corticobasal degeneration." Molecular Brain Research **104**(2): 210.

<http://www.sciencedirect.com/science/article/B6T07-46R9NFD-M/2/eedf67b1bf670b7bc868785515cd123d>

Deposition of hyperphosphorylated tau (p-tau) has been observed in several neurodegenerative diseases. The six isoforms of tau are divided into two main groups including three repeat (3R) and four repeat (4R) microtubule-binding domains. Using quantitative RT-PCR method and immunohistochemistry with phosphorylation dependent anti-tau antibody (AT8), we investigated the expression level of tau mRNA isoforms in the frontal cortex and globus pallidus of patients with progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) to determine whether altered expression patterns of tau mRNA isoforms correlate with p-tau accumulation. The 4R/3R ratios in frontal cortices of CBD and globus pallidus of PSP and CBD were significantly higher than the control ($P < 0.05$). There was no correlation between the expression patterns of tau mRNA isoforms and p-tau accumulation. Our findings suggest that neurodegeneration of PSP and CBD could be regulated by alternative splicing of tau mRNA to yield high 4R/3R ratio. In addition, the lack of correlation between the expression pattern of tau mRNA isoforms and p-tau accumulation suggests that not only alternative splicing of tau mRNA, but also other factors such

as post-transcriptional or translational modifications may play a role in the pathogenesis of specific neurodegeneration in PSP and CBD.

Temkin, R., D. Lowe, et al. (1997). "Expression of glutamate receptor subunits in [alpha]-motoneurons." Molecular Brain Research **52**(1): 38.

<http://www.sciencedirect.com/science/article/B6T07-3S0VGXX-4/2/bfbf6b98fa3f96c81270eadbf8ea63d2>

Whole-cell recordings from 6.5 day embryonic chick [alpha]-motoneurons indicated the presence of AMPA, kainate, and NMDA glutamate receptor subtypes in each motoneuron tested. AMPA consistently evoked a desensitizing response, while kainate could evoke either a desensitizing or non-desensitizing whole-cell response. In excised membrane patches, desensitizing AMPA responses appeared to be colocalized with non-desensitizing kainate responses. Desensitizing kainate responses were seen in some patches which were not responsive to AMPA, suggesting that kainate selective subunits and AMPA selective subunits localize separately on the motoneuron membrane. To determine which of the known glutamate receptor subunits might underlie these responses, we used RT-PCR amplification to detect subunits present in mRNA isolated from adult rat spinal cord and from a highly enriched motoneuron population from embryonic chick. Sequencing of the the amplified cDNA was used to verify the identity of the products and of the alternative splice variants of GluR1-4. In rat spinal cord, all subunits that we attempted to detect, including AMPA selective subunits GluR1-4, kainate selective subunits GluR5-7 and KA1-2, and NMDA subunit NR1 were present. The isolated motoneurons also contained AMPA subunits GluR1, 2, and 4, and kainate subunits GluR6 and 7. The GluR2 and 4 subunits were specifically processed by splicing, present primarily as the flip splice form.

Thiriet, N., S. Jayanthi, et al. (2001). "Methamphetamine increases expression of the apoptotic c-myc and -myc genes in the mouse brain." Molecular Brain Research **90**(2): 202.

<http://www.sciencedirect.com/science/article/B6T07-4378WBM-J/2/63718ddccbc53a2ff4abc10caa1c54c9>

To clarify the possible mechanisms by which the recreational drug, methamphetamine (METH), induces apoptosis, we investigated its effects on the expression of Myc apoptotic genes. This paper presents the characterization of c-myc and -myc gene transcription in the striatum and the cortex. In addition, the expression of the corresponding proteins was also evaluated. Our observations reveal that c-myc and -myc were up-regulated by METH at both the mRNA and protein levels. Thus, myc transcription factors might be responsible for some aspects of METH-induced apoptotic processes.

Vega-Saenz de Miera, E. C. (2004). "Modification of Kv2.1 K⁺ currents by the silent Kv10 subunits." Molecular Brain Research **123**(1-2): 91.

<http://www.sciencedirect.com/science/article/B6T07-4BWYVG9-1/2/0cb2d44aabbf32201c130742c5db41b2f>

Human and rat Kv10.1a and b cDNAs encode silent K⁺ channel pore-forming subunits that modify the electrophysiological properties of Kv2.1. These alternatively spliced variants arise by the usage of an alternative site of splicing in exon 1 producing an 11-amino acid insertion in the

linker between the first and second transmembrane domains in Kv10.1b. In human, the Kv10s mRNA were detected by Northern blot in brain kidney lung and pancreas. In brain, they were expressed in cortex, hippocampus, caudate, putamen, amygdala and weakly in substantia nigra. In rat, Kv10.1 products were detected in brain and weakly in testes. In situ hybridization in rat brain shows that Kv10.1 mRNAs are expressed in cortex, olfactory cortical structures, basal ganglia/striatal structures, hippocampus and in many nuclei of the amygdala complex. The CA3 and dentate gyrus of the hippocampus present a gradient that show a progression from high level of expression in the caudo-ventro-medial area to a weak level in the dorso-rostral area. The CA1 and CA2 areas had low levels throughout the hippocampus. Several small nuclei were also labeled in the thalamus, hypothalamus, pons, midbrain, and medulla oblongata. Co-injection of Kv2.1 and Kv10.1a or b mRNAs in *Xenopus* oocytes produced smaller currents than in the Kv2.1 injected oocytes and a moderate reduction of the inactivation rate without any appreciable change in recovery from inactivation or voltage dependence of activation or inactivation. At higher concentration, Kv10.1a also reduces the activation rate and a more important reduction in the inactivation rate. The gene that encodes for Kv10.1 mRNAs maps to chromosome 2p22.1 in human, 6q12 in rat and 17E4 in mouse, locations consistent with the known synteny for human, rat and mouse chromosomes.

Vidovic, M., D. Cohen, et al. (1994). "Identification of [alpha]2 adrenergic receptor gene expression in sympathetic neurones using polymerase chain reaction and in situ hybridization." Molecular Brain Research **22**(1-4): 49.

<http://www.sciencedirect.com/science/article/B6T07-485P8V9-7/2/bd244e5ed546c1f24e5bb27ef530f474>

[alpha]2 Adrenergic receptors are involved in mediating pre- and postsynaptic responses in the sympathetic nervous system. In this study, the expression of [alpha]2 genes was examined by the amplification of mRNA, extracted from adult rat superior cervical ganglion through reverse transcription and subsequent amplification of appropriate target sequence using polymerase chain reaction and sequence specific oligonucleotide primers for the three [alpha]2 receptor genes. Results from these studies have shown that the major [alpha]2 adrenergic mRNA transcript was the one that encodes the [alpha]2A receptor. Nucleotide sequence of the 312 base-pair (bp) [alpha]2A cDNA was homologous to the RG20 adrenergic receptor, the rat homologue of the human [alpha]2A receptor. The 312 bp [alpha]2A cDNA was used as a probe in Northern blot analysis of the mRNA from superior cervical ganglion and brain. A 3.9 kb mRNA transcript was present in these extracts. To confirm that the [alpha]2A gene expression was in the sympathetic neurones we have used the 312 bp [alpha]2A cDNA, biotinylated, as a probe for in situ hybridization studies and have demonstrated that the [alpha]2A mRNA was found only in the cell bodies of sympathetic neurones.

Vlajkovic, S. M., G. D. Housley, et al. (1999). "Evidence for alternative splicing of ecto-ATPase associated with termination of purinergic transmission." Molecular Brain Research **73**(1-2): 85.

<http://www.sciencedirect.com/science/article/B6T07-3Y8W5P6-B/2/8b9a7c9cdad40462b235ba641dbbdeb7>

Ectonucleotidases provide the signal termination mechanism for purinergic transmission, including fast excitatory neurotransmission by ATP in the CNS. This study provides evidence for ectonucleotidase expression in the rat cochlea, brain and other tissues. In addition to detection of rat ecto-ATPase and ecto-ATPDase in these tissues, we identify a novel ecto-ATPase splice variant arising from the loss of a putative exon (193 bp) in the C-terminal coding region. This is the first evidence of alternative splicing in the ecto-ATPase gene family. Splicing of the 193-bp

putative exon containing a stop codon extends the open reading frame and provides translation of an additional 50 amino acids compared with the isoform isolated earlier from the rat brain (rEATPaseA; GenBank accession #Y11835). The splice variant (rEATPaseB; GenBank accession #AF129103) encodes 545 amino acids with a predicted protein molecular mass of 60 kDa. rEATPaseB contains a long cytoplasmic tail (62 amino acids) with three potential protein kinase CK2 phosphorylation sites not present in rEATPaseA. Co-expression of two ecto-ATPase isoforms with different regulatory sites suggests that the extracellular ATP signal levels may be differently influenced by intracellular feedback pathways.

Vrana, S. L., B. W. Kluttz, et al. (1995). "Application of quantitative RT-PCR to the analysis of dopamine receptor mRNA levels in rat striatum." Molecular Brain Research **34**(1): 127.

<http://www.sciencedirect.com/science/article/B6T07-3Y3YTVF-F/2/98cf9d53054de9f0b819899f034fa323>

A quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) procedure has been developed which selectively amplifies and quantifies the two isoforms of the dopamine D2 receptor. Variability is corrected by the inclusion of a D2 dopamine receptor mRNA standard within each reaction. The internal standard was generated by introducing a point mutation within a D2 cDNA clone that created a unique restriction site within the amplified region. An in vitro transcribed RNA for the internal mutant control is added to the RNA isolated from brain tissue and the mixture is subjected to RT-PCR, digestion with the restriction enzyme, separation of the products by PAGE, and quantification by direct analysis of radioactivity incorporated during the PCR step. The standard is amplified, in the same reaction as the experimental RNA, using the same primers and RT-PCR conditions. In this manner, the effects of contaminants of the RNA preparation which could affect the amplification procedure are assessed. To insure that the amplification is linear, the number of PCR cycles is minimized. This adaptation avoids 'competitive PCR' and provides for a linear response. Moreover, to obviate non-specific co-amplification, primer annealing steps are performed at or above the melting temperature for the primers, thus increasing signal-to-noise ratios. Finally, primer pairs have been designed which permit amplification of specific fragments for each of the five rat dopamine receptor subtypes. These fragments have unique sizes and so can be differentiated when simultaneously amplified in the same RNA preparations.

Vreugdenhil, E., B. Engels, et al. (2001). "Multiple transcripts generated by the DCAMKL gene are expressed in the rat hippocampus." Molecular Brain Research **94**(1-2): 67.

<http://www.sciencedirect.com/science/article/B6T07-453JRSN-G/2/3421de967d5e63f37f5a5d0557a9c25a>

We have recently cloned a novel Doublecortin CaMK-like kinase (rDCAMKL) cDNA, and a related cDNA called CaMK-related peptide (CARP) from the rat hippocampus. These genes are structurally highly similar to the human DCAMK-1 gene and doublecortin, a gene associated with X-linked lissencephaly and subcortical band heterotopia. Here we report on the genomic organization of the murine DCAMKL gene and its products. Our results show that DCAMKL and CARP are alternative splice products of the same gene. The DCAMKL gene also generates three alternatively-spliced rDCAMKL transcripts of which we have cloned the corresponding cDNAs and which potentially generate different DCAMKL proteins. In situ hybridization experiments show that the different rDCAMKL transcripts are all expressed in the adult rat hippocampus. We conclude that alternative splicing of the DCAMKL gene may generate different but similar proteins in the adult rat hippocampus thereby regulating different but overlapping aspects of DCAMKL controlled neuronal plasticity.

Wheeler-Schilling, T. H., K. Marquardt, et al. (2001). "Identification of purinergic receptors in retinal ganglion cells." Molecular Brain Research **92**(1-2): 177.

<http://www.sciencedirect.com/science/article/B6T07-43KH3K7-X/2/8919e6542604351cb17bb140df46e1bf>

P2X receptors are ligand-gated ion channels activated by adenosine triphosphate and expressed in a broad variety of tissues. The present study demonstrates the expression of various types of purinergic P2X receptors in identified retinal ganglion cells (RGCs) of the adult rat retina. Single-cell reverse transcription polymerase chain reaction (SC-RT-PCR) resulted in a positive amplification signal for all P2X receptor subunit mRNAs examined (P2X3-5, P2X7). Immunohistochemistry with P2X3,4 receptor subunit-specific antibodies showed a labelling of neurons in the ganglion cell layer and inner nuclear layer. Our data suggest that extracellular ATP acts directly on RGCs via several types of P2X receptors and may provide neuromodulatory influences on information processing in the retina.

Yakubov, E., M. Gottlieb, et al. (2004). "Overexpression of genes in the CA1 hippocampus region of adult rat following episodes of global ischemia." Molecular Brain Research **127**(1-2): 10.

<http://www.sciencedirect.com/science/article/B6T07-4CS4R0W-1/2/08d92b277db9947228e27d7947943976>

Ischemic stress is associated with marked changes in gene expression in the hippocampus--albeit little information exists on the activation of nonabundant genes. We have examined the expression of several known genes and identified novel ones in the adult rat hippocampus after a mild, transient, hypovolemic and hypotensive, global ischemic stress. An initial differential screening using a prototype array to assess gene expression after stress followed by a suppression subtractive hybridization protocol and cDNA microarray revealed 124 nonoverlapped transcripts predominantly expressed in the CA1 rat hippocampus region in response to ischemic stress. About 78% of these genes were not detected with nonsubtracted probes. Reverse transcription polymerase chain reaction (RT-PCR) and in situ hybridization on these 124 transcripts confirmed the differential expression of at least 83. Most robustly expressed were gene sequences NFI-B, ATP1B1, RHOGAP, PLA2G4A, BAX, CASP3, P53, MAO-A, FRA1, HSP70.2, and NR4A1 (NUR77), as well as sequence tags of unknown function. New stress-related genes of similar functional motifs were identified, reemphasizing the importance of functional grouping in the analysis of multiple gene expression profiles. These data indicate that ischemia elicits expression of an array of functional gene clusters that may be used as an index for stress severity and a template for target therapy design.

Yamauchi, Y., S. Hongo, et al. (1999). "Molecular cloning and characterization of a novel developmentally regulated gene, Bdm1, showing predominant expression in postnatal rat brain." Molecular Brain Research **68**(1-2): 149.

<http://www.sciencedirect.com/science/article/B6T07-3WF80M0-H/2/ad984017fe304d20149e873af6594cd6>

Postnatal development, such as synapse refinement, is necessary for the establishment of a mature and functional central nervous system (CNS). Using differential display analysis, we identified a novel gene, termed Bdm1, that is more abundantly expressed in the adult brain than

in the embryonic brain. The full-length Bdm1 cDNA is 2718 base pairs long and contains an open reading frame of 1059 base pairs encoding a 38-kDa protein. Northern blot analysis revealed that expression of Bdm1 mRNA in the brain was weak on embryonic days and increased in the early postnatal period. Bdm1 mRNA was significantly expressed in the brain and heart, but there was no or little expression in other tissues. During the differentiation of mouse carcinoma cells P19 to neuron-like cells by retinoic acid, Bdm1 mRNA was up-regulated almost parallel to neurofilament mRNA. Expression of Bdm1 mRNA was observed appreciably in PC12 cells after neuronal differentiation but not in the nonneural cell lines examined. In situ hybridization demonstrated that Bdm1 was expressed widely in the olfactory bulb, cerebral cortex, hippocampus, cerebellum, thalamus, and medulla oblongata. Taken together, these data suggest that Bdm1 gene plays a role in the early postnatal development and function of neuronal cells.

Yarovinsky, T. O., D. W. Rickman, et al. (2000). "Expression of the protein tyrosine phosphatase, phosphatase of regenerating liver 1, in the outer segments of primate cone photoreceptors." Molecular Brain Research **77**(1): 95.

<http://www.sciencedirect.com/science/article/B6T07-408C11J-B/2/e98ca4b686685dec7073cf29fef97f7f>

Foveal cone photoreceptors are morphologically distinct and, presumably, express unique transcripts. We have identified a cDNA clone encoding the protein tyrosine phosphatase (PTP), phosphatase of regenerating liver 1 (PRL-1) in a screen for genes that are enriched in monkey fovea. PRL-1 was originally isolated as an immediate early gene in regenerating liver [R.H. Diamond, D.E. Cressman, T.M. Laz, C.S. Abrams, R. Taub, PRL-1, a unique nuclear protein tyrosine phosphatase, affects cell growth, *Mol. Cell Biol.* 14 (1994) 3752-3762]. On cDNA Southern blots of human and monkey retina, radiolabeled PRL-1 cDNA hybridized to a single mRNA species of about 2.5 kb that was most intense in fovea-enriched samples. The monkey PRL-1 deduced amino acid sequence is identical to human, rat and mouse PRL-1. Affinity-purified antibodies directed against PRL-1 preferentially labeled cone photoreceptor cells and a subpopulation of bipolar cells in monkey retina. Immunoreactivity in cones was confined to red and green, but not to blue, cones and was restricted to the outer segments. Immunolocalization also revealed that PRL-1 protein expression was non-nuclear, suggesting that its function in the retina may be unrelated to its role in other tissues where it is expressed primarily in nuclei. Although both foveal and extrafoveal cones were PRL-1 reactive, the high abundance of PRL-1 mRNAs detected in monkey fovea correlates with the high concentration of cones in the fovea. The PRL-1 gene is located on chromosome 6q within an interval that also contains the genes that cause two hereditary retinal dystrophies. These studies demonstrate novel expression of the PRL-1 gene in the neural retina and suggest the phosphatase activity of PRL-1 may modulate normal cone photoreceptor cell function.

Yu, G. and M. Fahnstock (2002). "Differential expression of nerve growth factor transcripts in glia and neurons and their regulation by transforming growth factor- β 1." Molecular Brain Research **105**(1-2): 115.

<http://www.sciencedirect.com/science/article/B6T07-46RMXRT-1/2/c866d6edec1590e2b7f656609a1fceb>

Nerve growth factor (NGF) influences neuronal development, function, and response to injury. Using reverse transcriptase polymerase chain reaction, we find that mouse and rat cortex and spinal cord, and both neurons and glia in culture, express NGF mRNA. In the mouse, NGF is regulated by at least two promoters that govern synthesis of four different transcripts, A through D, that are all expressed in the mouse tissues and cells examined. In contrast, rat NGF

expression varies with tissue and with cell type: transcript C is expressed strongly in brain but weakly in spinal cord, and transcript D is undetectable in rat central nervous system (CNS). In addition to species- and tissue-specific expression, NGF transcripts also exhibit cell type-specific expression: transcripts B, C and D are expressed in rat astrocytes but poorly or not at all in rat neurons, identifying glia as an important source of NGF in rat. NGF increases sharply after injury. TGF- β 1, which also increases immediately after injury, induces NGF mRNA and protein in rat and mouse glia but not in neurons. Furthermore, transcripts A, B and D, but not C, are upregulated by TGF- β 1 in mouse glia, whereas in rat glia, the major responsive transcript is C. Thus, there may be multiple TGF- β 1-responsive elements in the NGF promoters located upstream of exons 1 and 3 that may differ between mouse and rat. Moreover, NGF transcripts are differentially expressed in a species-, cell type-, and inducer-specific manner. These results have implications for the use of mice versus rats as models for the study of NGF regulation following CNS injury.

Zahniser, N. R., K. J. Buck, et al. (1992). "GABAA receptor function and regional analysis of subunit mRNAs in long-sleep and short-sleep mouse brain." Molecular Brain Research **14**(3): 196.

<http://www.sciencedirect.com/science/article/B6T07-4859N7W-83/2/3c89a9a28a0ca007e12e536eb68909ce>

The greater sensitivity of long-sleep (LS), as compared with short-sleep (SS), mice to ethanol is due in part to differences in GABAA receptor function in specific brain regions. To determine if differences in subunit composition of GABAA receptors contribute to this differential sensitivity, we measured α 1 and γ 2 subunit mRNAs with Northern analysis and in situ hybridization and γ 2S, γ 2L and α 6 subunit mRNAs with polymerase chain reaction (PCR) amplification. No differences in mRNAs in whole brain were apparent by Northern analysis. In situ hybridization revealed that α 1 and γ 2 subunit mRNAs were co-localized in many brain regions but that they still had distinct patterns of hybridization. However, the few differences observed between LS and SS mice in the levels of hybridization for these subunits did not show a regional distribution consistent with ethanol sensitivity differences. Similar ratios of γ 2L and γ 2S subunit mRNAs were found in LS and SS mouse cerebral cortex and hippocampus, and both mouse lines expressed essentially only γ 2L subunit mRNA in cerebellum, mRNA for the α 6 subunit was detected only in cerebellum and also was qualitatively similar between LS and SS mice. Studies of muscimol-stimulated $^{36}\text{Cl}^-$ uptake by cortical membrane vesicles confirmed earlier findings that ethanol does not enhance function of GABAA receptors in SS mice when assayed at 30°C. However, at 34°C ethanol did increase this function in SS mice although the enhancement remained greater in LS mice. These functional results, together with the results showing similar levels of α 1, γ 2S, γ 2L and α 6 subunits in LS and SS mice, suggest that the ethanol-insensitivity of SS mouse GABAA receptors cannot be due solely to lack of subunits required for ethanol action and further suggest that differences in catalytic mechanisms affecting post-translational processing may account for some genetic differences in ethanol sensitivity of GABAA receptors.

Zhou, C. J., S. Kikuyama, et al. (2000). "Cellular distribution of the splice variants of the receptor for pituitary adenylate cyclase-activating polypeptide (PAC1-R) in the rat brain by in situ RT-PCR." Molecular Brain Research **75**(1): 150.

<http://www.sciencedirect.com/science/article/B6T07-3YDG207-M/2/b936854c9bb30c28fc579da093b12b73>

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a pleiotropic neuropeptide and its specific receptor (the PAC1 receptor) is widely distributed in the rat brain. It has been reported

that alternative splicing of the region encoding the third intracellular loop of the PAC1 receptor generates six isoforms which are differentially coupled to signal transduction pathways, but the precise distribution and localization of these splice isoforms in the brain remain to be determined. Using the initial specific primer pairs which correspond to the 'hip' or 'hop' types of receptors for the solution-phase reverse transcription-polymerase chain reaction (RT-PCR), we demonstrated that the major splice variants of the PAC1 receptor in various regions of the rat brain are the short splice isoform 'PAC1-R-s' which does not contain either the 'hip' or 'hop' cassette and the another splice isoform, 'PAC1-R-hop', which contains the 'hop' cassette. With an innovative molecular histochemical technique, in situ RT-PCR, we determined that these two splice isoforms are both intensely expressed in the mitral cells of the olfactory bulb, the Purkinje cells of the cerebellum, the pyramidal cells of the hippocampus and neocortex, and many neurons in the nuclei of hypothalamus and thalamus as well as other regions. The initial mapping of the cell type-specific expression of these two splice variants of the PAC1 receptor provides the basis for a better understanding of the functional significance of the PAC1-R and its ligand PACAP in various brain regions.

Zohar, O., S. Cavallaro, et al. (2003). "Quantification and distribution of [beta]-secretase alternative splice variants in the rat and human brain." Molecular Brain Research **115**(1): 63.

<http://www.sciencedirect.com/science/article/B6T07-48W2NRV-G/2/55e42fc06ad77283f1245e650dac4168>

[beta]-Amyloid (A[beta]) is formed by sequential cleaving of the amyloid precursor protein by two proteolytic enzymes, [beta]- and [gamma]-secretases. [beta]-Secretase (BACE) is a type I transmembrane aspartic proteinase that is highly expressed in the mammalian brain. Four alternative splice variants of BACE are currently known and each encodes for a protein isoform with a different enzymatic activity. In Alzheimer's disease (AD) patients, the enzymatic activity and protein levels of BACE are increased in the neocortex, suggesting their differential expression may have a role in A[beta] plaque formation. We have determined the differential expression of BACE mRNA and its splice variants in eight regions of the rat and two of the human brain. In humans, the frontal cortex which shows A[beta] deposition in AD, expressed three-fold more BACE than the cerebellum and four fold more than the rats' frontal cortex both of which do not form A[beta] plaques. The highest BACE levels of rats were found in the frontal cortex and less in other areas. Although most human and rat brain regions expressed all four BACE variants, the human cerebellum did not express the I-457 BACE variant. Human and rat frontal cortex expressed high levels of the I-501 and I-457 variants, but I-432 was highly expressed only in the rat. Species-specific differences were evident between human and rat brain areas, suggesting that BACE transcript variants may have different evolutionary conservation. Differential expression of BACE variants may explain the broad spectrum of phenotypic abnormalities and possible pathogenetic mechanisms underlying Alzheimer's disease.

Molecular Cell (10)

Chen, L., Q.-w. Xie, et al. (1998). "Alkyl Hydroperoxide Reductase Subunit C (AhpC) Protects Bacterial and Human Cells against Reactive Nitrogen Intermediates." Molecular Cell **1**(6): 795.

<http://www.sciencedirect.com/science/article/B6WSR-4194JJS->

3/2/35de22fb5a1d917aa36f89256338f5fc

In *Salmonella typhimurium*, *ahpC* encodes subunit C of alkyl hydroperoxide reductase, an enzyme that reduces organic peroxides. Here, we asked if *ahpC* could protect cells from reactive nitrogen intermediates (RNI). *Salmonella* disrupted in *ahpC* became hypersusceptible to RNI. *ahpC* from either *Mycobacterium tuberculosis* or *S. typhimurium* fully complemented the defect. Unlike protection against cumene hydroperoxide, protection afforded by *ahpC* against RNI was independent of the reducing flavoprotein, *AhpF*. *Mycobacterial ahpC* protected human cells from necrosis and apoptosis caused by RNI delivered exogenously or produced endogenously by transfected nitric oxide synthase. Resistance to RNI appears to be a physiologic function of *ahpC*. *ahpC* is the most widely distributed gene known that protects cells directly from RNI, and provides an enzymatic defense against an element of antitubercular immunity.

Gomez-Gomez, L. and T. Boller (2000). "FLS2: An LRR Receptor-like Kinase Involved in the Perception of the Bacterial Elicitor Flagellin in *Arabidopsis*." *Molecular Cell* **5**(6): 1003.

<http://www.sciencedirect.com/science/article/B6WSR-41BDB49-B/2/e8aba504658307793b2b1b05e719ce8f>

Flagellin, the main protein of the bacterial flagella, elicits defence responses and alters growth in *Arabidopsis* seedlings. Previously, we identified the *FLS1* locus, which confers flagellin insensitivity in *Ws-0*. To identify additional components involved in flagellin perception, we screened for flagellin insensitivity mutants in the flagellin-sensitive accession *La-er*. Here, we describe the identification of a new locus, *FLS2*, by a map-based strategy. The *FLS2* gene is ubiquitously expressed and encodes a putative receptor kinase. *FLS2* shares structural and functional homologies with known plant resistance genes and with components involved in the innate immune system of mammals and insects.

Hedengren, M., BengtAsling, et al. (1999). "Relish, a Central Factor in the Control of Humoral but Not Cellular Immunity in *Drosophila*." *Molecular Cell* **4**(5): 827.

<http://www.sciencedirect.com/science/article/B6WSR-4194KC3-J/2/d79a79181e73b7f4117cbadaf59861d4>

The NF- κ B-like Relish gene is complex, with four transcripts that are all located within an intron of the *Nmdmc* gene. Using deletion mutants, we show that Relish is specifically required for the induction of the humoral immune response, including both antibacterial and antifungal peptides. As a result, the Relish mutants are very sensitive to infection. A single cell of *E. cloacae* is sufficient to kill a mutant fly, and the mutants show increased susceptibility to fungal infection. In contrast, the blood cell population, the hematopoietic organs, and the phagocytic, encapsulation, and melanization responses are normal. Our results illustrate the importance of the humoral response in *Drosophila* immunity and demonstrate that Relish plays a key role in this response.

Ho, Y., F. Elefant, et al. (2002). "A Defined Locus Control Region Determinant Links Chromatin Domain Acetylation with Long-Range Gene Activation." *Molecular Cell* **9**(2): 291.

<http://www.sciencedirect.com/science/article/B6WSR-4593XFV-F/2/9218bb3c7bbc149f0e3e614b96bb4f13>

Gene activation in higher eukaryotes is often under the control of regulatory elements quite distant from their target promoters. It is unclear how such long-range control is mediated. Here we show that a single determinant of the human growth hormone locus control region (hGH LCR) located 14.5 kb 5' to the hGH-N promoter has a critical, specific, and nonredundant role in facilitating promoter trans factor binding and activating hGH-N transcription. Significantly, this same determinant plays an essential role in establishing a 32 kb acetylated domain that encompasses the entire hGH LCR and the contiguous hGH-N promoter. These data support a model for long-range gene activation via LCR-mediated targeting and extensive spreading of core histone acetylation.

Kestila, M., U. Lenkkeri, et al. (1998). "Positionally Cloned Gene for a Novel Glomerular Protein--Nephrin--Is Mutated in Congenital Nephrotic Syndrome." Molecular Cell **1**(4): 575.

<http://www.sciencedirect.com/science/article/B6WSR-4194JG6-B/2/3ab00abeba6977440e35505c7d58706a>

Congenital nephrotic syndrome of the Finnish type (NPHS1) is an autosomal-recessive disorder, characterized by massive proteinuria in utero and nephrosis at birth. In this study, the 150 kb critical region of NPHS1 was sequenced, revealing the presence of at least 11 genes, the structures of 5 of which were determined. Four different mutations segregating with the disease were found in one of the genes in NPHS1 patients. The NPHS1 gene product, termed nephrin, is a 1241-residue putative transmembrane protein of the immunoglobulin family of cell adhesion molecules, which by Northern and in situ hybridization was shown to be specifically expressed in renal glomeruli. The results demonstrate a crucial role for this protein in the development or function of the kidney filtration barrier.

Kim, J. H., H. Li, et al. (2003). "CoCoA, a Nuclear Receptor Coactivator which Acts through an N-Terminal Activation Domain of p160 Coactivators." Molecular Cell **12**(6): 1537.

<http://www.sciencedirect.com/science/article/B6WSR-4C5R9M2-S/2/4c54ae3d2fed715d79f9dca15f1a73>

The p160 coactivators bind to and potentiate transcriptional activation by nuclear receptors by recruiting secondary coactivators such as the histone acetyltransferases p300 and CBP and the protein methyltransferase CARM1. The function of the highly conserved N-terminal basic-helix-loop-helix/Per-Arnt-Sim (bHLH-PAS) domain of p160 coactivators is unknown. This region is required for coactivator synergy among p160, p300, and CARM1 coactivators. We identified a coactivator, coiled-coil coactivator (CoCoA), which binds to this domain and thereby enhances transcriptional activation by the estrogen receptor and other nuclear receptors. Endogenous CoCoA was found simultaneously with p160 coactivators on the promoter of an endogenous estrogen-responsive gene. Reduction of endogenous cellular CoCoA levels inhibited the estrogen-stimulated expression of transiently transfected and endogenous genes. Moreover, CoCoA cooperated synergistically with GRIP1, CARM1, and p300 to enhance ER-mediated transcription. Thus, the N-terminal region of p160 coactivators contains an additional activation domain which contributes to coactivator function by recruitment of CoCoA.

Otake, L. R., P. Scamborova, et al. (2002). "The Divergent U12-Type Spliceosome Is Required for Pre-mRNA Splicing and Is Essential for Development in Drosophila." Molecular Cell **9**(2): 439.

<http://www.sciencedirect.com/science/article/B6WSR-4593XFV-X/2/3dd2dd770bd00acb18e1a1395411e6a5>

A minor class of pre-mRNA introns whose excision requires a spliceosome containing U11, U12, U4atac/U6atac, and U5 snRNPs has been identified in plants, insects, and vertebrates. We have characterized single loci that specify the U6atac and U12 snRNAs of *Drosophila melanogaster*. P element-mediated disruptions of the U6atac and U12 genes cause lethality during the third instar larval and embryonic stages, respectively, and are rescued by U6atac and U12 transgenes. The P element disruption of U6atac results in excision defects of U12-type introns from several transcripts including an alternative U12-dependent spliced isoform of *prospero*, a homeodomain protein required for CNS development. Thus, we demonstrate the requirement for the U12 spliceosome in the development of a metazoan organism.

Pokholok, D. K., N. M. Hannett, et al. (2002). "Exchange of RNA Polymerase II Initiation and Elongation Factors during Gene Expression In Vivo." Molecular Cell **9**(4): 799.

<http://www.sciencedirect.com/science/article/B6WSR-45PS8XV-F/2/324a0ffc7b0731e8253197ee164edbc2>

We have systematically explored the in vivo occupancy of promoters and open reading frames by components of the RNA polymerase II transcription initiation and elongation apparatuses in yeast. RNA polymerase II, Mediator, and the general transcription factors (GTFs) were recruited to all promoters tested upon gene activation. RNA polymerase II, TFIIS, Spt5, and, unexpectedly, the Paf1/Cdc73 complex, were found associated with open reading frames. The presence of the Paf1/Cdc73 complex on ORFs in vivo suggests a novel function for this complex in elongation. Elongator was not detected under any conditions tested, and further analysis revealed that the majority of elongator is cytoplasmic. These results suggest a revised model for transcription initiation and elongation apparatuses in living cells.

Romanow, W. J., A. W. Langerak, et al. (2000). "E2A and EBF Act in Synergy with the V(D)J Recombinase to Generate a Diverse Immunoglobulin Repertoire in Nonlymphoid Cells." Molecular Cell **5**(2): 343.

<http://www.sciencedirect.com/science/article/B6WSR-4195G0J-G/2/8d63159299e7d06247845e7b21338474>

Immunoglobulin (Ig) and T cell receptor (TCR) genes are assembled during lymphocyte maturation through site-specific V(D)J recombination events. Here we show that E2A proteins act in concert with RAG1 and RAG2 to activate Ig V[kappa]1J but not Ig[lambda] V[lambda]III-J[lambda]1 rearrangement in an embryonic kidney cell line. In contrast, EBF, but not E2A, promotes V[lambda]III-J[lambda]1 recombination. Either E2A or EBF activate IgH DH4J recombination but not V(D)J rearrangement. The Ig coding joints are diverse, contain nucleotide deletions, and lack N nucleotide additions. Ig[kappa] VJ recombination requires the presence of the E2A transactivation domains. These observations indicate that in nonlymphoid cells a diverse Ig repertoire can be generated by the mere expression of the V(D)J recombinase and a transcriptional regulator.

Schwaller, J., E. Parganas, et al. (2000). "Stat5 Is Essential for the Myelo- and Lymphoproliferative Disease Induced by TEL/JAK2." Molecular Cell **6**(3): 693.

<http://www.sciencedirect.com/science/article/B6WSR-41FKPV5-M/2/072a12f9ff9072bb19eb63a7bd829c06>

STAT5 is activated in a broad spectrum of human hematologic malignancies. We addressed whether STAT5 activation is necessary for the myelo- and lymphoproliferative disease induced by TEL/JAK2 using a genetic approach. Whereas mice transplanted with bone marrow transduced with retrovirus expressing TEL/JAK2 develop a rapidly fatal myelo- and lymphoproliferative syndrome, reconstitution with bone marrow derived from Stat5ab-deficient mice expressing TEL/JAK2 did not induce disease. Disease induction in the Stat5a/b-deficient background was rescued with a bicistronic retrovirus encoding TEL/JAK2 and Stat5a. Furthermore, myeloproliferative disease was induced by reconstitution with bone marrow cells expressing a constitutively active mutant, Stat5a, or a single Stat5a target, murine oncostatin M (mOSM). These data define a critical role for Stat5a/b and mOSM in the pathogenesis of TEL/JAK2 disease.

Molecular Genetics and Metabolism (18)

Azevedo, L., L. Stolnaja, et al. (2003). "New polymorphic sites within ornithine transcarbamylase gene: population genetics studies and implications for diagnosis." Molecular Genetics and Metabolism **78**(2): 152.

<http://www.sciencedirect.com/science/article/B6WNG-47X6S94-1/2/11fc286299737ac059bd2d52ba70de11>

Ornithine transcarbamylase (OTC) deficiency, transmitted as an X-linked trait, is the most common disorder of the urea cycle. At least 3.5% out of more than 230 mutations consist of large gene deletions, involving one or more exons. Only in 78% of OTC patients the diagnosis was confirmed on DNA level. We analysed OTC intragenic polymorphisms and haplotypes, in an attempt to contribute to the clarification of unresolved cases, in three populations (Czech, Portuguese, and Mozambican) and identified six novel nucleotide changes, all of them occurring with frequency higher than 12.5% in Europeans. Five of these polymorphisms occur with a significant frequency also in Africans. The number and frequency of haplotypes defined with the newly reported markers differ in individual populations.

Brun-Heath, I., A. Taillandier, et al. (2005). "Characterization of 11 novel mutations in the tissue non-specific alkaline phosphatase gene responsible for hypophosphatasia and genotype-phenotype correlations." Molecular Genetics and Metabolism **84**(3): 273.

<http://www.sciencedirect.com/science/article/B6WNG-4F2B9SB-6/2/ec682aa4d56c22c0f84f25a8296136cc>

Hypophosphatasia is an inherited disorder caused by mutations in the bone alkaline phosphatase gene. We report here 11 new mutations responsible for hypophosphatasia. Four of them were deletions or insertions resulting in frameshift, two affected a donor splice site and five were missense mutations. Site-directed mutagenesis and transfection experiments of missense mutations showed that the mutations resulted in loss of most enzymatic activity, confirming the disease-causing role of these mutations. Analysis of the 3D model of tissue non-specific alkaline

phosphatase showed that among the five missense mutations, one affected a residue in the crown domain and four affected residues located in the calcium-binding region. Alignment of the protein sequences of the calcium-binding region from 11 species showed that the four residues coordinating the calcium ion and the residues affected by the missense mutations described here are conserved in vertebrates. Together, our results confirm the functional role of the calcium site and suggest that its function is likely to be specific to vertebrates.

Canto, P., S. Razo, et al. (2004). "Screening for mutations in the WNT-4 gene in patients with 46,XX true hermaphroditism." Molecular Genetics and Metabolism **83**(4): 341.

<http://www.sciencedirect.com/science/article/B6WNG-4DJBR5K-1/2/e3a52f4a6ba5fd0844e8da97cf4afe56>

We investigated if eight SRY-negative 46,XX true hermaphrodites presented mutations in WNT-4, in blood leukocytes and/or gonadal tissue, as the cause of their disorder. We designed the sequences of the reverse primer of exon 1 and the primers of exons 2-5. Direct sequencing of all five exons demonstrated no mutant alleles in any of the patients. The possibility of the existence of causative mutations in the untranslated regions of WNT-4, or within introns cannot be ruled out.

Chen, R., T. Tsuji, et al. (2002). "Mutation analysis of the G4.5 gene in patients with isolated left ventricular noncompaction." Molecular Genetics and Metabolism **77**(4): 319.

<http://www.sciencedirect.com/science/article/B6WNG-47902N4-7/2/abaaeda6ef5c006a0bf4bf2b04ff15de>

Chistiakov, D. A., K. V. Savost'anov, et al. (2004). "Screening of SNPs at 18 positional candidate genes, located within the GD-1 locus on chromosome 14q23-q32, for susceptibility to Graves' disease: a TDT study." Molecular Genetics and Metabolism **83**(3): 264.

<http://www.sciencedirect.com/science/article/B6WNG-4D99S00-1/2/758744069bcd8b00f7fd0ff5a546e8e8>

Graves' disease (GD) is a complex autoimmune thyroid disorder with a strong genetic component. Genome-wide screens resolved several susceptibility loci that contribute to the development of GD. One of the susceptibility loci (GD-1 locus) was mapped on chromosome 14q31. However, a susceptibility gene located within the GD-1 locus remains undefined. Here we screen eighteen single nucleotide polymorphisms (SNPs), each is situated at a corresponding positional candidate gene, located within the GD-1 susceptibility locus on chromosome 14q23-q32, for predisposition to GD using the transmission disequilibrium test in 126 simplex Russian families affected with GD. Among SNPs tested, a significant preferential transmission of the Ala allele (41 transmissions vs. 17 nontransmissions, corrected $P = 0.031$) of the Thr92Ala SNP within the DIO2 gene, encoding type II iodothyronine deiodinase, from parents to affected children was found in a Russian family data set. The Thr92Ala SNP of the DIO2 gene and the D727E substitution of the thyrotropin receptor (TSHR) gene have been found to be in pair-wise linkage disequilibrium. The A92/E727 haplotype showed significant preferential transmission from parents to affected sibling (17 transmissions vs. 8 nontransmissions, $P = 0.039$) in simplex families. This suggests that the Thr92Ala variant of the DIO2 gene is associated or may be in linkage disequilibrium with a functional DIO2 polymorphism which involves in the development of

GD in a Russian population.

Costa, C., J. M. Costa, et al. (2003). "Mutational spectrum and DNA-based prenatal diagnosis in carnitine-acylcarnitine translocase deficiency." Molecular Genetics and Metabolism **78**(1): 68.

<http://www.sciencedirect.com/science/article/B6WNG-47HBVK0-6/2/ce52054863525b69d2a7023c6c30351f>

Carnitine-acylcarnitine translocase (CAC) deficiency is a rare autosomal recessive disorder of long-chain fatty acid oxidation with a severe outcome. We report mutation analysis in a cohort of 12 patients. Twelve mutations were identified of which 9 have not been reported so far (G28C, D32N, R178Q, P230R, D231H, 179delG, 802delG, 69-70insTGTGC, and 609-1g > a). Altogether, including our results, 22 mutations of the CAC gene have been published to date in 23 patients demonstrating the allelic heterogeneity of CAC deficiency. DNA-based prenatal diagnosis was performed for the first time in pregnancies at risk for CAC deficiency. Two fetuses were affected and one pregnancy was terminated by family decision. Two other fetuses had normal genotype and five others were heterozygotes. All the offspring of these seven pregnancies are alive and apparently healthy.

Khoo, J. P., T. Nicoli, et al. (2004). "Congenic mapping and genotyping of the tetrahydrobiopterin-deficient hph-1 mouse." Molecular Genetics and Metabolism **82**(3): 251.

<http://www.sciencedirect.com/science/article/B6WNG-4CHRGJ-1/2/d96fb93ab859086c9e1e68fd4146d59b>

The hph-1 ENU-mutant mouse provides a model of tetrahydrobiopterin deficiency for studying hyperphenylalaninaemia, dopa-response dystonia, and vascular dysfunction. We have successively localized the hph-1 mutation to a congenic interval of 1.6-2.8 Mb, containing the GCH gene encoding GTP cyclohydrolase I (GTP-CH I). We used these data to establish a PCR method for genotyping wild type, hph-1 and heterozygote mice, and found that heterozygote animals have partial tetrahydrobiopterin deficiency. These new findings will extend the utility of the hph-1 mouse in studies of GTP-CH I deficiency.

Malvagia, S., A. Morrone, et al. (2004). "New mutations in the PPBG gene lead to loss of PPCA protein which affects the level of the [beta]-galactosidase/neuraminidase complex and the EBP-receptor." Molecular Genetics and Metabolism **82**(1): 48.

<http://www.sciencedirect.com/science/article/B6WNG-4C40SRM-4/2/84c7b0d2a2fb73b548d0e740ba5faa12>

We describe the clinical findings, and the molecular and biochemical studies in an Italian family with recurrent hydrops fetalis due to galactosialidosis (GS). GS is a rare lysosomal storage disorder caused by a deficiency of the protective protein/cathepsin A (PPCA). This protein forms a high-molecular-weight complex with the hydrolases [beta]-galactosidase (GLB1) and neuraminidase (NEU1). By virtue of this association these two enzymes are correctly compartmentalized in lysosomes and protected against rapid proteolytic degradation. Controversial data show that PPCA is also present in a second complex, including the Elastin Binding Protein (EBP) the EBP-receptor, which is involved in elastogenesis, and NEU1. We investigated the potential role of the PPCA in both complexes. Two new genetic lesions (c60delG

and IVS2 + 1 G > T) that lead to a frameshift and a premature stop codon were detected in the PPCA cDNA and genomic DNA of the patient. The deleterious effect of such mutations was confirmed by the complete absence of the PPCA protein on Western blots. Thus, we examined the effect of the loss of PPCA on the two protein complexes in the patient's fibroblasts. Interestingly, a reduced amount of both GLB1 and EBP proteins was detected. These data confirm that PPCA is present in two functional complexes one with GLB1 and NEU1 in the lysosomal lumen and the other with EBP at the cell surface. The reduction in GLB1 and EBP confirms that PPCA is essential for their integrity.

Matsuura, T., Y. Chinen, et al. (2002). "Two newly identified genomic mutations in a Japanese female patient with fructose-1,6-bisphosphatase (FBPase) deficiency." Molecular Genetics and Metabolism **76**(3): 207.

<http://www.sciencedirect.com/science/article/B6WNG-46952MC-6/2/9cf0c83f085468b5440066f37ec4a89a>

Meng, X.-L., J.-S. Shen, et al. (2005). "GALC transduction leads to morphological improvement of the twitcher oligodendrocytes in vivo." Molecular Genetics and Metabolism **84**(4): 332.

<http://www.sciencedirect.com/science/article/B6WNG-4FB3X5V-4/2/0dfca261ace4cd5a5774bf0ae4c4a0ab>

Globoid cell leukodystrophy (GLD, Krabbe disease) is a severe demyelinating disease caused by a genetic defect of [beta]-galactocerebrosidase (GALC). To date treatment to GLD is limited to hematopoietic stem cell transplantation. Experimental approaches by means of gene therapy in twitcher mouse, an authentic murine model of human GLD, showed significant but only marginal improvements of the disease. To clarify whether the introduction of GALC could provide beneficial effects on the oligodendrocytes in GLD, we transduced twitcher oligodendrocytes by stereotactically injecting recombinant retrovirus encoding GALC-myc-tag fusion gene into the forebrain subventricular zone of neonatal twitcher mouse. In vivo effects of exogenous GALC on twitcher oligodendrocytes were studied histologically by combined immunostaining for the myc-epitope and the oligodendroglial specific marker, [pi] form of glutathione-S-transferase, at around 40 days of age. We show here that GALC transduction led to dramatic morphological improvement of the twitcher oligodendrocytes comparing with those in untreated twitcher controls. This study provided direct in vivo evidence that GALC transduction could prevent or correct aberrant morphology of oligodendrocytes in GLD which may be closely related to the dysfunction and/or degeneration of oligodendrocytes and the demyelination in this disease.

Robitaille, J., C. Brouillette, et al. (2004). "Plasma concentrations of apolipoprotein B are modulated by a gene-diet interaction effect between the LFABP T94A polymorphism and dietary fat intake in French-Canadian men." Molecular Genetics and Metabolism **82**(4): 296.

<http://www.sciencedirect.com/science/article/B6WNG-4CS4K95-3/2/ffc024757d9753481681148c5df2add>

Hyperapobetalipoproteinemia is a common feature of the metabolic syndrome and could result from the interaction between genetic and dietary factors. The objective of this study was to verify whether dietary fat intake interacts with the T94A polymorphism of the liver fatty acid-binding protein (LFABP) gene to modulate plasma apolipoprotein (apo) B levels. Dietary fat and saturated

fat intakes were obtained by a dietitian-administered food frequency questionnaire and the LFABP T94A genotype was determined by a PCR-RFLP based method in 623 French-Canadian men recruited through the Chicoutimi Lipid Clinic (279 T94/T94, 285 T94/A94, and 59 A94/A94). The LFABP T94A polymorphism was not associated with plasma apo B levels when fat intake was not taken into consideration. However, in a model including the polymorphism, fat intake expressed as a percentage of total energy intake, the interaction term and covariates, the variance in apo B concentrations was partly explained by the LFABP T94A polymorphism (5.24%, $p=0.01$) and by the LFABP T94A * fat interaction (6.25%, $p=0.005$). Results were similar when saturated fat replaced fat intake in the model (4.49%, $p=0.02$ for LFABP T94A and 6.43%, $p=0.004$ for the interaction). Moreover, in men consuming more than 30% of energy from fat, the odds ratio for having plasma apo B levels above 1.04 g/L for A94 carriers was of 0.40 ($p=0.02$) compared to T94/T94 homozygotes. Results were similar for carriers of the A94 allele consuming more than 10% of energy from saturated fat (OR: 0.32, $p=0.005$). In conclusion, T94/T94 exhibit higher apo B levels whereas carriers of the A94 allele seem to be protected against high apo B levels when consuming a high fat and saturated fat diet. These findings reinforce the importance to take into account gene-diet interactions in the prevention and management of the metabolic syndrome.

Sellner, L., E. Edkins, et al. (2005). "Detection of mutations in the glycine decarboxylase gene in patients with nonketotic hyperglycinaemia." Molecular Genetics and Metabolism **84**(2): 167.

<http://www.sciencedirect.com/science/article/B6WNG-4DVW1HS-1/2/3423a6f2b6e9fdd0792b2bd7ab764e61>

Nonketotic hyperglycinaemia (NKH) is an autosomal recessive disorder of glycine metabolism caused by a deficiency in the mitochondrial glycine cleavage enzyme. The majority of cases are caused by mutations in the P-protein, one of the four components of the glycine cleavage enzyme, also known as glycine decarboxylase (GLDC). Previous studies searching for causative mutations in NKH patients have only looked for a limited number of specific mutations or only screened part of the gene, and in many cases either no mutation or only one mutation was found, which is of limited use for prenatal diagnosis. In this study, we describe the screening of the entire GLDC gene in 3 NKH families by D-HPLC analysis of all 25 exons, identifying two point mutations and two large deletions (exon 8 and exons 2-15) using a combination of D-HPLC analysis, long range PCR, Southern blot and sequencing. For complete prenatal testing both mutations need to be identified, and we suggest that screening of the entire gene as well as deletional analysis should be considered in those subjects where only one mutation has been identified.

Stefanelli, C. B., A. Rosenthal, et al. (2004). "Novel troponin T mutation in familial dilated cardiomyopathy with gender-dependant severity." Molecular Genetics and Metabolism **83**(1-2): 188.

<http://www.sciencedirect.com/science/article/B6WNG-4CS4K95-1/2/d27a8b2257de41cb0e0eb2c16981cdbc>

Mutations in sarcomeric proteins can lead to either hypertrophic or dilated cardiomyopathy depending on their effects on the structural and functional properties of the contractile unit of the heart. Mutations in cardiac troponin T, which binds the calcium-responsive troponin complex to [alpha]-tropomyosin, have been shown to result in cardiac hypertrophy or cardiac dilatation and heart failure, depending on the nature of the specific mutation. In this study, we report the identification of a novel cardiac troponin T mutation (A171S) leading to dilated cardiomyopathy and sudden cardiac death. In contrast to prior described mutations, the A171S mutation results in a significant gender difference in the severity of the observed phenotype with adult males (over 20 years of age) demonstrating more severe ventricular dilatation [left ventricular end diastolic

dimension (LVEDD) 7.1 vs. 5.1 cm; $P=0.01$, t test] and left ventricular dysfunction [left ventricular shortening fraction (LVSF) 21 vs. 34%; $P=0.04$, t test] than adult females. The described mutation substitutes a hydrophilic amino acid for a hydrophobic one in a highly conserved domain involved in the interaction between troponin T and [alpha]-tropomyosin. Interestingly, four previously described mutations within 12 amino acids of A171 lead to a hypertrophic phenotype, suggesting that further characterization of the functional consequences of the A171S mutation may lead to a better understanding of the pathophysiology of DCM and of the functional differences between HCM- and DCM-causing mutations in cardiac troponin T.

Tang, N. L. S., J. Hui, et al. (2003). "A novel mutation (G233D) in the glycogen phosphorylase gene in a patient with hepatic glycogen storage disease and residual enzyme activity." Molecular Genetics and Metabolism **79**(2): 142.

<http://www.sciencedirect.com/science/article/B6WNG-48PVDMC-1/2/b52fe98eac74d9e6b68aca10e6128e54>

We identified a novel mutation in the glycogen phosphorylase gene (PGYL) in a Chinese patient with glycogen storage disease (GSD) type VI. The patient presented with gross hepatomegaly since the age of two without history of any hypoglycemic attack. Otherwise, he was largely asymptomatic. Liver tissue enzyme assays revealed a mild deficiency of total glycogen phosphorylase. Both PGYL and PHKA2 genes were sequenced. The patient was homozygous of a missense mutation G233D in PGYL. This location forms a hairpin turn secondary structure and the small glycine residue is completely conserved in all the orthologous proteins from *Escherichia coli* to mammals. This is the sixth reported mutation of this form of GSD.

Wolf, B., K. Jensen, et al. (2002). "Seventeen novel mutations that cause profound biotinidase deficiency." Molecular Genetics and Metabolism **77**(1-2): 108.

<http://www.sciencedirect.com/science/article/B6WNG-46SG1G2-F/2/6b07eed527fb63d9cfc9387eee9966bb>

We report 17 novel mutations that cause profound biotinidase deficiency. Six of the mutations are due to deletions, whereas the remaining 11 mutations are missense mutations located throughout the gene and encode amino acids that are conserved in mammals. Our results increase the total number of different mutations that cause biotinidase deficiency to 79. These additional mutations will undoubtedly be helpful in identifying structure/function relationships once the three-dimensional structure of biotinidase is determined.

Wolford, J. K., P. B. Colligan, et al. (2003). "Variants in the interleukin 6 receptor gene are associated with obesity in Pima Indians." Molecular Genetics and Metabolism **80**(3): 338.

<http://www.sciencedirect.com/science/article/B6WNG-49JPP0K-1/2/988336d31cad93f165bc256bb464f0df>

Circulating levels of the cytokine interleukin 6 (IL-6) are elevated in obesity, correlate with body mass index (BMI), and predict the development of type 2 diabetes mellitus (T2DM). A promoter polymorphism in the IL6 gene is associated with obesity, altered levels of insulin sensitivity, and T2DM. IL-6 exerts its effects by binding to the IL-6 receptor (IL-6R) and levels of IL-6R have been correlated with BMI. It is possible that IL6R variants may also be related to obesity, but to our

knowledge, no study has yet examined this relationship. The objective of this study was to examine the relationship between genetic variants in the IL6R gene and obesity in Pima Indians, a population prone to excess adiposity. We sequenced 6 kb of the IL6R gene, corresponding to all exons, exon-intron boundaries, and 2 kb of promoter in 30 Pima Indians. We identified six single nucleotide polymorphisms (SNPs) in the IL6R gene: a predicted Asp → Ala substitution at position 358, a variant in the 3'-untranslated region, and 4 intronic SNPs. All SNPs were in strong linkage disequilibrium (D' [ges] 0.90) and varied in minor allele frequency from 0.33 to 0.48. Association between IL6R genotype and BMI (kg/m²) was assessed in approximately 700 nondiabetic, full-heritage Pima Indians. For each SNP, individuals carrying the variant allele had a higher mean BMI compared to those with the wild-type allele (range: [37.3 ± 7.2-38.2 ± 7.0] vs. [35.5 ± 7.3-36.0 ± 7.5]; $P=0.02-0.004$). Our findings suggest that genetic variants in the IL6R gene may play a role in susceptibility to obesity. Assessment of these SNPs in other populations will be useful to determine the magnitude of obesity risk.

Wolford, J. K., J. D. Gruber, et al. (2003). "A C-reactive protein promoter polymorphism is associated with type 2 diabetes mellitus in Pima Indians." Molecular Genetics and Metabolism **78**(2): 136.

<http://www.sciencedirect.com/science/article/B6WNG-47X1VRJ-5/2/124f377eb2b157a589e424654e002637>

Linkage analysis has identified a susceptibility locus for type 2 diabetes mellitus (T2DM) on chromosome 1q21-q23 in several populations. Results from recent prospective studies indicate that increased levels of C-reactive protein (CRP), a marker of immune system activation, are predictive of diabetes, independent of adiposity. Because CRP is located on 1q21, we considered it a potential positional candidate gene for T2DM. We therefore evaluated CRP and the nearby serum amyloid P-component, APCS, which is structurally similar to CRP, as candidate diabetes susceptibility genes. Approximately 10.9 kb of the CRP-APCS locus was screened for polymorphisms using denaturing high performance liquid chromatography and direct sequencing. We identified 27 informative polymorphisms, including 26 single nucleotide polymorphisms (SNPs) and 1 insertion/deletion, which were divided into 7 linkage disequilibrium clusters. We genotyped representative SNPs in ~1300 Pima samples and found a single variant in the CRP promoter (SNP 133552) that was associated with T2DM ($P=0.014$), as well as a common haplotype (CGCG) that was associated with both T2DM ($P=0.029$) and corrected insulin response, a surrogate measure of insulin secretion in non-diabetic subjects ($P=0.050$). Linkage analyses that adjusted for the effect of these polymorphisms indicated that they do not in themselves account for the observed linkage with T2DM on chromosome 1q. However, these findings suggest that variation within the CRP locus may play a role in diabetes susceptibility in Pima Indians.

Wolford, J. K., Y. L. Konheim, et al. (2003). "Association of a F479L variant in the cytosolic phospholipase A2 gene (PLA2G4A) with decreased glucose turnover and oxidation rates in Pima Indians." Molecular Genetics and Metabolism **79**(1): 61.

<http://www.sciencedirect.com/science/article/B6WNG-48JK1HJ-1/2/b4641a51943f060141a9e799314e2f44>

Phospholipase A2, Group IVA (PLA2G4A) belongs to the class of cytosolic calcium-dependent phospholipases (cPLA2s) that preferentially cleave arachidonic acid (AA) from membrane glycerophospholipids. AA and AA metabolites play key roles in glucose disposal and insulin secretion. PLA2G4A is located on Chromosome 1q, where a number of groups have reported linkage to type 2 diabetes mellitus. We have screened the PLA2G4A gene and identified a C→G variant, which predicts a phenylalanine to leucine substitution. In logistic regression analyses

adjusted for age, sex, ethnicity, and birth year, we found a trend toward association between this SNP and diabetes [OR=1.53 (0.97-2.40); p=0.06]. Individuals with the variant genotype had lower mean basal endogenous glucose output (1.8+/-0.03 vs. 1.9+/-0.01 mg/kg EMBS/min; p=0.04) and lower mean basal glucose oxidation (1.2+/-0.11 vs. 1.4+/-0.03 mg/kg EMBS/min; p=0.005) compared to individuals with the wild-type genotype. During a low dose insulin infusion, non-diabetic individuals with the variant genotype had a lower mean glucose oxidation (1.9+/-0.11 vs. 2.0+/-0.03 mg/kg EMBS/min; p=0.04) and total glucose turnover rate (2.5+/-0.22 vs. 2.6+/-0.06 mg/kg EMBS/min; p=0.01) compared to subjects with the wild-type genotype. In addition, under basal conditions, individuals with the variant genotype had a higher mean lipid oxidation rate compared to individuals with the wild-type genotype (0.77+/-0.25 vs. 0.67+/-0.23 mg/kg EMBS/min; p=0.02). These results provide evidence supporting a role for the eicosanoid biosynthesis pathway in type 2 diabetes mellitus pathophysiology.

Molecular Immunology (48)

Armour, K. L., P. R. Tempest, et al. (1994). "Sequences of heavy and light chain variable regions from four bovine immunoglobulins." *Molecular Immunology* **31**(17): 1369.

<http://www.sciencedirect.com/science/article/B6T9R-476N7JP-5B/2/c8f240fb549edf1d8042f60a1d29070f>

Oligodeoxyribonucleotide primers based on the 5' ends of bovine IgG1/2 and [lambda] constant (C) region genes, together with primers encoding conserved amino acids at the N-terminus of mature variable (V) regions from other species, have been used in cDNA and polymerase chain reactions (PCRs) to amplify heavy and light chain V region cDNA from bovine heterohybridomas. The amino acid sequences of VH and V[lambda], from four bovine immunoglobulins of different specificities are presented.

Bates, E. E. M., M. C. Dieu, et al. (1998). "CD40L activation of dendritic cells down-regulates DORA, a novel member of the immunoglobulin superfamily." *Molecular Immunology* **35**(9): 513.

<http://www.sciencedirect.com/science/article/B6T9R-3VR19S9-2/2/1f521e6c275a7ba8fb94940fc5b2d5cf>

Using a cDNA subtraction technique, a novel member of the immunoglobulin superfamily was isolated from human Dendritic cells (DC). This cDNA which we named DORA, for Down-Regulated by Activation encodes a protein belonging to the CD8 family of receptors containing a single V type loop domain with an associated J chain region, a transmembrane region containing an atypical tyrosine residue and a cytoplasmic domain containing three putative tyrosine phosphorylation sites. The hDORA gene has been localised to chromosome 16. From database searches a rat cDNA was identified that encoded a polypeptide with 63% identity to hDORA. The expression of the human cDNA was studied in detail. Northern blot analysis revealed 1.0 kb and 2.5 kb mRNAs in peripheral blood lymphocytes, spleen and lymph node, while low levels were observed in thymus, appendix, bone marrow and fetal liver. No signal was noted in non-immune system tissues. By RT-PCR analysis of hDORA revealed expression in cells committed to the myeloid lineage but not in CD34+ precursors or B cells and low expression in T cells. Expression was also observed in DC, purified ex vivo or generated in vitro from either monocytes or CD34+

progenitors. This was down-regulated following activation both by PMA and Ionomycin treatment and also by CD40L engagement. In situ hybridisation performed on tonsil sections showed the presence of hDORA in cells within Germinal Centers. This structure and expression suggests a function as a co-receptor, perhaps in an antigen uptake complex, or in homing or recirculation of DC.

Breiteneder, H., R. Friedl-Hajek, et al. (1996). "Sequence comparisons of the CDR3 hyper-variable loops of human T cell receptors specific for three major T cell epitopes of the birch pollen allergen Bet v 1." Molecular Immunology **33**(13): 1039.

<http://www.sciencedirect.com/science/article/B6T9R-3W3171M-4/2/4875f1e3cd492f3b007286c6cc07d071>

We have analysed the T cell receptor (TCR) [alpha] and [beta] chain sequences of 16 human CD4+ T cell clones (TCCs) specific for three important epitopes of the major birch pollen allergen Bet v 1. The TCCs were raised from the peripheral blood of eight patients with birch pollen allergy, showing allergic rhino-conjunctivitis and allergic asthma. The TCCs from these individuals were specific for Bet v 1-derived peptides: amino acids (aa)77-92 (epitope 1), aa93-108 (epitope 2) and aa113-126 (epitope 3). The DNA sequence analysis of the TCRAV and BV regions revealed heterogeneous repertoires for recognition of the peptides. Multiple combinations of AV/AJ and BV/BJ were used. However, some inter-individual restriction was evident. A limited selection of AV8 and the normally infrequently used BV1S4 was obvious in TCCs specific for epitope 1. The TCRBV13 was more frequent in TCCs recognizing epitope 3. A very narrow distribution in length could be seen in the CDR3 sequences of the [beta] chain of TCRs with specificity for epitopes 1 and 2. Inter-individual positional micro-restriction was observed for the aa motif LR in the [beta]CDR3 (epitope 1), for the aa residue M in the [alpha]CDR3 and for the aa residue G in the [beta]CDR3 (epitope 3). Our results illustrate clearly that each antigenic peptide derived from a single allergen, is capable of selecting different characteristics in the responding repertoire of TCRs, thus increasing the complexity of allergen-recognition by T lymphocytes. Therefore, our findings limit the potential use of TCR targeted therapeutical strategies in Type I allergy.

Campbell, M. J., A. D. Zelenetz, et al. (1992). "Use of family specific leader region primers for PCR amplification of the human heavy chain variable region gene repertoire." Molecular Immunology **29**(2): 193.

<http://www.sciencedirect.com/science/article/B6T9R-476TRCP-2M/2/50891e6db13d28a7af83f515babb8bcd>

We have designed a set of six, non-degenerate oligonucleotide primers, corresponding to the 5' leader regions of each of the six human VH gene families. A general strategy for family specific polymerase chain reaction amplification is described using these primers and a conserved 3' primer corresponding to frame work 3, JH, or constant region. This strategy was used to isolate and sequence novel human germline VH genes belonging to the VH2 and VH4 families. Under certain conditions, chimeric VH sequences were created by a "jumping polymerase chain reaction", combining DNA segments from different germline genes, but this could be avoided by limiting the number of amplification cycles. PCR amplification with these family specific primers will facilitate studies of the repertoire of germline VH genes as well as studies on VH gene usage in normal and aberrant (B cell malignancies, autoimmune diseases, etc.) B cell populations.

Chu, C. C. and W. E. Paul (1998). "Expressed genes in interleukin-4 treated B cells identified by cDNA representational difference analysis." Molecular Immunology **35**(8): 487.

<http://www.sciencedirect.com/science/article/B6T9R-3VS7KGS-7/2/e9c0bc6d725535c05602f3ff8b0113cc>

Interleukin-4 (IL-4) stimulates B cell growth and differentiation, such as inducing mature B cells to switch to IgG1 and IgE production. To further characterize IL-4 effects on B cells, we used a sensitive PCR-based subtraction approach to isolate genes expressed in IL-4 treated cells. Our approach combined an adaptation of the genomic representational difference analysis (RDA) method to cDNA analysis with a physical separation method (magnetic bead depletion). This cDNA RDA technique allowed us to perform subtraction on the relatively small number of highly characterized, purified B cells that can be conveniently prepared. In the hopes of removing genes responsible for general cell growth, we subtracted cDNA made from lipopolysaccharide (LPS)-stimulated B cells from cDNA from LPS+IL-4 stimulated B cells. Two rounds of subtraction resulted in greater than 100-fold enhancement of expected IL-4-induced C[gamma]1 cDNA. At that point, we cloned this subtraction library and analysed 154 randomly picked clones for sequence similarities. From these clones, 37 individual genes were obtained. Most of these genes (30) could be functionally identified by sequence similarity. These included genes encoding C[gamma]1 (1), cytoskeletal components (4) and products involved in DNA replication (3), metabolism (5), signal transduction (4), transcription (4), translation (6) and transport (3). Only 7 genes had no similarity to known sequences in the GenBank, EMBL or Swiss Prot databases. One unknown gene (designated Fig1 for IL-Four Induced Gene1) and one gene with homology to the human transcription factor E4BP4 were confirmed by Northern blot analysis to be induced 10-20-fold by IL-4 treatment. This list of expressed genes in LPS+IL-4 treated B cells may shed further insight on the action and mechanism of IL-4 stimulation of cells.

De Jonge, J., J. Brissinck, et al. (1995). "Production and characterization of bispecific single-chain antibody fragments." Molecular Immunology **32**(17-18): 1405.

<http://www.sciencedirect.com/science/article/B6T9R-3YKM941-D/2/c65a23570a406a7aa010afbd03567de6>

We report the construction, expression and purification of a bispecific single-chain Fv antibody fragment produced in *Escherichia coli*. The protein possesses a dual specificity: the single-chain FvB1 portion is directed to the Idiotypic of BCL1 lymphoma cells, the single-chain Fv2C11 moiety binds to the CD3 marker on T cells. The two domains are joined by a flexible peptide linker. Using Immobilized Metal Affinity Chromatography, the recombinant protein was purified from bacterial insoluble membrane fractions. After refolding of the bispecific protein, it was affinity-purified. As demonstrated by flow cytometry, both binding sites are retained in the refolded protein. Retargeted cytotoxicity and T cell proliferation assays further prove the biological activity and specificity of the bispecific single-chain Fv. Thus, these bispecific molecules show a potential anti-tumor activity.

Delgado-Cervino, E., G. Fontan, et al. (2005). "C5 complement deficiency in a Spanish family: Molecular characterization of the double mutation responsible for the defect." Molecular Immunology **42**(1): 105.

<http://www.sciencedirect.com/science/article/B6T9R-4D39YGS-2/2/6a66908554ddc4cbd4489fb02eee4749>

The complement C5 deficiency is a recessive autosomal defect associated with recurrent infectious episodes, generally caused by Gram-negative micro-organisms. To date, only two mutations responsible for C5 deficiency have been characterized, both in heterozygosis. In this paper, we evaluate by immunochemical methods the C5 deficiency in a six-member family, in which one member suffered from meningococcal sepsis and several pneumonia episodes; and a second one with two bacterial meningitis episodes and frequent tonsillitis, pneumonia and herpetic episodes. We also characterize the molecular basis of this deficiency. No C5 protein was found in the serum from three of the children. They were found to be homozygous for a double mutation in the exon 40 of the C5 gene. The parents and the other children have half-normal levels of C5, and they were heterozygotes for the double mutation. This mutation modifies the reading frame, leading to a premature stop codon, and the resulting protein lacks 50 amino acids. As a result, homozygotes and heterozygotes have a total or a partial C5 deficiency respectively. This is the first report of a whole molecular characterization of C5 deficiency.

Duber, S., H. Engel, et al. (2003). "Germline transcripts of immunoglobulin light chain variable regions are structurally diverse and differentially expressed." Molecular Immunology **40**(8): 509.

<http://www.sciencedirect.com/science/article/B6T9R-49NXFW9-2/2/5f591eca774e5a72921731bc4f4478f5>

The murine pre-B cell line R2-bfl, which can be induced to differentiate in vitro, was used to study germline transcription of variable regions of the light chain loci. RNA from these cells was subjected to a 3'-RACE and germline transcripts from 17 individual V[kappa] gene segments belonging to 12 V[kappa] families were characterized. Germline transcripts of all three V[lambda] regions were similarly analyzed. The synchronous differentiation of R2-bfl cells was then used to investigate the order of appearance of germline transcripts of the V and JC clusters of both light chain loci. This was taken as indicator for accessibility of a particular locus to rearrangement. Germline transcripts of the JC[kappa] cluster and the V[kappa] family most proximal to JC[kappa] was detectable already at day 0, while transcripts of the most distal V[kappa] family became apparent after initiation of differentiation at day 1. Transcripts of the JC[lambda] cluster could be found at day 2, whereas transcripts of the V[lambda] region were already present at day 1. Thus, the [lambda] locus becomes accessible to rearrangement later during development than [kappa], confirming and extending our previous findings. The V and JC clusters open at the same stage of development although slight asynchronicities were found for the V[lambda] and the distal V[kappa] gene segments.

Duenas, M., A.-C. Malmborg, et al. (1996). "Selection of phage displayed antibodies based on kinetic constants." Molecular Immunology **33**(3): 279.

<http://www.sciencedirect.com/science/article/B6T9R-3VXNH65-6/2/ad9122b1eaa05721972a0bb4b86dc239>

The display of antibody fragments on the surface of filamentous bacteriophages and the selection of binders from antibody libraries have provided powerful tools to generate human antibodies. We reported recently a new concept (SAP system) for the selection of specific phages by linking antigenic recognition and phage replication, using a soluble fusion protein containing the antigen and a fragment of the M13 coat protein 3. In this investigation, a model library has been composed using six different antibody fragments which were characterized individually regarding their k_{ass} , k_{diss} and K_a . All Fab fragments were specific for a 15 amino acid region of the V3 loop of gp120 (HIV-1). We demonstrated that the SAP system could discriminate between the kinetic parameters of each clone, using different selection strategies. Phages expressing high affinity clones were selected preferentially using low doses of antigen but clones of lower affinity

also could be selected by increasing the antigen concentration or using a preselection procedure. Phages expressing antibody fragment with high association or low dissociation rate constants were retrieved by utilizing short contact times between antigen and antibody or antigen-chase conditions.

Engel, H., H. Ruhl, et al. (2001). "Germ-line transcripts of the immunoglobulin [lambda] J-C clusters in the mouse: characterization of the initiation sites and regulatory elements." Molecular Immunology **38**(4): 289.

<http://www.sciencedirect.com/science/article/B6T9R-43YH4VJ-4/2/d4dad1a87493936fa0aff19b34d5f134>

Transcription of unrearranged immunoglobulin gene segments strongly correlates with their accessibility to the V(D)J recombination machinery. The regulatory mechanisms governing this germ-line transcription are still poorly defined. In order to identify new regulatory elements, we first carried out a detailed characterization of the transcription initiation sites for the J-C germ-line transcripts, using rapid amplification of 5' cDNA ends, assisted by a template switching mechanism at the 5'-end of the RNA. Transcripts were observed that initiated heterogeneously, starting up to 293 ([lambda]1), 116 bp ([lambda]2) and 79 bp ([lambda]3) upstream from the respective J[lambda] gene segment. Additional RT-PCR analysis revealed the existence of germ-line transcripts of [lambda] and also of [kappa] that initiate even more upstream of these transcription initiation sites, although their frequencies were low. Promoter activity was detected in vitro 5' of J[lambda]2, with the minimal promoter activity mapping to the region between positions -35 and -120. In addition, computer analysis allowed the prediction of a nuclear scaffold/matrix attachment (S/MAR) region between the two J-C gene clusters at each hemi-locus. This region between the [lambda]1/[lambda]3 clusters binds to the nuclear matrix in vitro, and J-C [lambda]1 germ-line transcription initiates a short distance downstream from this S/MAR element.

Evans, M. J., S. A. Rollins, et al. (1995). "In vitro and in vivo inhibition of complement activity by a single-chain Fv fragment recognizing human C5." Molecular Immunology **32**(16): 1183.

<http://www.sciencedirect.com/science/article/B6T9R-3YKM94S-M/2/236cb667619cb911f4436d986d360267>

Complement activation has been implicated in the pathogenesis of several human diseases. Recently, a monoclonal antibody (N19-8) that recognizes the human complement protein C5 has been shown to effectively block the cleavage of C5 into C5a and C5b, thereby blocking terminal complement activation. In this study, a recombinant N19-8 scFv antibody fragment was constructed from the N19-8 variable regions, and produced in both mammalian and bacterial cells. The N19-8 scFv bound human C5 and was as potent as the N19-8 monoclonal antibody at inhibiting human C5b-9-mediated hemolysis of chicken erythrocytes. In contrast, the N19-8 scFv only partially retained the ability of the N19-8 monoclonal antibody to inhibit C5a generation. To investigate the ability of the N19-8 scFv to inhibit complement-mediated tissue damage, complement-dependent myocardial injury was induced in isolated mouse hearts by perfusion with Krebs-Henseleit buffer containing 6% human plasma. The perfused hearts sustained extensive deposition of human C3 and C5b-9, resulting in increased coronary artery perfusion pressure, end-diastolic pressure, and a decrease in heart rate until the hearts ceased beating approximately 10 min after the addition of plasma. Hearts treated with human plasma supplemented with either the N19-8 monoclonal antibody or the N19-8 scFv did not show any detectable changes in cardiac performance for at least 1 hr following the addition of plasma. Hearts treated with human plasma alone showed extensive deposition of C3 and C5b-9, while

hearts treated with human plasma containing the N19-8 scFv showed extensive deposition of C3, but no detectable deposition of C5b-9. Administration of a 100 mg bolus dose of N19-8 scFv to rhesus monkeys inhibited the serum hemolytic activity by at least 50% for up to 2 hr. Pharmacokinetic analysis of N19-8 scFv serum levels suggested a two-compartment model with a T_{1/2}[α] of 27 min. Together, these data suggest the recombinant N19-8 scFv is a potent inhibitor of the terminal complement cascade and may have potential in vivo applications where short duration inhibition of terminal complement activity is desirable.

Fayen, J., J.-H. Huang, et al. (1995). "Class I MHC alpha 3 domain can function as an independent structural unit to bind CD8[α]." *Molecular Immunology* **32**(4): 267.

<http://www.sciencedirect.com/science/article/B6T9R-3YKM9C5-57/2/77ee6f49ed8b8d57f96ffcc3a048a733>

Functional interactions between CD8-dependent cytotoxic T cells and their targets require physical contact between CD8 and a non-polymorphic determinant on the [α]3 domain of the class I MHC molecule. We developed a cell-free assay to directly monitor this molecular interaction, specifically excluding the participation of other cellular proteins and lipids. This assay employed a soluble CD8 derivative and a plate-bound HLA-A2.1 derivative, [α]3/MaIE, in which the [α]3 domain has been expressed independently of its neighboring polypeptide domains on the native class I MHC molecule and [β]2-microglobulin ([β]2-m). These proteins were produced using eukaryotic and prokaryotic expression systems, respectively. Our data demonstrated specific, saturable binding between soluble CD8[α] (sCD8[α]) and [α]3/MaIE, and the K_d of this interaction was determined to be 4.5 x 10⁻⁷ M. Monoclonal antibodies (mAb) directed against either CD8 or the [α]3 domain of class I MHC inhibited binding; mAb directed against other sites on class I MHC and [β]2-m did not. Our data suggest that the interaction between CD8[α] and the [α]3 domain of class I MHC does not require the participation of neighboring class I sequences or [β]2-m.

Garlisi, C. G., K. J. Pennline, et al. (1993). "Cytokine gene expression in mice undergoing chronic graft-versus-host disease." *Molecular Immunology* **30**(7): 669.

<http://www.sciencedirect.com/science/article/B6T9R-476F814-CT/2/484a90c634d7d87d0aeb7f5d81695561>

Chronic graft-versus-host disease (GVHD) can be induced in B6D2F1 mice by injection of parental DBA/2 lymphoid cells. Stimulation of donor T cells by host MHC antigens leads to the stimulation of host B cells. Little is known of the lymphokines produced during such a reaction. This study was designed to directly measure the levels of mRNA for interferon-gamma (IFN-[γ]), interleukin 2 (IL-2), IL-4, IL-5, and IL-10, as well as several other genes, using semiquantitative polymerase chain reaction (PCR). Semiquantitative PCR was reproducible and signals generated were dependent on the amount of specific RNA or cDNA in each reaction. Early during the progression of GVHD (2 days after the first injection of parental cells) there was little increase in IL-10 mRNA, a slight increase in IL-4 mRNA, and a dramatic increase in IL-2 mRNA. In addition, IL-2 bioactivity was demonstrated in supernatants from GVH splenocytes cultured in vitro for 24 h. Later in the response (1 week after the second and final injection of parental cells) IL-4 mRNA levels were elevated as they were earlier while IL-10 mRNA levels were dramatically increased. IL-2 mRNA levels were no different in mice undergoing GVHD than in normal mice at this time. IFN-[γ] mRNA was detectable both early and late, although at similar levels in normal mice and mice undergoing GVHD. At both times examined, IL-4 was below the limits of detection by bioassay and IFN-γ, IL-4, IL-5 and IL-10 were below the limits of detection by ELISA. Further studies showed that a majority of the IL-4 and IL-10 mRNA found

elevated in GVH mice were produced by Thy1.2+ T cells, with small amounts from B220+ B cells. In addition, the detectable IFN- γ mRNA found in GVH mice at this later time also was produced by Thy1.2+ T cells, with small amounts from B220+ B cells.

Gerritsma, J. S. J., A. F. Gerritsen, et al. (1996). "Interleukin-1 α enhances the biosynthesis of complement C3 and factor B by human kidney proximal tubular epithelial cells in vitro." Molecular Immunology **33**(10): 847.

<http://www.sciencedirect.com/science/article/B6T9R-3W3NC30-2/2/bccf62f4675878f38b8728d982c6cabd>

Local production in tubular cells of complement has been shown to occur in several kidney diseases by in situ hybridization, but the regulation at the local site during an inflammation is still unknown. In the present study, we demonstrate that human proximal tubular epithelial cells (PTEC) are able to produce complement components C3 and Factor B under non-stimulated conditions in vitro. The basal production of both was increased by 0.5 ng/ml interleukin-1 α (IL-1 α) for C3: from 95.5 $\pm$ 4.0 ng/106 cells to 416.5 $\pm$ 4.9 ng/106, and for Factor B: from 271 $\pm$ 7.0 ng/106 cells to 457.5 $\pm$ 7.0 ng/106 cells. In contrast cytokines such as TNF- α , IFN- γ , IL-10 and IL-15 had no detectable effect. The upregulation by IL-1 α was dose- and time-dependent. The response to IL-1 α was shown to be mediated via the IL-1 receptor, as the addition of recombinant interleukin-1 receptor antagonist inhibited the IL-1 α induced complement production by more than 80%. IL-1 α enhanced mRNA expression of both C3 and Factor B as demonstrated by RT-PCR and dot-blot analysis. This indicated that IL-1 α upregulated the expression of the C3 and Factor B at the transcriptional level. We hypothesize that in vivo the production of C3 and Factor B at the local site during an inflammatory response in the kidney may be regulated by IL-1 α produced by inflammatory cells.

Glas, A. M., S.-C. Huang, et al. (1999). "Motif-specific probes identify individual genes and detect somatic mutations." Molecular Immunology **36**(9): 599.

<http://www.sciencedirect.com/science/article/B6T9R-3X3K84V-C/2/914ff9f0ecaf9d5375370c74ef007601>

This report describes the correlation between motif-specific hybridization and nucleotide sequence as an approach to the identification of individual human VH genes using motif-specific oligonucleotide probes, complementary to specific motifs within individual VH genes. The sensitivity of the hybridization and post washing processes permits discrimination of single nucleotide differences between probe and target. This feature is used both to identify individual genes, as well as to detect mutations in genes by sequential hybridization with multiple probes. In addition to the general strategy, specific details are provided for the identification of 12 VH3 genes and 14 VH4 genes.

Goetz, F. W., D. B. Iliev, et al. (2004). "Analysis of genes isolated from lipopolysaccharide-stimulated rainbow trout (*Oncorhynchus mykiss*) macrophages." Molecular Immunology **41**(12): 1199.

<http://www.sciencedirect.com/science/article/B6T9R-4CX00DG-6/2/d407318e2c79792bab9fc991fe9f67d0>

A primary cell culture system was used to obtain differentiated rainbow trout (*Oncorhynchus*

mykiss) macrophages that were stimulated with Escherichia coli lipopolysaccharide (LPS-10 [μg/ml]) for 12 h in vitro. Messenger RNA from the LPS-stimulated cells was used to create two cDNA libraries from which a total of 1048 sequences were analyzed. A large number of cDNAs were obtained that could be related to immune function including structural proteins, proteases and antiproteases, regulators of transcription and translation, cell death regulators, receptors, lectins and immunoglobulins, cytokines and chemokines, cell surface antigens, signal transduction proteins, antimicrobial peptides, and enzymes involved in eicosanoid synthesis. Selected genes that were analyzed by RT-PCR and real time PCR and found to be upregulated by LPS, included vascular cell adhesion molecule, the CCAAT/enhancer binding protein [beta], the inhibitor of NF-κB [alpha], CD209, a major histocompatibility class II-invariant chain protein, cyclin L1, acute phase serum amyloid A, and prostaglandin endoperoxide synthase 2.

Harmsen, M. M., R. C. Ruuls, et al. (2000). "Llama heavy-chain V regions consist of at least four distinct subfamilies revealing novel sequence features." *Molecular Immunology* **37**(10): 579.

<http://www.sciencedirect.com/science/article/B6T9R-423J9MV-D/2/3d258e477290b8170df37edebdc63b9>

In addition to conventional antibodies (Abs), camelids possess Abs consisting of only heavy chains. The variable domain of such a heavy-chain Ab (VHH) is fully capable of antigen (Ag) binding. Earlier analysis of 47 VHHs showed sequence features unique to VHH domains. These include the presence of characteristic amino acid substitutions in positions which, in conventional VH domains are involved in interdomain interactions, and the presence of a long third complementarity-determining region (CDR3) which is frequently constrained by an interloop disulphide bond. Here, we describe a large (152) set of Lama glama VHH cDNAs. Based on amino acid sequence similarity, these and other published camelid VHHs were classified into four subfamilies. Three subfamilies are absent in dromedaries, which have been the primary source of VHHs thus far. Comparison of these subfamilies to conventional VH regions reveals new features characteristic of VHHs and shows that many features earlier regarded as characteristic of VHHs in general are actually subfamily specific. A long CDR3 with a concomitant putative additional disulphide bond is only observed in two VHH subfamilies. Furthermore, we identified new VHH-characteristic residues at positions forming interdomain sites in conventional VH domains. The VHH subfamilies also differ from each other and conventional VH domains in the canonical structure of CDR1 and CDR2, mean CDR3 length, and amino acid residue variability. Since different VHH-characteristic residues are observed in all four subfamilies, these subfamilies must have evolved independently from classical VH domains.

Haruta, H., H. Tachibana, et al. (1999). "Serum starvation induced secondary V[lambda]J[lambda] rearrangement in a human plasma B cell line." *Molecular Immunology* **36**(3): 177.

<http://www.sciencedirect.com/science/article/B6T9R-3WNMGP5-3/2/a4f7ce64eeef04a3448b655607a5d9c4>

HB4C5 is a human antibody producing plasma B cell line that expresses the recombination activating gene-1 (RAG-1) and RAG-2 constitutively, but undergoes few secondary immunoglobulin gene rearrangements when cultured in fetal bovine serum-containing medium. Here, we found that depletion of serum from the culture media induces secondary V[lambda]J[lambda] rearrangement in this cell line. To investigate the induction mechanism of secondary V[lambda]J[lambda] rearrangement, we assessed the expression levels of RAG-1 and RAG-2 products, V[lambda] germline transcription level and the amount of V[lambda] signal broken ends (SBE) in HB4C5 cells cultured in serum-supplemented or serum-free medium. Western-blot analysis showed that the expression level for the RAG-1 and RAG-2 proteins was

not affected by the serum depletion. V[lambda] germline transcript was found to be constitutively expressed in HB4C5 cell line and this transcription level was not affected by the lack of serum. On the other hand, the amount of V[lambda] SBE was shown to be increased in HB4C5 cells cultured in serum-free medium, suggesting that this increased formation of V[lambda] SBE at least partly contributed to the enhanced occurrence of secondary V[lambda]J[lambda] rearrangement in HB4C5 cells cultured in the serum-free condition. These results indicate that expression of RAG proteins and V[lambda] germline transcription is not enough to undergo secondary V[lambda]J[lambda] rearrangement in this cell line.

Hexham, J. M., D. Dudas, et al. (2001). "Influence of relative binding affinity on efficacy in a panel of anti-CD3 scFv immunotoxins." *Molecular Immunology* **38**(5): 397.

<http://www.sciencedirect.com/science/article/B6T9R-448BFWG-8/2/0a3bb431e521d910f1cbee2984b51cb8>

The in vitro cell killing potency of an immunotoxin reflects the aggregate of several independent biochemical properties. These include antigen binding affinity; internalization rate, intracellular processing and intrinsic toxin domain potency. This study examines the influence of antigen binding affinity on potency in various immunotoxin fusion proteins where target antigen binding is mediated by single chain antibody variable region fragments (scFv). Firstly, the relationship between affinity and potency was examined in a panel of four scFv immunotoxins generated from different anti-CD3 monoclonal antibodies fused to the 38 kDa fragment of *Pseudomonas aeruginosa* exotoxin A (PE38). Of these four scFv-PE38 immunotoxins, the one derived from the anti-CD3 monoclonal antibody UCHT1 has highest cell killing potency. Analysis of these four scFv-PE38 immunotoxins indicated a correlation between antigen binding affinity and immunotoxin potency in the cell killing assay with the exception of the scFvPE38 immunotoxin derived from the antibody BC3. However this scFv appeared to suffer a greater drop in affinity (~100 x), relative to the parent Mab than did the other three scFvs used in this study (2-10 x). Secondly, the scFv(UCHT1)-PE38 immunotoxin was then compared with a further panel of scFv(UCHT1)-derived immunotoxins including a divalent PE38 version and both monovalent and divalent *Corynebacterium diphtheriae* toxin (DT389) fusion proteins. When the scFv-UCHT1 domain was amino-terminally positioned relative to the toxin, as in the scFv(UCHT1)-PE38, an approximately 10-fold higher antigen-binding affinity was observed than with the C-terminal fusion, used in the DT389-scFv(UCHT1) molecule. Despite this lower antigen-binding activity, the DT389-scFv immunotoxin had a 60-fold higher potency in the T-cell-killing assay. Thirdly, a divalent form of the DT389-scFv construct, containing tandem scFv domains, had a 10-fold higher binding activity, which was exactly reflected in a 10-fold increase in potency. Therefore, when comparing immunotoxins in which scFvs from different antibodies are fused to the same toxin domain (DT or PE) a broad correlation appears to exist between binding affinity and immunotoxin potency. However, no correlation between affinity and potency appears to exist when different toxin domains are combined with the same scFv antibody domain.

Hildebrand, W. H., R. M. Horton, et al. (1992). "Nucleotide sequence analysis of H-2Df and the spontaneous in vivo H-2Dfm2 mutation." *Molecular Immunology* **29**(1): 61.

<http://www.sciencedirect.com/science/article/B6T9R-476F92G-SK/2/91261c1600604a210ec4faab2eb5efa6>

The nucleotide sequence of the standard H-2Df allele and the spontaneous in vivo H-2Dfm2 mutation are reported here. Locus-specific sequences in the 5' and 3' untranslated regions of the mouse MHC class I H-2D-region genes were used to design primers for the specific amplification and cloning of H-2D-region cDNA from standard B10.M/Sn H-2f and mutant BIO.M-H-2fm2/Mob

mice. A partial Df genomic clone and direct Df and Dfm2 mRNA sequence analysis confirmed the authenticity of the cDNA clones. Interestingly, H-2Df contains a proline in the [alpha]-helix of the [alpha] 1 domain at amino acid position 62; no other known class I molecule has a proline at this position. The H-2Dfm2 mutation, however, replaces this unique proline in Df with the H-2 and HLA consensus arginine at position 62. Although a point mutation cannot be ruled out, the single nucleotide change in the H-2Dfm2 mutation is flanked by a stretch of 47 nucleotide bases with an identical counterpart in H-2Kf, a finding consistent with a recombinatorial event between H-2Kf and H-2Df.

Hohman, V. S., S. E. Stewart, et al. (1997). "Sequence and expression pattern of J chain in the amphibian, *Xenopus laevis*." Molecular Immunology **34**(14): 995.

<http://www.sciencedirect.com/science/article/B6T9R-3S84H6J-3/2/5a1aa3434e026704c80bd0d3381cf918>

We have determined the cDNA sequence encoding J chain, a polypeptide accessory molecule associated with polymeric Ig, from the anuran amphibian, *Xenopus laevis* (South African clawed frog). The translated polypeptide consists of 164 amino acid residues, including the signal sequence, and is somewhat longer than the corresponding sequence in mouse and cow, the two mammalian species in which the signal sequence of J chain has been determined. J chain in several mammalian species (human, mouse, cow and rabbit) has eight Cys residues. In the human chain, two of these Cys residues, the second and third in the sequence, have been shown to form disulfide bridges to heavy chains in IgM or IgA; the remaining Cys residues form intrachain disulfide bonds. The *Xenopus* J chain contains only seven of these Cys residues. Ser is found at the position corresponding to the third Cys in mammalian J chains. Northern blot analysis, performed on RNA isolated from various organs of 3-month old frogs, indicated that the highest level of expression was in the intestine. Transcripts corresponding to J chain were also detected in the spleen and at very low levels in the thymus.

Honda, T., T. Nishizawa, et al. (2005). "Molecular cloning and expression analysis of a macrophage-colony stimulating factor receptor-like gene from rainbow trout, *Oncorhynchus mykiss*." Molecular Immunology **42**(1): 1.

<http://www.sciencedirect.com/science/article/B6T9R-4D5KRGS-1/2/9b125861dfd893a238d982563a2059ee>

The M-CSF and its receptor (M-CSFR, CSF-1R or c-fms proto-oncogene) system were initially implicated as essential in mammals for normal monocyte development as well as for pregnancy. To allow a comparison with the M-CSF and M-CSFR system of an oviparous animal, we cloned a M-CSFR-like gene from rainbow trout (*Oncorhynchus mykiss*). The gene was cloned from a cDNA library of head kidney. It contained an open reading frame encoding 967 amino acids with a predicted size of 109 kDa. The putative amino acid sequence of rainbow trout M-CSFR showed 54% amino acid identity to fugu (*Takifugu rubripes*) M-CSFR, 52% to zebrafish (*Danio rerio*) M-CSFR and 40% to mouse (*Mus musculus*) and human (*Homo sapiens*) M-CSFR. The M-CSFR-like gene was constitutively expressed in head kidney, kidney, intestine, spleen and blood. The gene was detected especially in the ovary of immature female rainbow trout. These results suggest that a M-CSFR-like receptor may be involved in female reproductive tracts even in an oviparous animal like fish.

Irigoyen, M., C. Kowal, et al. (1996). "Molecular mapping of the 8.12 sle-associated idiotype specificity at the single amino acid level." Molecular Immunology **33**(16): 1255.

<http://www.sciencedirect.com/science/article/B6T9R-3W3FCV0-P/2/48a058283ecf18134665d24e70ef259d>

The 8.12 idiotype is expressed in elevated titer in the serum of patients with systemic lupus erythematosus and is a marker for a subpopulation of anti-DNA antibodies that possess a V[lambda]II encoded light chain. This study utilized a eukaryotic expression system to identify the structural basis for expression of this idiotype. Reversion of the 8.12+ DSC light chain to the hs1v215.23/DPL11 germline gene reveals that the 8.12 idiotype is encoded in the germline. The 8.12+ DSC and the 8.12- AS17 light chains, both belonging to the V[lambda]II family, were subjected to site directed mutagenesis, to localize amino acids important for expression of the 8.12 idiotype. Point mutations were performed in CDR1, CDR2, FR3 and CDR3, in positions where the 8.12+ DSC differs from the 8.12- AS17. Amino acids in CDR1 and the CDR2 proximal region of FR3, but not the J proximal region of CDR3, play a crucial role in 8.12 reactivity. The 3-D structure of Mcg, a human IgG1, with which DSC shares a sequence homology of 92.3% has been examined to visualize the effect of each of the mutations and to identify the surface on DSC that comprises the idiotype.

Ji, Y., S. Desravines, et al. (1999). "Junctional diversity in *Xenopus* immunoglobulin light chains." Molecular Immunology **36**(17): 1159.

<http://www.sciencedirect.com/science/article/B6T9R-3YCM594-4/2/553c04f359afb559d4b7595b8fed1375>

Xenopus cDNA sequences encoding the homolog of mammalian kappa ([kappa]) light (L) chains were isolated from isogenic tadpole and adult individuals to investigate whether there existed stage-specific immunoglobulin L chain expression and somatic diversification. In the course of these studies rearrangements to a sixth JL gene segment and a pseudogene (JL[psi]) were found, and it is suggested that the order of these gene segments with respect to the L chain constant (C) region exon is: JL6-JL1-JL2-JL3-JL4-JL5-JL[psi]-CL. The cDNA junctional diversity was analyzed; few N and P regions were found and almost all the CDR3 were 9 codons in length. There were restricted patterns of recombination site resolution, and this is attributed to some constraint in JL coding end processing.

Kimura, T., T. Hosoi, et al. (2000). "Epitope mapping of monoclonal antibodies specific for a macrophage lectin: a calcium-dependent epitope is in the carbohydrate recognition domain." Molecular Immunology **37**(3-4): 151.

<http://www.sciencedirect.com/science/article/B6T9R-40J1DSX-7/2/6b53b75769203f5a1a3442c4dbab2300>

Mouse macrophage galactose/N-acetylgalactosamine-specific calcium-type lectin (mMGL) has a calcium-dependent conformational epitope which is a ligand-induced binding site. A monoclonal antibody (mAb) specific for this epitope (LOM-11) stabilize lectin activity. We performed mapping for this conformational epitope using trypsin fragments that contain a carbohydrate recognition domain (CRD) and chimeric recombinant proteins between mMGL and a human counterpart of this molecule. Binding site for the mAb LOM-11 was mapped within the C-terminal 59 amino acids of CRD. Binding sites for all four mAbs that block carbohydrate ligand binding were also mapped in the C-terminal half of CRD. These results indicated that the calcium-dependent site

potentially involved in protein-protein interaction, regulatory or for coordinated binding, is mapped within CRD in addition to the independent carbohydrate binding site, and that both of the distinct sites may have spatial proximity.

Larsen, J. N., P. Stroman, et al. (1992). "PCR based cloning and sequencing of isogenes encoding the tree pollen major allergen Car b I from *Carpinus betulus*, hornbeam." Molecular Immunology **29**(6): 703.

<http://www.sciencedirect.com/science/article/B6T9R-476F8Y3-PR/2/4fd27350e93ca14843bf20903905eecd>

Cloning of the gene encoding the major allergen, Car b I, from *Carpinus betulus* (hornbeam) pollen was performed using the Polymerase Chain Reaction (PCR) to specifically amplify the gene of interest using single stranded cDNA as template. Specific primers, deduced from the aminoterminal sequence of the purified protein, were tailored to facilitate direct expression of plasmic clones, and the large fraction of positive clones obtained, revealed the presence of isogenic variation. Three clones were characterized in detail by antibody based assays and nucleotide sequencing. The recombinant allergens were shown by crossed immunoelectrophoresis (CIE) to precipitate with monospecific polyclonal rabbit antibodies raised against purified Bet v I, by crossed radioimmuno-electrophoresis (CRIE) to bind tree pollen allergic patient serum IgE, and by immunoblotting to bind murine monoclonal antibodies, raised against purified Car b I from pollen. Car b I is encoded by a 159-triplets open reading frame. The molecular masses ($M_r = 17272$, 17355 and 17217 Da, respectively), the amino acid composition, and the aminoterminal sequence of the predicted polypeptides agree well with data obtained by analysis of the protein purified from pollen. The deduced amino acid sequences show pronounced homology (73, 75 and 74% identities respectively) to Bet v I, the major allergen from *Betula verrucosa* (white birch) pollen. Soluble recombinant Car b I, without a fusion partner, was produced in *Escherichia coli* with an immunochemical reactivity closely resembling that of the native pollen allergen. The tree pollen major allergens therefore constitute an ideal system for the study of allergenic epitopes.

Leung, S.-o., D. M. Goldenberg, et al. (1995). "Construction and characterization of a humanized, internalizing, B-cell (CD22)-specific, leukemia/lymphoma antibody, LL2." Molecular Immunology **32**(17-18): 1413.

<http://www.sciencedirect.com/science/article/B6T9R-3YKM941-F/2/a131d152e333c7db2be85fd30125b8c3>

The murine monoclonal antibody, LL2, is a B-cell (CD22)-specific IgG2a which has been demonstrated to be clinically significant in the radioimmunodetection of non-Hodgkin's B-cell lymphoma. The antibody carries a variable region-appended glycosylation site in the light chain, and is rapidly internalized upon binding to Raji target cells. Humanization of LL2 was carried out in order to develop LL2 as a diagnostic and immunotherapeutic suitable for repeated administration. Based on the extent of sequence homology, and with the aid of computer modeling, we selected the EU framework regions (FR) 1, 2 and 3, and the NEWM FR4 as the scaffold for grafting the heavy chain complementarity determining regions (CDRs), and the REI FRs for that of light chains. The light chain glycosylation site, however, was not included. Construction of the CDR-grafted variable regions was accomplished by a rapid and simplified method that involved long DNA oligonucleotide synthesis and the polymerase chain reaction (PCR). The humanized LL2 (hLL2), lacking light chain variable region glycosylation, exhibited immunoreactivities that were comparable to that of chimeric LL2 (cLL2), which was shown previously to have antigen-binding properties similar to its murine counterpart, suggesting that the

VK-appended oligosaccharides found in mLL2 are not necessary for antigen binding. Moreover, the hLL2 retained its ability to be internalized into Raji cells at a rate similar to its murine and chimeric counterparts.

Lim, P. L., D. T. M. Leung, et al. (1994). "Structural analysis of a phosphorylcholine-binding antibody which exhibits a unique carrier specificity for *Trichinella spiralis*." Molecular Immunology **31**(14): 1109.

<http://www.sciencedirect.com/science/article/B6T9R-476N7KP-5R/2/aef1fc93d5bb2cb2030ae56434542282>

A phosphorylcholine (PC)-binding IgG (Mab2) antibody produced by a hybridoma derived from a BALB/c mouse which had been immunized against *Trichinella spiralis* was found to bind to the immunizing antigen (TSC) but not to other PC-associated antigens such as pneumococcal antigen (PNC) and PC-conjugated ovalbumin (PC-OVA). Sequence analysis of the protein revealed the presence of a heavy chain (VH) which was very similar (differing in only four amino acids) to that of the M511 myeloma protein, and a light chain (VL) which was completely identical to that of the M167 myeloma protein. Several M511/M167+ proteins, including the prototypic M511 protein and PC-binding proteins of other families (TEPC 15 and W3207), were examined in their binding to the various PC-associated antigens. These were found to be largely indiscriminate although subtle differences were observed for some antigens with some of the antibodies. A comparison of the VH sequences of Mab2 and these proteins revealed that of the differences seen, the single most important substitution in Mab2 which could contribute to the unique specificity of the molecule is the glycine residue at 49H. None of the other proteins, including other PCV-binding proteins published to-date, which utilize the same VH segment (99 in total), has this substitution.

Liu, Y., Y. Endo, et al. "Ficolin A and ficolin B are expressed in distinct ontogenic patterns and cell types in the mouse." Molecular Immunology In Press, Corrected Proof

<http://www.sciencedirect.com/science/article/B6T9R-4F60N8C-2/2/e8a0b873e1423f76e072cd735fc32c1c>

Ficolins are a group of proteins characterized by the presence of collagen-like and fibrinogen-like domains. Two of three human ficolins, L-ficolin and H-ficolin, are serum lectins that form complexes with mannose-binding lectin-associated serine proteases (MASPs) and play important roles in the lectin complement pathway. The other human ficolin, M-ficolin, is a non-serum-type ficolin that is expressed in monocytes. Little is known about the physiological roles of ficolins. In this study, we delineated the ontogeny and cell types that express ficolins in mice. RT-PCR analysis showed that the expression pattern of ficolin A expression was closely similar to that of Masps, suggesting that these molecules may function in coordination as components of the lectin complement pathway. The cell types that express ficolin A mRNA in both adult liver and spleen were identified as macrophages by in situ hybridization. Ficolin B exhibited a distinct ontogeny pattern that switched from embryonic liver to postnatal bone marrow and spleen. The cells that express ficolin B mRNA were identified as belonging to the myeloid cell lineage by magnetic sorting and by subsequent RT-PCR in bone marrow cells. Thus, the different spatial-temporal expression patterns of ficolins A and B suggest that these molecules play distinct roles in the prenatal and postnatal stages.

Louie, K. A., J. Ochoa-Garay, et al. (1996). "H-2Ld-alloreactive T cell hybridomas utilize diverse V[alpha]

and V[beta] T cell receptor chains." Molecular Immunology **33**(9): 747.

<http://www.sciencedirect.com/science/article/B6T9R-3W2YC96-2/2/a42b6126b984fb5af00fcda8951b9381>

We have sequenced the TCRs from Ld-specific alloreactive T cell hybridomas, whose reactivities we have found to be quite representative of those of a primary dm2 anti-BALB/cJ mixed lymphocyte reaction. We find V[beta]6, V[beta]7, V[beta]8 and V[beta]10 gene segments. V[alpha] usage is diverse, although closely related to that from peptide-specific Ld-restricted CTLs. V[alpha]-V[beta] selection provides evidence of preferential pairing. Amino acid frequency analysis shows that the [alpha]CDR2 region is rich in charged amino acids, in contrast to the [beta]CDR2 region. Our data suggests the [beta] chain may be more immunoglobulin-like than the [alpha] chain, and that charge complementarity may be important in TCR-MHC interactions. We do not consider our results to be contradictory to those previously reported but rather they may represent an early, more diverse response.

Mazzucchelli, R., M. Amadio, et al. (2004). "Establishment of an ex vivo model of monocytes-derived macrophages differentiated from peripheral blood mononuclear cells (PBMCs) from HIV-1 transgenic rats." Molecular Immunology **41**(10): 979.

<http://www.sciencedirect.com/science/article/B6T9R-4CX00BV-2/2/54329d4d1705dbf3eff3c7f932e2f156>

It has been developed an HIV type 1 transgenic rat model (HIV-1 Tg Rat) which contains a gag-pol-deleted HIV-1 provirus regulated by the viral LTR promoter. Although it harbors a non infectious provirus, efficient viral expression occurs in different tissues and disease manifestations as well as immune-response alterations and pathologies similar to humans can be observed. Regulation of HIV-1 expression is influenced by various cellular factors and it is well known that macrophages are one of the major reservoir of HIV-1 infection and a vehicle for virus spread to other tissues. Purpose of our work was to establish an antigen presenting cells (monocyte-derived macrophages, MDM) ex vivo model from these HIV-1 transgenic rats. This model can be used to study function of HIV-infected MDM and their behavior like HIV-1 reservoir. Ultimately, these studies may be helpful in defining approaches to control HIV-1 spread.

McDermott, M. J., E. Weber, et al. (2000). "Identification, cloning, and characterization of a major cat flea salivary allergen (Cte f 1)." Molecular Immunology **37**(7): 361.

<http://www.sciencedirect.com/science/article/B6T9R-41MB06R-4/2/852d94531cd112cace98f8cb458b2200>

An 18 kDa protein isolated from saliva of the cat flea, *Ctenocephalides felis*, elicits a positive intradermal skin test (IDST) in 100 and 80% of experimental and clinical flea allergic dogs, respectively. Using solid-phase enzyme-linked immuno assay (ELISA), this protein detected IgE in 100 and 80% of experimental and clinical flea allergic dogs, respectively. A cDNA (pFSI) encoding a full-length Cte f 1 protein was isolated from a *C. felis* salivary gland cDNA library, using a combination of PCR and hybridization screening. This cDNA is 658 bp in length, and contains an open reading frame of 528 bp. The open reading frame encodes a protein of 176 amino acids, consisting of an 18 amino acid signal sequence and a 158 amino acid mature protein. The calculated molecular weight and pI of the mature protein are 18106 Da and 9.3, respectively. The protein, named Cte f 1, is the first novel major allergen described for canine flea allergy. Recombinant Cte f 1 (rCte f 1) was expressed in *Escherichia coli*, *Pichia pastoris* and

baculovirus infected *Trichoplusia ni* cells. Approximately, 90% of the rCte f 1 expressed in *E. coli* accumulated in insoluble inclusion bodies, which could be refolded to a soluble mixture of disulfide isomers with partial IgE binding activity. Small quantities of an apparently correctly refolded form of rCte f 1, which had IgE binding activity equal to the native antigen, was isolated from the soluble fraction of *E. coli* cells. However, *P. pastoris* and baculovirus infected insect cells expressed and secreted a fully processed, correctly refolded and fully active form of rCte f 1. Mass spectrometry analysis of the active forms of rCte f 1 confirmed that eight intact disulfide bonds were present, matching the number observed in the native allergen. The relative ability of rCte f 1 to bind IgE in the serum of flea allergic animals, produced in these three expression systems, matched that of the native allergen. Competition ELISA demonstrated that approximately 90% of the specific IgE binding to native Cte f 1 could be blocked by the different forms of rCte f 1.

McKenzie, S. E., M. A. Keller, et al. (1992). "Characterization of the 5'-flanking transcriptional regulatory region of the human Fc[gamma] receptor gene, Fc[gamma] RIIA." *Molecular Immunology* **29**(10): 1165.

<http://www.sciencedirect.com/science/article/B6T9R-476F8VT-P6/2/ea05ad0fd81ee1363994365d7316a3ff>

The human Fc[gamma] receptor gene Fc[gamma] RIIA is expressed in platelets, neutrophils, monocytes and macrophages. Understanding the regulation of expression of Fc[gamma] RIIA will enhance our knowledge of regulated gene expression and immune function in these cells. We cloned a 3.65 kb region of the 5' end of the Fc[gamma] RIIA gene and characterized 3.4 kb of previously unreported sequence of the 5'-flanking region. Primer extension studies and RNase protection analyses of mRNA from HEL, K562 and U937 cells revealed multiple transcription start sites. One transcription start site mapped to a 5'-untranslated (5'UT) exon approximately 1 kb 5' to the ATG translation initiation codon, while a second start site mapped near the ATG codon. Reverse transcription combined with PCR (RT-PCR) employing an oligonucleotide in the putative 5'UT exon and an antisense oligonucleotide in the translated region yielded products which confirm that transcription starts in this 5'UT exon 881 bp upstream of the ATG codon. Sequence analysis of the RT-PCR products showed two related RNA splice products which use alternative 3'-consensus AG splice acceptor sites. Fc[gamma] RIIA mRNA thus has three distinct potential 5'UT regions, two alternatively spliced forms from the start site in the 5'UT exon and the third from the start site near the ATG codon. Comparisons of the human Fc[gamma] RIIA 5'-flanking region with human Fc[gamma] RI and mouse Fc[gamma] RII[beta] genes as well as with other genes expressed in megakaryocytes, neutrophils and monocytes reveal structural similarities and shared promoter elements.

Nye, S. H., C. M. Pelfrey, et al. (1995). "Purification of immunologically active recombinant 21.5 kDa isoform of human myelin basic protein." *Molecular Immunology* **32**(14-15): 1131.

<http://www.sciencedirect.com/science/article/B6T9R-3YKM95C-1G/2/acebd3809819e1cd467d8f2edfd52a47>

We have designed and expressed in bacteria a recombinant fetal form of human myelin basic protein (21.5 kDa isoform; rhMBP21.5), a candidate autoantigen in multiple sclerosis. An exon 2 insertion, carboxy-terminal histidine tag and preferred bacterial codons differentiate the MBP21.5 gene from that encoding the adult, brain-derived form of human MBP (18.5 kDa isoform; hMBP18.5). MBPs were expressed at high levels in *E. coli* and extracted from whole cells by simultaneous acid solubilization and mechanical disruption. A nearly two-fold increase in recombinant protein was detected in strains harboring MBP genes with bacterial preferred codons

compared to genes containing human codons. The recombinant molecules were purified in two steps, first by reversed-phase chromatographic separation and then by metal affinity chromatography. Dimeric forms of recombinant MBP21.5 were detected under physiological conditions, however, substitution of a serine for the single cysteine at amino acid residue 81 resulted in only monomer formation. All forms of recombinant MBPs induced proliferative responses of human T lymphocytes specific for epitopes in MBP18.5 kDa. In contrast, human T cell lines that recognize an exon 2-encoded epitope of MBP responded to the 21.5 kDa isoform of MBP, but not the 18.5 kDa isoform.

Ohlin, M. and C. A. K. Borrebaeck (1998). "Insertions and deletions in hypervariable loops of antibody heavy chains contribute to molecular diversity." Molecular Immunology **35**(4): 233.

<http://www.sciencedirect.com/science/article/B6T9R-3W1RG0F-5/2/19248871758071a9ac32a653c3ef3fb1>

Antibody diversity, a molecular feature which allows these proteins to specifically interact with a diverse set of targets, is created at the genetic level by a variety of means. These include germline gene segment recombination, junctional diversity and single basepair (bp) substitution. We here demonstrate that a human high affinity antibody specific for an exogenous protein antigen carry three amino acid residues immediately adjacent to the first hypervariable loop of the heavy chain. These additional residues are shown not to be encoded by the germline repertoire. We also describe the characteristics of insertions and deletions, not found in any known germline sequence, within the first and second hypervariable loops of other previously described antibody-encoding genes. These findings demonstrate that insertions or deletions of entire codons provide yet another approach by which the human antibody repertoire is diversified in vivo. Since these major genetic modifications occur within or immediately adjacent to loops contributing to the antigen-binding site, they are likely to affect the binding properties of the mutated antibodies.

Press, J. L., C. A. Giorgetti, et al. (1991). "A new germline VH36-60 gene is used in the neonatal primary and adult memory response to (T,G)-A--L." Molecular Immunology **28**(11): 1217.

<http://www.sciencedirect.com/science/article/B6T9R-476F6XV-M/2/bdde81062bc8524a99ff3bbfbb822edc>

Our previous studies of the neonatal primary response to (T,G)-A--L showed that the majority of anti-(T,G)-A--L antibodies bind the copolymer L-Glu:L-Tyr (GT), share idiotype (Id), and use the H10 germline VH gene from the VHJ558 family and a V[kappa] 1 gene. We also identified two hybridomas from different neonatal donors that produced GT +, Id + antibodies using a V[kappa] 1 gene with a VH gene from the VH36-60 family. In the study reported here, we show that both neonatal hybridomas use the same germline VH gene from the VH36-60 gene family. However, the VH gene sequence is different from previously identified germline genes of the VH36-60 gene family. To determine whether the expressed heavy chain gene had undergone somatic mutation, we isolated the corresponding germline gene from kidney DNA. Sequence analysis of this gene shows that it is a new member of the VH36-60 family which is not mutated in the neonatal antibodies. Furthermore, the deduced amino acid sequences of the two neonatal antibodies are identical not only in the VH region but also in the VH-D-JH joins, suggesting that there is a strong selection for CDRIII among neonatal anti-(T,G)-A--L antibodies using this germline gene (designated here as VH3A1) with a V[kappa] 1 gene. Also, the VH gene from the VH36-60 family that we showed previously was used by an adult memory B cell clone specific for (T,G)-A--L, can now be identified as a rearrangement of the VH3A1 germline gene. Elucidation of the germline variable region genes that are used in the antigen-specific neonatal response will help us understand the mechanisms that shape the preimmune B cell repertoire during B cell

development.

Rogerson, B. J. (1994). "Mapping the upstream boundary of somatic mutations in rearranged immunoglobulin transgenes and endogenous genes." Molecular Immunology **31**(2): 83.

<http://www.sciencedirect.com/science/article/B6T9R-476N7PY-6R/2/01f412e96ad7caac1bfde0a9c8c0d364>

Mammalian B-cell specific somatic hypermutation contributes to affinity maturation of the antibody response. This mutator activity is highly focused on rearranged immunoglobulin variable regions, but the underlying mechanism remains to be elucidated. In an effort to gain insights into the mechanism of somatic hypermutation, the precise distribution and frequency of mutations upstream of murine immunoglobulin genes was determined by examining the same variable gene segments when mutated in different B-cell lines. Immunoglobulin sequences analysed included [kappa] light chain transgenes bearing mutated V[kappa]24 variable regions, and the endogenous V[kappa] gene isolated from myeloma MOPC167, which also exhibits mutations in the variable region. In addition, mutated endogenous VH1 gene segments of the S107 heavy chain variable gene family were also examined. For both VH1 and V[kappa]24, somatic mutations were generally not found upstream of the leader intron, even in genes which exhibited a high mutation frequency in the variable region itself. The 5' somatic mutation boundary identified in immunoglobulin transgenes overlaps the boundary observed in endogenous genes, suggesting that both share cis -elements required for defining the mutable domain. Furthermore, the location of this 5' boundary appears not to change when these immunoglobulin genes are examined in different cell lines. These data may be indicative of a defined start site for immunoglobulin mutator activity.

Roos, A., L. H. Bouwman, et al. (2003). "Functional characterization of the lectin pathway of complement in human serum." Molecular Immunology **39**(11): 655.

<http://www.sciencedirect.com/science/article/B6T9R-476K74Y-1/2/904213914d11e347e2abf2f2abbd995e>

Mannan-binding lectin (MBL) is a major initiator of the lectin pathway (LP) of complement. Polymorphisms in exon 1 of the MBL gene are associated with impaired MBL function and infections. Functional assays to assess the activity of the classical pathway (CP) and the alternative pathway (AP) of complement in serum are broadly used in patient diagnostics. We have now developed a functional LP assay that enables the specific quantification of autologous MBL-dependent complement activation in human serum. Complement activation was assessed by ELISA using coated mannan to assess the LP and coated IgM to assess the CP. Normal human serum (NHS) contains IgG, IgA and IgM antibodies against mannan, as shown by ELISA. These antibodies are likely to induce CP activation. Using C1q-blocking and MBL-blocking mAb, it was confirmed that both the LP and the CP contribute to complement activation by mannan. In order to quantify LP activity without interference of the CP, LP activity was measured in serum in the presence of C1q-blocking Ab. Activation of serum on coated IgM via the CP resulted in a dose-dependent deposition of C1q, C4, C3, and C5b-9. This activation and subsequent complement deposition was completely inhibited by the C1q-blocking mAb 2204 and by polyclonal Fab anti-C1q Ab. Evaluation of the LP in the presence of mAb 2204 showed a strong dose-dependent deposition of C4, C3, and C5b-9 using serum from MBL-wildtype (AA) but not MBL-mutant donors (AB or BB genotype), indicating that complement activation under these conditions is MBL-dependent and C1q-independent. Donors with different MBL genotypes were identified using a newly developed oligonucleotide ligation assay (OLA) for detection of MBL exon 1 polymorphisms. We describe a novel functional assay that enables quantification of autologous

complement activation via the LP in full human serum up to the formation of the membrane attack complex. This assay offers novel possibilities for patient diagnostics as well as for the study of disease association with the LP.

Suzuki, K., T. Hirose, et al. (1998). "The Fc receptor (FcR) [gamma] subunit is essential for IgE-binding activity of cell-surface expressed chimeric receptor molecules constructed from human high-affinity IgE receptor (Fc[epsilon]RI) [alpha] and FcR[gamma] subunits." Molecular Immunology **35**(5): 259.

<http://www.sciencedirect.com/science/article/B6T9R-3V4JNSC-G/2/fbe51b106b6e7f33951ee5a28055f40d>

The Fc receptor (FcR) [gamma] subunit was originally discovered as a homodimeric subunit of the high-affinity IgE receptor (Fc[epsilon]RI). But it was recently found to be a common signal-generating subunit of Fc receptors including IgG Fc receptors (Fc[gamma]Rs) and IgA Fc receptor (Fc[alpha]R), and furthermore to generate a signal also with stimuli through non-immune receptors. In addition, it plays an essential role in cell-surface expression of the Fc[epsilon]RI and the Fc[gamma]RIIIA isoform and also regulates cell-surface expression and ligand-binding affinity of the Fc[gamma]RI. In this report, we addressed the possibility that the FcR[gamma] could affect the correct folding of the IgE-binding region of the Fc[epsilon]RI[alpha] subunit by using the chimeric receptor molecules constructed from human Fc[epsilon]RI[alpha] and FcR[gamma]. Furthermore, we demonstrated that the seven amino acid residues in the C-terminal region on the extracellular domain of the Fc[epsilon]RI[alpha] were essential for maintaining the IgE-binding activity of the Fc[epsilon]RI[alpha] exodomain on the cell membrane and/or may affect the correct folding of the [alpha] subunit itself within the cell.

Talken, B. L., K. Peterson, et al. (1994). "A polymorphic residue in the amino terminal [alpha] 1 hemi-domain of the mouse Ld class I molecule affects its assembly and surface expression." Molecular Immunology **31**(15): 1169.

<http://www.sciencedirect.com/science/article/B6T9R-4754VTW-6/2/7957b555a0701bba81bd3c81a27ad864>

In comparison to Dd and most other mouse major histocompatibility complex class I molecules, the Ld molecule is poorly expressed on the cell surface, has a lower affinity for [beta]2-microglobulin and is trafficked more slowly to the cell surface. Previous studies using Ld-Dd exon-shuffled constructs and the chimeric Ddml molecules suggested that the Ld[alpha] 1 domain was responsible for this phenotype. Two constructs, one containing an Ld-Dd hemi-exon-shuffled [alpha] 1 exon and the other containing a Dd-Ld hemi-exon-shuffled [alpha] 1 exon, were inserted into either Ld or Dd to replace the intact [alpha] 1 exon. These constructs were transfected into mouse L cells. Flow cytometric analyses of the resulting transfectants indicate that the Dd-Ld [alpha] 1/Ld molecules, similar to the Dd [alpha] 1/Dd [alpha] 2/Ld molecules, were expressed at a higher level on the cell surface than either the Ld-Dd [alpha] 1/Ld molecules or intact Ld molecules. Analyses of the molecules in lysates suggested that a higher proportion of the Dd-Ld [alpha] 1/Ld molecules, like the Dd [alpha] 1/Dd [alpha] 2/Ld molecules, as compared to the Ld-Dd [alpha] 1/Ld and intact Ld molecules were assembled as detected by [alpha] 2 domain-reactive monoclonal antibodies. Pulse-chase and lysate stability studies suggested that the lower steady state levels of assembled Ld-Dd [alpha] 1 molecules resulted from a slower assembly rate rather than instability. Collectively, these studies suggest that residues in the amino terminal half of the Ld [alpha] 1 domain are responsible for its inefficient assembly, probably leading to its low cell surface expression. To determine which polymorphic residues in the amino terminal [alpha] 1 hemi-domain might influence this phenotype, several Ld point mutants, in which a Dd amino

terminal [alpha] 1 hemi-domain residue was substituted into the corresponding position of Ld, were analysed. These analyses suggested that, while the residue at position 9 has only a slight effect on [beta]2-microglobulin association, it has a striking effect on assembly and cell surface expression.

Tosi, G., S. Brunelli, et al. (1994). "The complex interplay of the DQB1 and DQA1 loci in the generation of the susceptible and protective phenotype for insulin-dependent diabetes mellitus." Molecular Immunology **31**(6): 429.

<http://www.sciencedirect.com/science/article/B6T9R-476N815-96/2/94d3a72747a5815b824c1d915488004d>

IDDM patients of North East Italian region were molecularly typed for their HLA-DQB1 and DQA1 loci by using allele specific oligonucleotide probes and PCR amplified genomic DNA. IDDM status strongly correlated with DQB1 alleles carrying a non-aspartic acid residue in position 57 of DQ[beta] chain and DQA1 alleles with an arginine residue in position 52 of DQ[alpha] chain. Genotype analysis revealed that individuals with two DQB1 alleles having a non-aspartic residue in position 57 and two DQA1 alleles with an arginine residue in position 52 had the highest relative risk of disease: they constituted 41% of IDDM patients as compared to 0% of controls. Heterozygosity either at residue 57 of DQB1 or residue 52 of DQA1 was sufficient to abrogate statistical significance for disease association, although 43.6% of IDDM patients were included in these two groups as compared to 21.6% of normal controls. On the other hand the presence of two DQB1 alleles with aspartic acid in position 57 was sufficient to confer resistance to disease irrespective of the DQA1 genotype. Based on the number of possible susceptible heterodimers an individual can form, it was found that 85% of IDDM cases could form two or more heterodimers (two in cis and two in trans), but no IDDM case was found to form one susceptible heterodimer in cis. These results demonstrate that the complete HLA-DQ genotype, more than single DQB1 or DQA1 alleles or DQB1-DQA1 haplotypes, is associated with the highest risk of disease. Screening of the population for preventive purposes and/or early signs of IDDM should then take advantage of this result and "susceptible homozygous" individuals should be followed very closely and considered the first group of choice for possible new therapeutical trials.

Tosi, G., G. Mantero, et al. (1993). "HLA-DQB1 typing of north east Italian IDDM patients using amplified DNA, oligonucleotide probes and a rapid DNA-enzyme immunoassay (DEIA)." Molecular Immunology **30**(1): 69.

<http://www.sciencedirect.com/science/article/B6T9R-476KP7N-3B/2/a1b4ffb24d6dfe6a8d5b9336ab4718c8>

We report on HLA-DQB1 typing in IDDM patients of north east Italian region using an enzymatic method based on the detection of hybridization reaction between PCR amplified DNA from whole blood and allele specific oligonucleotides by an antibody directed against double stranded DNA (DNA-enzyme immunoassay or DEIA). The method is reliable, simple and sensitive as the classical radioactive method with the advantage of using a universal non radioactive detection reagent. Nineteen families, each including one subject with juvenile insulin-dependent diabetes mellitus (IDDM) were analyzed. A strong association between absence of an aspartic acid (Asp) in position 57 of DQB1[beta] chain in homozygous conditions and susceptibility to IDDM was found. In contrast with some previous observations, however, no significant association was found between Asp/non-Asp heterozygous genotype and IDDM. No patients were found with an homozygous Asp/Asp genotype, known to be protective in caucasoid population. Of particular interest was the DQB1 alleli distribution in our population sample. The non-Asp allele most frequently found in IDDM subjects was the DQB1 0201 allele and this finding was statistically

significant (Pc value <0.05, relative RISK = 5.01). No significant association was found for any other allele including the DQB1 0302 (Pc VALUE = not significant although with relative RISK = 3.28) previously reported as the most frequent allele in IDDM caucasoid patients.

Viquez, O. M., K. N. Konan, et al. (2004). "Genomic organization of peanut allergen gene, Ara h 3." Molecular Immunology **41**(12): 1235.

<http://www.sciencedirect.com/science/article/B6T9R-4D5KX9F-1/2/4f3282834c09c81a345542f5b993b6ae>

Type 1 hypersensitivity to peanut proteins is a well-recognized health problem. Several peanut seed storage proteins have been identified as allergens. Ara h 3, a glycinin protein, is one of the important peanut allergens. Although amino acid and cDNA sequences are available for Ara h 3, there is not information at the genomic level. The objectives of this study were to isolate, sequence, and characterize the genomic clone of peanut allergen, Ara h 3. A peanut genomic library was screened, using two [32P] end-labeled oligonucleotide probes designed based on cDNA sequences of Ara h 3 and Ara h 4. Four positives lambda FIX II clones were obtained after four rounds of screenings. Digestion with Sac I resulted in two fragments of 1.5 and 10 kb hybridizing to the probes. Both fragments were subcloned into p-Bluescript vector and sequenced. The Ara h 3 gene spans 3.5 kb and consists of four exons, three introns, 5' and 3' flanking regions. The open reading frame is 2008 bp long and can encode a polypeptide of 538 amino acids residues. Sequences analogous to a TATA-box (TATAAAT), CAAT-box (AGGA), G-box (TCCTACGTGTCC) and several cis-elements were found in the promoter region. In the 3' downstream region, three polyadenylation signals (AATAAA) were identified.

Viquez, O. M., K. N. Konan, et al. (2003). "Structure and organization of the genomic clone of a major peanut allergen gene, Ara h 1." Molecular Immunology **40**(9): 565.

<http://www.sciencedirect.com/science/article/B6T9R-49SN9FS-1/2/70aeeae21914d97e4d22e480075c58>

Peanut is one of the most allergenic foods. It contains multiple seed storage proteins identified as allergens, which are responsible for triggering IgE-mediated allergic reactions. Ara h 1 is a major peanut allergen recognized by over 90% of peanut sensitive population. The objectives of this study were to isolate, sequence, and determine the structure and organization of at least one genomic clone encoding Ara h 1. Two 100 bp oligonucleotides were synthesized and used as probes to screen a peanut genomic library constructed in a Lambda FIX II vector. After three rounds of screening, four putative positive clones were selected and their DNA digested with SacI. A unique 12-13 kb insert fragment was released, confirmed positive by Southern hybridization, subcloned into a pBluescript vector, and sequenced. Sequence analysis revealed a full-length Ara h 1 gene of 4447 bp with four exons of 721, 176, 81 and 903 bp and three introns of 71, 249 and 74 bp. The deduced amino acid encodes a protein of 626 residues that is identical to the Ara h 1 cDNA clone P41b. Several well characterized elements for promoter strength were found in the promoter region of Ara h 1 and include two TATA-boxes (TATATAAATA and TTATATATAT) at positions -89 and -348, respectively; a CAAT-box (CAAT) at position -133, a GC-box (CGGGACCGGGCCGG GCCTTCGGGCCGGGCCGGGT) at position -475, two G-boxes (TAACACGTACAC and ATGGACGTGAAA) at positions -264 and -1808, respectively; two RY elements (CATGCAC and CATGCAT) at positions -235 and -278, respectively; and other cis-element sequences. In the 3' UTR, a poly-A signal (AATAAA) was found at +2350, two additional stop codons (TAA) at +2303 and +2306, and TTTG/CTA/G motifs. Three introns and a potentially strong promoter could explain the high expression of the Ara h 1 gene. Amino acid sequence comparisons revealed high sequence similarity with other plant vicilins, member of the cupin

superfamily.

Wade, W. F., I. Khrebtukova, et al. (1995). "Truncated MHC class II cytoplasmic and transmembrane domains: Effect on plasma membrane expression." Molecular Immunology **32**(6): 433.

<http://www.sciencedirect.com/science/article/B6T9R-3YKM99P-47/2/184d6ef30644a17cf09d6fb4de9ffabd>

Plasma membrane (PM) expression of major histocompatibility complex (MHC) class II molecule is required for the interaction of antigen (Ag) presenting cells and T lymphocytes. Class II molecules composed of an alpha and a beta chain are highly polymorphic which facilitates their interaction with Ag and Ag-specific T cells. Recently, we have focused on the less polymorphic sequences of class II molecules, the transmembrane (TM) and cytoplasmic (Cy) domains, in an attempt to understand what their function might be. Using site-directed mutagenesis to create truncations in the TM and Cy domains of I-Ak's alpha or beta chain, or both, we have identified some of the sequence requirements for efficient surface expression of I-Ak molecules. Ak[beta]TM mutants that are not expressed at the PM are not transported past the medial-Golgi as indicated by in situ staining and Western blot analysis of endoglycosidase-H-treated immunoprecipitates. The lack of transport of TM class II mutants is not due to lack of association with the invariant chain (Ii). Class II molecules with Cy domain truncations in both chains are not efficiently transported to the PM and also have a percentage of molecules that are endoglycosidase-H sensitive. In situ staining of class II in cells expressing Cy domain truncated class II molecules revealed a discrete vesicular pattern compared to the staining of transfectants that expressed wildtype class II molecules. The immunofluorescence data along with the endoglycosidase-H data indicate the Cy domains are required for efficient transport. Immunoprecipitation studies using a panel of I-Ak conformation-specific antibodies revealed that the truncation of the Cy domains of both chains did not effect the conformation of class II. However, further truncation of the Ak[beta] chain into the TM domain resulted in lack of transport past the ER/medial-Golgi and diminished expression (stability) of mutant class II proteins within the cells. The alpha/beta chains of the TM mutants that did associate bound a panel of conformation sensitive antibodies except for one, 3F12. We conclude that the Cy domain of the alpha and beta chains of MHC class II, as well as sequences in the TM domains of the Ak[beta] chain are required for efficient class II PM expression. The reason for the lack of PM expression of TM mutants may be the inability to access a transport competent conformation as defined by the 3F12-specific epitope, while truncation of the Ak[alpha]Cy domains is proposed to prevent complete masking of the ER retention sequence of the Ii chain.

Walter, W., M. Loos, et al. (1999). "Differential expression of alternative H2-M isoforms in B cells, dendritic cells and macrophages by proinflammatory cytokines." Molecular Immunology **36**(11-12): 733.

<http://www.sciencedirect.com/science/article/B6T9R-3XP0H37-4/2/54458ccf5d200e36efd2fe78f2aa0a62>

Major histocompatibility (MHC) class II heterodimers bind peptides generated by degradation of endocytosed antigens and display them on the surface of antigen presenting cells (APCs) for recognition by CD4⁺ T cells. Efficient loading of MHC class II molecules with peptides is catalyzed by the MHC class II-like molecule H2-M. The coordinate regulation of MHC class II and H2-M expression is a prerequisite for efficient MHC class II/peptide assembly in APCs determining both the generation of the T cell repertoire in the thymus and cellular immune responses in the periphery. Here we show that expression of H2-M and MHC class II genes is coordinately and cell type-specific regulated in splenic B cells, splenic dendritic cells (DCs) and

peritoneal macrophages (M[Phi]) in response to proinflammatory and immunoregulatory cytokines, including GM-CSF, IFN-[gamma], TGF-[beta]2, IL-4, IL-10 and viral IL-10. In addition, ratio-RT-PCR expression analysis of the duplicated H2-M[beta]-chain loci demonstrates for the first time that Mb1 and Mb2 genes are differentially expressed in individual APC types. Mb2 is preferentially expressed in IL-4, GM-CSF, IL-10, vIL-10 and IFN-[gamma] stimulated splenic B cells, whereas splenic DCs express both Mb genes at almost equal levels. In contrast, peritoneal M[Phi] express predominantly Mb2 but stimulation with IFN-[gamma] induces a switch towards Mb1 expression. These data suggest a common mechanism that regulates coordinate expression of H2-M and MHC class II genes in professional APCs. Differential expression of Mb1 and Mb2, and by consequence alternative H2-M isoforms (M[alpha][beta]1 or M[alpha][beta]2), may influence the nature of the peptide repertoire presented by different APC types.

Willett, B. J. and J. C. Neil (1995). "cDNA cloning and eukaryotic expression of feline CD9." Molecular Immunology **32**(6): 417.

<http://www.sciencedirect.com/science/article/B6T9R-3YKM99P-45/2/aae906e5949b72b1a91c502166c13d77>

A monoclonal antibody (vpg15) has been described which can block infection with feline immunodeficiency virus (FIV) and which recognizes the feline homologue of CD9. In order to study the role of feline CD9 in infection with FIV we have molecularly cloned a cDNA encoding feline CD9 by R.A.C.E (rapid amplification of cDNA ends). The amino acid sequence of feline CD9 displays 95.1, 93.8 and 90.7% homology to human, murine and bovine CD9, respectively. Although feline CD9 appears most homologous to human CD9, it has two important features in common with bovine and murine CD9: the presence of a histidine residue at position 192 which is absent from the corresponding position (194) in human CD9; and the absence of two asparagine residues which are found at positions 51 and 52 of human CD9. Feline CD9 is unique in that it lacks a potential N-linked glycosylation site in the first extracellular loop, a feature common to CD9 of other species. Despite the high degree of sequence homology, significant cross-species variation occurred in the two predicted extracellular loops, notably between amino acids 169 to 180 of the second loop. When feline CD9 was expressed on human and murine cells, it was recognized by both the conformation-dependent feline CD9-specific antibody, vpg15, and the cross-species reactive anti-human CD9 antibody, FMC56, confirming that the feline CD9 clone encoded a protein which was synthesized, transported to the cell surface and expressed in a similar conformation to native feline CD9. However, although the vpg15 antibody did not recognize human CD9 when expressed on human epithelial cells, it reacted with human CD9 when expressed on murine fibroblast cells. It is possible therefore, that the conformational epitope recognized by the vpg15 epitope is sensitive to either species- or tissue-specific post-translational modification.

Yamamoto, R., T. Isobe, et al. "Porcine TCR CD3[zeta]-chain and [eta]-chain." Molecular Immunology **In Press, Corrected Proof** <http://www.sciencedirect.com/science/article/B6T9R-4FPX2GX-1/2/b43d1a5cff78c561526636cf1540b34d>

Complete porcine CD3[zeta]-chain cDNA sequence was obtained for the first time, and its genomic nucleotide sequence was investigated from exon 2 down to CD3[eta]-chain exon 8. The sequence of porcine CD3[zeta]-chain showed homologous amino acid sequence with human and murine counterparts, in contrast to CD3[eta]-chain exon 8 with diversity among animals previously investigated. CD3[eta]-chain peptide is an alternative splice form of CD3[zeta]-chain exon 7 splicing to CD3[eta]-chain exon 8 instead of CD3[zeta]-chain exon 8. The genomic sequences revealed that the splice acceptor sequences of CD3[eta]-chain exon 8 of all animals investigated to be completely uniform. Further, CD3[eta]-chain exon 8 amino acid sequences

retained the unique characters of having high proline (Pro) and positively charged amino acid content except for rats and mice. Although the biological role of CD3[eta]-chain remains to be enigmatic, these evidences suggests the evolutionary pressure to maintain its sequence.

Molecular Phylogenetics and Evolution (49)

Arnedo, M. A., J. Coddington, et al. (2004). "From a comb to a tree: phylogenetic relationships of the comb-footed spiders (Araneae, Theridiidae) inferred from nuclear and mitochondrial genes." Molecular Phylogenetics and Evolution **31**(1): 225.

<http://www.sciencedirect.com/science/article/B6WNH-49CN2XB-6/2/0d2acf3be8260aefca161ee5393996b9>

The family Theridiidae is one of the most diverse assemblages of spiders, from both a morphological and ecological point of view. The family includes some of the very few cases of sociality reported in spiders, in addition to bizarre foraging behaviors such as kleptoparasitism and araneophagy, and highly diverse web architecture. Theridiids are one of the seven largest families in the Araneae, with about 2200 species described. However, this species diversity is currently grouped in half the number of genera described for other spider families of similar species richness. Recent cladistic analyses of morphological data have provided an undeniable advance in identifying the closest relatives of the theridiids as well as establishing the family's monophyly. Nevertheless, the comb-footed spiders remain an assemblage of poorly defined genera, among which hypothesized relationships have yet to be examined thoroughly. Providing a robust cladistic structure for the Theridiidae is an essential step towards the clarification of the taxonomy of the group and the interpretation of the evolution of the diverse traits found in the family. Here we present results of a molecular phylogenetic analysis of a broad taxonomic sample of the family (40 taxa in 33 of the 79 currently recognized genera) and representatives of nine additional araneoid families, using approximately 2.5 kb corresponding to fragments of three nuclear genes (Histone 3, 18SrDNA, and 28SrDNA) and two mitochondrial genes (16SrDNA and Col). Several methods for incorporating indel information into the phylogenetic analysis are explored, and partition support for the different clades and sensitivity of the results to different assumptions of the analysis are examined as well. Our results marginally support theridiid monophyly, although the phylogenetic structure of the outgroup is unstable and largely contradicts current phylogenetic hypotheses based on morphological data. Several groups of theridiids receive strong support in most of the analyses: latrodectines, argyrodines, hadrotarsines, a revised version of spintharines and two clades including all theridiids without trace of a colulus and those without colular setae. However, the interrelationships of these clades are sensitive to data perturbations and changes in the analysis assumptions.

Barker, F. K. (2004). "Monophyly and relationships of wrens (Aves: Troglodytidae): a congruence analysis of heterogeneous mitochondrial and nuclear DNA sequence data." Molecular Phylogenetics and Evolution **31**(2): 486.

<http://www.sciencedirect.com/science/article/B6WNH-49NVGDS-7/2/be744d24eafd71e40b03b7e0806e4e3a>

The wrens (Aves: Troglodytidae) are a group of primarily New World insectivorous birds, the

monophyly of which has long been recognized, but whose intergeneric relationships are essentially unknown. In order to test the monophyly of the group, and to attempt to resolve relationships among genera within it, sequences from the mitochondrial cytochrome b gene and the fourth intron of the nuclear [beta]-fibrinogen gene were obtained from nearly all genera of wrens, from their relatives as suggested by traditional taxonomy and DNA-DNA hybridization analyses, and from additional passerines. Maximum likelihood analysis of the two data sets yielded maximal congruence between independently derived estimates of relationship, outperforming a variety of weighted parsimony methods. Hierarchical likelihood ratio tests indicated that the two gene regions differed significantly in every estimated parameter of sequence evolution, and combined analysis of the two data sets was accomplished using a heterogeneous-model Bayesian approach. Independent and simultaneous analyses of both data sets supported monophyly of the wrens (excluding one recently added member, the monotypic genus *Donacobius*) and a sister-group relationship between wrens and the gnatcatchers (*Poliophtila*). Additionally, strong support was found for paraphyly of the genus *Thryothorus*, and for a sister-group relationship between the genera *Cistothorus* and *Troglodytes*. Analyses of these data failed to resolve basal relationships within wrens, possibly due to ambiguity in rooting with a distant, species-poor outgroup. Analysis of the combined data for wrens alone yielded results which were largely congruent with relationships inferred using the complete data set, with the benefit of stronger support for relationships within the group. However, alternative rootings of this ingroup tree were weakly supported by nucleotide substitution data. Insertion-deletion events suggest that the genus *Salpinctes* may be sister to all other wrens.

Bickham, J. W., J. C. Patton, et al. (2004). "Molecular phylogenetics, karyotypic diversity, and partition of the genus *Myotis* (Chiroptera: Vespertilionidae)." Molecular Phylogenetics and Evolution **33**(2): 333.

<http://www.sciencedirect.com/science/article/B6WNH-4D2WMC3-4/2/68117290843c09cfbb228e5e217634af>

Nucleotide sequences of the mitochondrial cytochrome b gene are reported from bats of the genus *Myotis* including species of the endemic southern African subgenus *Cistugo*, *Myotis* (*Cistugo*) *sebrai* and *Myotis* (*Cistugo*) *lesueuri*. We also examined *Myotis* annectans from Southeast Asia, and *Myotis* *macropus* from Australia. The two species of *Cistugo* and *Myotis* annectans represent the only species of *Myotis* to differ in chromosome number from the common $2n = 44$ found in >40 species. Our results show that the two species of *Cistugo* are more divergent from the other species of *Myotis* than several other well-recognized genera and we recommend elevating *Cistugo* to full generic rank. *Myotis* annectans groups well within *Myotis*, clustering with other Southeast Asian and Japanese species, and thus represents the only species of *Myotis* known to have diverged from the common $2n = 44$ karyotype. *Myotis* *macropus* clusters within a clade that includes Southeast Asian species.

Burger, J., W. Rosendahl, et al. (2004). "Molecular phylogeny of the extinct cave lion *Panthera leo spelaea*." Molecular Phylogenetics and Evolution **30**(3): 841.

<http://www.sciencedirect.com/science/article/B6WNH-49S7XSK-2/2/282c38530dc3d082751bc69ab73bac42>

To reconstruct the phylogenetic position of the extinct cave lion (*Panthera leo spelaea*), we sequenced 1 kb of the mitochondrial cytochrome b gene from two Pleistocene cave lion DNA samples (47 and 32 ky B.P.). Phylogenetic analysis shows that the ancient sequences form a clade that is most closely related to the extant lions from Africa and Asia; at the same time, cave lions appear to be highly distinct from their living relatives. Our data show that these cave lion

sequences represent lineages that were isolated from lions in Africa and Asia since their dispersal over Europe about 600 ky B.P., as they are not found among our sample of extant populations. The cave lion lineages presented here went extinct without mitochondrial descendants on other continents. The high sequence divergence in the cytochrome b gene between cave and modern lions is notable.

Calcagnotto, D., S. A. Schaefer, et al. "Relationships among characiform fishes inferred from analysis of nuclear and mitochondrial gene sequences." *Molecular Phylogenetics and Evolution In Press, Corrected Proof* <http://www.sciencedirect.com/science/article/B6WNH-4FDJRVG-2/2/c9c2b46cb8831c6e1aa1da4a1fdd2e49>

Suprafamilial relationships among characiform fishes and implications for the taxonomy and biogeographic history of the Characiformes were investigated by parsimony analysis of four nuclear and two mitochondrial genes across 124 ingroup and 11 outgroup taxa. Simultaneous analysis of 3660 aligned base pairs from the mitochondrial 16S and cytochrome b genes and the nuclear recombination activating gene (RAG2), seven in absentia (sia), forkhead (fkh), and [alpha]-tropomyosin (trop) gene loci confirmed the non-monophyly of the African and Neotropical assemblages and corroborated many suprafamilial groups proposed previously on the basis of morphological features. The African distichodontids plus citharinids were strongly supported as a monophyletic Citharinoidei that is the sistergroup to all other characiforms, which form a monophyletic Characoidei composed of two large clades. The first represents an assemblage of both African and Neotropical taxa, wherein a monophyletic African Alestidae is sister to a smaller clade comprised of the Neotropical families Ctenolucidae, Lebiasinidae, and the African Hepsetidae, with that assemblage sister to a strictly Neotropical clade comprised of the Crenuchidae and Erythrinidae. The second clade within the Characoidei is strictly Neotropical and includes all other Characiformes grouped into two well supported major clades. The first, corresponding to a traditional definition of the Characidae, is congruent with some groupings previously supported by morphological evidence. The second clade comprises a monophyletic Anostomoidea that is sister to a clade formed by the families Hemiodontidae, Parodontidae, and Serrasalminidae, with that assemblage, in turn, the sistergroup of the Cynodontidae. Serrasalminidae, traditionally regarded as a subfamily of Characidae, was recovered as the sistergroup of (Anostomoidea (Parodontidae + Hemiodontidae)) and the family Cynodontidae was recovered with strong support as the sistergroup to this assemblage. Our results reveal three instances of trans-continental sistergroup relationships and, in light of the fossil evidence, suggest that marine dispersal cannot be ruled out a priori and that a simple model of vicariance does not readily explain the biogeographic history of the characiform fishes.

Carson, R. J. and G. S. Spicer (2003). "A phylogenetic analysis of the emberizid sparrows based on three mitochondrial genes." *Molecular Phylogenetics and Evolution* **29**(1): 43.

<http://www.sciencedirect.com/science/article/B6WNH-48KFF3P-2/2/47bc8c274a60106c3dba8f8504a28f5f>

Previous molecular phylogenetic studies have examined the taxonomic relationships among a number of typical emberizid sparrow genera. To help clarify these relationships, we sequenced a 1673 base pair fragment for the complete sequence of three mitochondrial genes: adenosine triphosphatase (Atp8 and Atp6) and cytochrome oxidase subunit III (COIII) for 38 sparrow species, along with *Passerina amoena* (Cardinalidae) and *Piranga ludoviciana* (Thraupidae) which were selected as the outgroups. Our analysis confirms the monophyly of traditional genera such as *Junco*, *Melospiza*, and *Zonotrichia*. Although *Calcarius* and *Plectrophenax* are often thought to be putative emberizids, all our analyses placed these genera basal to all other sparrows examined. As observed with *Calcarius*, *Spizella* did not form a monophyletic group, with

S. arborea being the sister-taxon to *Passerella iliaca*. Our analyses also suggest that *Aimophila ruficeps* is probably more closely related to the "brown towhees" (*Pipilo aberti*, *P. crissalis*, and *P. fuscus*) than its putative congeners. The genus *Ammodramus* was also not monophyletic, since it appears that *Passerculus sandwichensis* is more closely related to *A. henslowii* and *A. leconteii* than either one is related to its congener *A. savannarum*. Finally, our analyses exhibited other unsuspected associations, such as the sister-taxon relationships between *Amphispiza bilineata* and the *Chondestes grammacus/Calamospiza melanocorys* clade, and *Amphispiza belli* and *Pooecetes gramineus*.

Cheverson, Z. A., S. J. Hackett, et al. "Complex evolutionary history of a Neotropical lowland forest bird (*Lepidothrix coronata*) and its implications for historical hypotheses of the origin of Neotropical avian diversity." Molecular Phylogenetics and Evolution **In Press, Corrected Proof**
<http://www.sciencedirect.com/science/article/B6WNH-4FMBK98-4/2/dd9ecca5ecd445b7ad6894c87e554024>

Here we apply a combination of phylogeographic and historical demographic analyses to the study of mtDNA sequence variation within the Blue-crowned Manakin (*Lepidothrix coronata*), a widespread Neotropical bird. A high degree of phylogeographic structure allowed us to demonstrate that several vicariant events, including Andean uplift, the formation of riverine barriers, and climatically induced vegetational shifts, as well as a non-vicariant process, range expansion, have all acted, at varying spatial and temporal scales, to influence genetic structure within *L. coronata*, suggesting that current historical hypotheses of the origin of Neotropical avian diversity that focus on single vicariant mechanisms may be overly simplistic. Our data also support an origin (>2 mybp) that is substantially older than the late Pleistocene for the genetic structure within this species and indicate that phylogeographic patterns within the species are not concordant with plumage-based subspecific taxonomy. These data add to a growing body of evidence suggesting that the origin of several Neotropical avian species may have occurred in the mid-Pliocene, thus, geological arguments surrounding putative Pleistocene vicariant events, while interesting in their own right, may have little relevance to Neotropical avian diversification at the species level.

Cortes-Ortiz, L., E. Bermingham, et al. (2003). "Molecular systematics and biogeography of the Neotropical monkey genus, *Alouatta*." Molecular Phylogenetics and Evolution **26**(1): 64.
<http://www.sciencedirect.com/science/article/B6WNH-4783K6K-1/2/047833090815bf05218ec4905e7105c8>

Despres, L., L. Gielly, et al. (2003). "Using AFLP to resolve phylogenetic relationships in a morphologically diversified plant species complex when nuclear and chloroplast sequences fail to reveal variability." Molecular Phylogenetics and Evolution **27**(2): 185.
<http://www.sciencedirect.com/science/article/B6WNH-47TNSB8-2/2/67e6e755288629e3efff2e3d9290e75a>

Inferring phylogenetic relationships among closely related plant species is often difficult due to the lack of molecular markers exhibiting enough nucleotide variability at this taxonomic level. Moreover, gene tree does not necessarily represent the true species tree because of random sorting of polymorphic alleles in different lineages. A solution to these problems is to use many amplified fragment length polymorphisms (AFLP) distributed throughout the whole genome, to

infer cladistic and phenetic among-species relationships. Phylogenetic relationships among interfertile species of *Trollius* L. (Ranunculaceae) were investigated using nuclear DNA (ITS1 + 5.8S rRNA + ITS2) and chloroplast DNA (trnL intron and trnL-trnF intergene spacer) sequences, and AFLP markers. ITS sequences were not informative at the intrageneric level, but confirmed the sister relationship between *Trollius* and *Adonis* genera, and provided new information on the phylogenetic relationships among five Ranunculaceae genera. Chloroplast DNA was more informative among *Trollius* species, but not consistent with the sections previously described. AFLP proved to be a powerful tool to resolve the complex genetic relationships between the morphological entities constituting the genus *Trollius*. Although as much as 76.1% of the total AFLP variability was found within a priori defined morphological groups, the remaining 23.9% variability differentiating groups was sufficient to generate congruent and robust cladistic and phenetic trees. Several morphological traits, independent from those used to define groups, were mapped onto the molecular phylogeny, and their evolution discussed in relation to the absence/presence of pollinator-seed parasite *Chiastocheta* flies.

Dick, M. H., A. Herrera-Cubilla, et al. (2003). "Molecular phylogeny and phylogeography of free-living Bryozoa (Cupuladriidae) from both sides of the Isthmus of Panama." Molecular Phylogenetics and Evolution **27**(3): 355.

<http://www.sciencedirect.com/science/article/B6WNH-4840SR4-8/2/d39c006a1bb2682e98390aaa90427967>

Genetic data were used to identify Recent species of free-living bryozoans (Cupuladriidae) from both sides of the Isthmus of Panama, and to examine their phylogenetic relationships, species richness, and population structures. An approximately 480 bp fragment of the 16S mitochondrial rRNA gene was sequenced from 182 individuals from Panama, the Gulf of Mexico, and El Salvador. Ten haplotype groups (Cupuladria 4, 5, and 6; Discoporella 1, 2, 3A, 3B, 3C, 7, and 8) were identified. Genetic distances between haplotype groups (3.2-26.5%; K2P + [Gamma]) were 1-2 orders of magnitude greater than within groups (0.1-1.4%). Seven of the haplotype groups represent morphologically distinct species; Discoporellas 3A-C appear to be cryptic species. Phylogenetic analyses identified two pairs of transisthmian sister clades. An average divergence rate derived from other taxa suggests that Cupuladrias 4 and 5 diverged [ap]7 Ma, a Discoporella 7 clade diverged from a 3A-C clade [ap]11 Ma, and the 3A-C clade radiated [ap]6-4 Ma; these events all predated final closure of the isthmus? 3 Ma. The Caribbean side of the isthmus, with 5 species, is only marginally richer in cupuladriids than the Pacific side, with 4, but has greater phylogenetic depth. The Caribbean retains lineages stemming from a New World Miocene radiation that are not represented in the eastern Pacific; extant eastern Pacific cupuladriids share most recent common ancestry with only two of the Caribbean lineages. Species in the eastern Pacific tend to show shallow population structures, with high levels of gene flow between geographically separate populations, whereas Caribbean species tend to show deeper populations structures, with indications of restricted gene flow between Bocas del Toro/Gulf of Mosquitos and Costa Arriba/San Blas. The population structures derive from Pleistocene histories and may be of limited value in interpreting the macroevolutionary pattern, as our results provide no evidence of speciation on either side of the isthmus following closure in the late Pliocene.

Eberhard, J. R. and E. Bermingham "Phylogeny and comparative biogeography of Pionopsitta parrots and Pteroglossus toucans." Molecular Phylogenetics and Evolution **In Press, Corrected Proof**
<http://www.sciencedirect.com/science/article/B6WNH-4FR3NDB-1/2/29c3a5ca15b05b74361027e4b751f9cf>

Studies of Neotropical birds, and their distributions and areas of endemism, in particular, have been central in the formulation of hypotheses proposed to explain the high species diversity in the

Neotropics. We used mtDNA sequence data (ATPase 6 and 8, COI, and cyt b) to reconstruct the species-level phylogenies for two genera, *Pionopsitta* (Aves: Psittacidae) and *Pteroglossus* (Aves: Ramphastidae), compare our results with previous morphology-based phylogenetic analyses, and estimate the absolute timing of lineage and biogeographic divergences. Both the *Pionopsitta* and *Pteroglossus* phylogenies support a hypothesis of area relationships in which a divergence of the Serra do Mar (Atlantic Forest, Brazil) region of endemism is followed by the divergence of cis- and trans-Andean regions, then a split between the upper and lower Amazon basin, next the divergence of the Guyana area, and finally diversification of taxa in the upper Amazon basin's areas of endemism. Phylogenies of both genera support a hypothesis of area relationships that is similar to that proposed by Prum [XIX International Ornithological Congress (1988), 2562] for high-vagility species, but while they agree on the relative timing of area divergence (vicariance) events, they yield different absolute time estimates for those divergences when the typical avian mtDNA clock calibration is used. Taken at face value, the time estimates indicate that both genera began to diversify before the start of the Pleistocene, and that climatic and habitat shifts alone do not account for the diversification of these taxa.

Garza, J. C. and D. S. Woodruff (1992). "A phylogenetic study of the gibbons (*Hylobates*) using DNA obtained noninvasively from hair." Molecular Phylogenetics and Evolution 1(3): 202.

<http://www.sciencedirect.com/science/article/B6WNH-4DXK9Y9-4/2/8c14e9413b8620da324e88fa78947692>

Variation in a 252-nucleotide segment of the cytochrome b gene from 26 gibbons is described. DNA was extracted from hair, amplified, and directly sequenced. These sequences represent seven of the nine nominal species and three of the four hylobatid subgenera. Variation was observed at 55 sites, 42 of which are phylogenetically informative. Levels of transitional and transversal divergence between the taxa are similar to those reported for homologous mtDNA sequences in other mammals. Parsimony, maximum likelihood, and bootstrap analyses (1) support some traditional phylogenetic hypotheses (monophyly of the concolor gibbons), (2) suggest previously unrecognized affinities between the *lar* species group and *Hylobates klossi* and between *H. lar* and *H. agilis unko*, and (3) show that this segment does not contain information sufficient for completely resolving gibbon relationships at the subgeneric level. The study demonstrates the great potential of noninvasive DNA sampling for phylogenetic analyses of mammals.

Gentile, A., M. S. Rossi, et al. (2005). "Origin, divergence, and phylogeny of epichloe endophytes of native Argentine grasses." Molecular Phylogenetics and Evolution 35(1): 196.

<http://www.sciencedirect.com/science/article/B6WNH-4FFH20D-2/2/f8d7492acf2a57c846109cc68cb80970>

The epichloe endophytes are systemic, constitutive, and often vertically transmitted fungal symbionts of grass species in subfamily Pooideae. Prior studies indicate that several asexual epichloe endophytes (*Neotyphodium* species) have evolved directly from sexual (*Epichloe*) species, whereas others evolved by hybridization between two or more endophyte species. In this paper, we investigate the phylogenies of 27 *Neotyphodium* spp. isolates from 10 native grass species (in 4 tribes) in 22 populations throughout Argentina. Relationships among these fungi and a worldwide collection of epichloe endophytes were estimated by phylogenetic analysis of sequences from variable portions (mainly introns) of genes for [beta]-tubulin (*tub2*) and translation elongation factor 1-[alpha] (*tef1*). Most of the Argentine endophyte isolates were interspecific hybrids of *Epichloe festucae* and *E. typhina*. Only one isolate was a hybrid of a different ancestry, and three isolates were apparently non-hybrid endophytes. These results indicate that

interspecific hybridization, which promotes genetic variation, was common during the evolution of the endophytes of Argentine grasses.

Gomez-Zurita, J. (2004). "Molecular systematics and time-scale for the evolution of *Timarcha*, a leaf-beetle genus with a disjunct Holarctic distribution." Molecular Phylogenetics and Evolution **32**(2): 647.

<http://www.sciencedirect.com/science/article/B6WNH-4C2NKD5-1/2/91844db8219e55d1830a8d405d8525e0>

In recent years we have investigated the evolution of the Holarctic leaf-beetle genus *Timarcha* using molecular approaches, but to date several important questions remained unanswered, including its systematic arrangement in a temporal context, or the phylogenetic placement of the Nearctic taxa. Here I present a reanalysis of available genetic data together with newly generated data for key taxa (markers 16S rDNA, CO2, ITS-2, and 18S rDNA), including the Nearctic species (subgenus *Americanotimarcha*), using direct optimization-based phylogenetic reconstructions. Lineage ages are estimated using maximum likelihood branch-length estimates and the molecular clock calibration derived from several presumed vicariance events in the Mediterranean. Phylogenetic analyses and 18S rDNA divergences suggest the ancient divergence of the Nearctic and Palaearctic lineages, related to the North Atlantic opening in the middle Eocene. The diversification of the Palaearctic *Timarcha* seems closely related to the geological evolution of the Mediterranean area during the Tertiary, with Pleistocene climate changes affecting species ranges and lineage extinction, but not resulting in extensive speciation.

Hay, J. M., C. H. Daugherty, et al. (2003). "Low genetic divergence obscures phylogeny among populations of *Sphenodon*, remnant of an ancient reptile lineage." Molecular Phylogenetics and Evolution **29**(1): 1.

<http://www.sciencedirect.com/science/article/B6WNH-48Y069B-1/2/2e37be624691a1f854385553d1a4aa1d>

Tuatara (two species of *Sphenodon*) are the last representatives of a branch of an ancient reptilian lineage, *Sphenodontia*, that have been isolated on the New Zealand landmass for 82 million years. We present analyses of geographic variation in allozymes, mitochondrial DNA, nuclear DNA sequences, and one-way albumin immunological comparisons. These all confirm a surprisingly low level of genetic diversity within *Sphenodon* for such an ancient lineage. We hypothesise a recent extended population bottleneck, probably during the Pliocene/Pleistocene glaciation cycles, to explain the current paucity of variation. All data sets reveal clear genetic differentiation between the northern populations and those in Cook Strait, but offer conflicting views of the history and taxonomic relationships of the Cook Strait population on North Brother Island, currently recognised as *Sphenodon guntheri*. Allozymes show this population to be the most divergent of all tuatara populations, but preliminary mitochondrial DNA data indicate few differences between *S. guntheri* and Cook Strait *Sphenodon punctatus*. Interpretation of the trees is confounded by the lack of a suitable outgroup. As in other cases of conflicting nuclear and mitochondrial data sets, the different data sets likely reveal different aspects of the animals' evolutionary history, and introgression is not uncommon between species pairs.

Hyvonen, J., S. Koskinen, et al. (2004). "Phylogeny of the Polytrichales (Bryophyta) based on simultaneous analysis of molecular and morphological data." Molecular Phylogenetics and

Evolution **31**(3): 915.

<http://www.sciencedirect.com/science/article/B6WNH-4BBMT6T-1/2/bc37a00303f555e9b77f997d9cc9bc11>

Phylogenetic analyses of Polytrichales were conducted using morphology and sequence data from the chloroplast genes *rbcL* and *rps4* plus the *trnL-F* gene region, part of the mitochondrial *nad5* and the nuclear-encoded 18S rDNA. Our analyses included 46 species representing all genera of Polytrichales. Phylogenetic trees were constructed with simultaneous parsimony analyses of all sequences plus morphology and separate combinations of sequence data only. Results lend support for recognition of Polytrichales as a monophyletic entity. *Oedipodium griffithianum* appears as a sister taxon to Polytrichales or as a sister taxon of all mosses excluding Sphagnales and Andreaeales. Within Polytrichales, *Alophosia* and *Atrichopsis*, species without the adaxial lamellae (in *Atrichopsis* present but poorly developed on male gametophyte) otherwise typical of the group are sister to the remaining species followed by a clade including *Bartramiopsis* and *Lyellia*, species with adaxial lamellae covering only the central portion of the leaves. Six taxa with an exclusively Southern Hemisphere distribution form a grade between the basal lineages and a clade including genera that are mostly confined to the Northern Hemisphere.

Joseph, L., T. Wilke, et al. (2004). "Towards a phylogenetic framework for the evolution of shakes, rattles, and rolls in *Myiarchus* tyrant-flycatchers (Aves: Passeriformes: Tyrannidae)." Molecular Phylogenetics and Evolution **31**(1): 139.

<http://www.sciencedirect.com/science/article/B6WNH-49CN2XB-5/2/49a692ddb4c88198c0a1971faf9dd4aa>

A phylogeny of 19 of the 22 currently recognized species of *Myiarchus* tyrant-flycatchers is presented. It is based on 842 bp of mitochondrial DNA (mtDNA) sequences from the ATPase subunit 8 and ATPase subunit 6 genes. Except for the morphologically distinct *M. semirufus*, mtDNAs of the remaining 18 species fall into either of two clades. One comprises predominantly Caribbean and Central and North American taxa (Clade I), and the other is of predominantly South American taxa (Clade II). The phylogeny is only very broadly concordant with some vocal characters and also with the limited morphological diversity for which the group is well known. Paraphyly in several species (*M. swainsoni*, *M. tuberculifer*, *M. ferox*, *M. phaeocephalus*, *M. sagrae*, *M. stolidus*) suggests that morphological evolution, albeit resulting in limited morphological diversity, has been more rapid than that of mtDNA, or that current taxonomy is faulty, or both. A South American origin for *Myiarchus* is likely. Dispersal and vicariance both appear to have been involved in generating the present-day distribution of some species. Relatively recent dispersal events out of South America are inferred to have brought species of Clades I and II into broad sympatry. Jamaica has been colonized independently at least twice by members of Clades I and II. The phylogeny brings a historical perspective that in turn suggests that ecological study of closely related species from within each major clade where they are sympatric will be especially rewarding.

Kizirian, D., A. Trager, et al. (2004). "Evolution of Galapagos Island Lava Lizards (Iguania: Tropiduridae: Microlophus)." Molecular Phylogenetics and Evolution **32**(3): 761.

<http://www.sciencedirect.com/science/article/B6WNH-4CHRY76-1/2/6e102df9ebc94bffc074ddb3d459d03>

Nucleotide sequences of mitochondrial genes (ND1, ND2, COI, and tRNAs) were determined for 38 samples representing 15 taxa of tropidurid lizards from the Galapagos Islands and mainland South America. Phylogenetically informative characters (759 of 1956) were analyzed under Bayesian, maximum likelihood, and parsimony frameworks. This study supports the hypothesis that tropidurid lizards dispersed to the Galapagos on at least two separate occasions. One dispersal event involved an eastern Galapagos clade (*Microlophus habelii* and *M. bivittatus*, on Marchena and San Cristobal islands, respectively) the sister taxon of which is *M. occipitalis* from coastal Ecuador and Peru; the closest mainland relative of the western Galapagos clade was not unambiguously identified. The wide-ranging *M. albemarlensis* is revealed to be a complex of weakly divergent lineages that is paraphyletic with respect to the insular species *M. duncanensis*, *M. grayii*, and *M. pacificus*.

Lavoue, S. and J. P. Sullivan (2004). "Simultaneous analysis of five molecular markers provides a well-supported phylogenetic hypothesis for the living bony-tongue fishes (Osteoglossomorpha: Teleostei)." Molecular Phylogenetics and Evolution **33**(1): 171.

<http://www.sciencedirect.com/science/article/B6WNH-4CPVM0R-1/2/ceac61e394db4e7fae7f4c459ec66f4a>

Fishes of the Superorder Osteoglossomorpha (the "bonytongues") constitute a morphologically heterogeneous group of basal teleosts, including highly derived subgroups such as African electric fishes, the African butterfly fish, and Old World knifefishes. Lack of consensus among hypotheses of osteoglossomorph relationships advanced during the past 30 years may be due in part to the difficulty of identifying shared derived characters among the morphologically differentiated extant families of this group. In this study, we present a novel phylogenetic hypothesis for this group, based on the analysis of more than 4000 characters from five molecular markers (the mitochondrial cytochrome b, 12S and 16S rRNA genes, and the nuclear genes RAG2 and MLL). Our taxonomic sampling includes one representative of each extant non-mormyrid osteoglossomorph genus, one representative for the monophyletic family Mormyridae, and four outgroup taxa within the basal Teleostei. Maximum parsimony analysis of combined and equally weighted characters from the five molecular markers and Bayesian analysis provide a single, well-supported, hypothesis of osteoglossomorph interrelationships and show the group to be monophyletic. The tree topology is the following: (Hiodon alosoides, (Pantodon buchholzi, (((Osteoglossum bicirrhosum, Scleropages sp.), (Arapaima gigas, Heterotis niloticus))), ((Gymnarchus niloticus, Ivindomyrus opdenboschi), ((Notopterus notopterus, Chitala ornata), (Xenomystus nigri, Papyrocranus afer))))). We compare our results with previously published phylogenetic hypotheses based on morpho-anatomical data. Additionally, we explore the consequences of the long terminal branch length for the taxon *Pantodon buchholzi* in our phylogenetic reconstruction and we use the obtained phylogenetic tree to reconstruct the evolutionary history of electroreception in the Notopteroidei.

Liu, H. and A. T. Beckenbach (1992). "Evolution of the mitochondrial cytochrome oxidase II gene among 10 orders of insects." Molecular Phylogenetics and Evolution **1**(1): 41.

<http://www.sciencedirect.com/science/article/B6WNH-4DYVPKS-6/2/d24d2bdf5b50478cb857053f28947e5d>

We examine the complete nucleotide sequences of the mitochondrial cytochrome oxidase II gene of 13 species of insects, representing 10 orders. The genes range from 673 to 690 by in length, encoding 226 to 229 amino acids. Several insertion or deletion events, each involving one or two codons, can be observed. The 3' end of the gene is extremely variable in both length and sequence, making alignment of the ends unreliable. Using the first 639 nucleotide positions, for

which unambiguous alignments could be obtained, we examine the neighbor-joining trees based on nucleotide divergences and based on conserved subsets of that data, including transversion and amino acid and second codon position divergences. Each of these subsets produces different trees, none of which can be easily reconciled with trees constructed using morphology and the fossil record. Bootstrap analysis using second codon positions strongly supports affinities between the order Blattaria (cockroaches) and the order Isoptera (termites) and between a wasp and the published honeybee sequence (Order Hymenoptera). The divergence of insect orders is very ancient and may have occurred too rapidly for easy resolution using mitochondrial protein sequences. Unambiguous resolution of insect orders will probably require analysis of many additional taxa, using the COII gene and other conserved sequences.

Luo, J., D. Yang, et al. (2004). "Molecular phylogeny and biogeography of Oriental voles: genus *Eothenomys* (Muridae, Mammalia)." Molecular Phylogenetics and Evolution **33**(2): 349.

<http://www.sciencedirect.com/science/article/B6WNH-4CYWM12-4/2/3f623876e95346a6ddf5416c49aede69>

Oriental voles of the genus *Eothenomys* are predominantly distributed along the Southeastern shoulder of the Qinghai-Tibetan Plateau. Based on phylogenetic analyses of the mitochondrial cytochrome b gene (1143 bp) obtained from 23 specimens (eight species) of Oriental voles collected from this area, together with nucleotide sequences from six specimens (two species) of Japanese red-backed voles (*Eothenomys andersoni* and *Eothenomys smithii*) and five species of the closely related genus *Clethrionomys*, we revised the systematic status of *Eothenomys*. We also tested if vicariance could explain the observed high species diversity in this area by correlating estimated divergence times to species distribution patterns and corresponding paleogeographic events. Our results suggest that: (1) the eight species of Oriental voles form a monophyletic group with two distinct clades, and that these two clades should be considered as valid subgenera--*Eothenomys* and *Antelionomys*; (2) *Eothenomys eleusis* and *Eothenomys miletus* are not independent species; (3) Japanese red-backed voles are more closely related to the genus *Clethrionomys* than to continental Asian *Eothenomys* taxa; and (4) the genus *Clethrionomys*, as presently defined, is paraphyletic. In addition, the process of speciation of Oriental voles appears to be related to the Trans-Himalayan formation via three recent uplift events of the Qinghai-Tibetan Plateau within the last 3.6 million years, as well as to the effects of the mid-Quaternary ice age.

Mansion, G. and L. Struwe (2004). "Generic delimitation and phylogenetic relationships within the subtribe Chironiinae (Chironieae: Gentianaceae), with special reference to *Centaurium*: evidence from nrDNA and cpDNA sequences." Molecular Phylogenetics and Evolution **32**(3): 951.

<http://www.sciencedirect.com/science/article/B6WNH-4CJ46KB-2/2/923b3a969f96b65bf0dced47cc7d4e7e>

To better understand the evolutionary history of the genus *Centaurium* and its relationship to other genera of the subtribe Chironiinae (Gentianaceae: Chironieae), molecular analyses were performed using 80 nuclear ribosomal ITS and 76 chloroplast trnLF (both the trnL UAA intron and the trnL-F spacer) sequences. In addition, morphological, palynological, and phytochemical characters were included to a combined data matrix to detect possible non-molecular synapomorphies. Phylogenetic reconstructions support the monophyly of the Chironiinae and an age estimate of ca. 22 million years for the subtribe. Conversely, both molecular data sets reveal a polyphyletic *Centaurium*, with four well-supported main clades hereafter treated as separate genera. The primarily Mediterranean *Centaurium* s.s. is closely related to southern African endemics *Chironia* and *Orphium*, and to the Chilean species *Centaurium cachanlahuen*. The

resurrected Mexican and Central American genus *Gyrandra* is closely related to *Sabatia* (from eastern North America). Lastly, the monospecific genus *Exaculum* (Mediterranean) forms a monophyletic group together with the two new genera: *Schenkia* (Mediterranean and Australian species) and *Zeltnera* (all other indigenous American centauries). Several biogeographical patterns can be inferred for this group, supporting a Mediterranean origin followed by dispersals to (1) North America, Central America, and South America, (2) southern Africa (including the Cape region), and (3) Australia and Pacific Islands.

Markos, S. and B. G. Baldwin (2002). "Structure, molecular evolution, and phylogenetic utility of the 5' region of the external transcribed spacer of 18S-26S rDNA in *Lessingia* (Compositae, Astereae)." Molecular Phylogenetics and Evolution **23**(2): 214.

<http://www.sciencedirect.com/science/article/B6WNH-4627PGJ-B/2/8c6d9b365e492fbd04bc620aeddc37>

Marks, B. D., S. J. Hackett, et al. (2002). "Historical relationships among Neotropical lowland forest areas of endemism as determined by mitochondrial DNA sequence variation within the Wedge-billed Woodcreeper (Aves: Dendrocolaptidae: *Glyphorynchus spirurus*)." Molecular Phylogenetics and Evolution **24**(1): 153.

<http://www.sciencedirect.com/science/article/B6WNH-4698TKT-G/2/0fb9bf50aed09e6c4f9518016b4fb7e0>

Maxmen, A. B., B. F. King, et al. (2003). "Evolutionary relationships within the protostome phylum Sipuncula: a molecular analysis of ribosomal genes and histone H3 sequence data." Molecular Phylogenetics and Evolution **27**(3): 489.

<http://www.sciencedirect.com/science/article/B6WNH-4894317-1/2/14a8677a2ae2e3967cd007c7f17b3092>

The phylogenetic relationships of the members of the phylum Sipuncula are investigated by means of DNA sequence data from three nuclear markers, two ribosomal genes (18S rRNA and the D3 expansion fragment of 28S rRNA), and one protein-coding gene, histone H3. Phylogenetic analysis via direct optimization of DNA sequence data using parsimony as optimality criterion is executed for 12 combinations of parameter sets accounting for different indel costs and transversion/transition cost ratios in a sensitivity analysis framework. Alternative outgroup analyses are also performed to test whether they affected rooting of the sipunculan topology. Nodal support is measured by parsimony jackknifing and Bremer support values. Results from the different partitions are highly congruent, and the combined analysis for the parameter set that minimizes overall incongruence supports monophyly of Sipuncula, but nonmonophyly of several higher taxa recognized for the phylum. Mostly responsible for this is the split of the family Sipunculidae in three main lineages, with the genus *Sipunculus* being the sister group to the remaining sipunculans, the genus *Phascolopsis* nesting within the *Golfingiiformes*, and the genus *Siphonosoma* being associated to the *Phascolosomatidea*. Other interesting results are the position of *Phascolion* within *Golfingiidae* and the position of *Antillesoma* within *Aspidosiphonidae*. These results are not affected by the loci selected or by the outgroup chosen. The position of *Apionsoma* is discussed, although more data would be needed to better ascertain its phylogenetic affinities. Monophyly of the genera with multiple representatives (*Themiste*, *Aspidosiphon*, and *Phascolosoma*) is well supported, but not the monophyly of the genera

Nephasoma or Golfingia. Interesting phylogeographic questions arise from analysis of multiple representatives of a few species.

Morris, D. C., M. P. Schwarz, et al. (2002). "Phylogenetics of Australian Acacia thrips: the evolution of behaviour and ecology." Molecular Phylogenetics and Evolution **25**(2): 278.

<http://www.sciencedirect.com/science/article/B6WNH-470M3Y9-1/2/79ecef87b39a47604f03bfa1025d1fb>

The species of thrips found on Acacia constitute a major component of the Australian thrips fauna, with at least 235 species in more than 30 genera, many of these being in the process of description as new. These thrips exhibit social behaviours, ranging from solitary and colonial species to a variety of more complex social organisations. Furthermore, the domiciliary habits of these species include domicile construction, gall induction, and opportunistic use of abandoned galls and domiciles. This suite of thrips also includes a variety of inquiline and kleptoparasitic taxa. To understand how these various traits have evolved and interact in this diverse group, we have reconstructed a phylogeny for 42 species of thrips associated with Acacia around Australia. We obtained DNA sequence data from two nuclear genes (Elongation Factor-1[alpha] and wingless) and one mitochondrial gene (cytochrome oxidase I) and analysed these using maximum parsimony and maximum likelihood methods. A phylogeny resulting from such analysis allows inference of evolutionary transitions in domiciliary habits, social organisations, and parasitic behaviours. Gall induction and parasitic behaviour are postulated to each have a single origin, with no losses of either trait. Once parasitism evolved a remarkable radiation followed that allowed exploitation of very diverse hosts. Our data do not allow hypotheses of single versus multiple origins of domicile building to be resolved while opportunistic gall use appears to have arisen several times.

Morrison, C. L., R. Rios, et al. (2004). "Phylogenetic evidence for an ancient rapid radiation of Caribbean sponge-dwelling snapping shrimps (Synalpheus)." Molecular Phylogenetics and Evolution **30**(3): 563.

<http://www.sciencedirect.com/science/article/B6WNH-49CN2XB-2/2/d5feac373acf096efc1b2459d5287e21>

A common challenge in reconstructing phylogenies involves a high frequency of short internal branches, which makes basal relationships difficult to resolve. Often it is not clear whether this pattern results from insufficient or inappropriate data, versus from a rapid evolutionary radiation. The snapping shrimp genus Synalpheus, which contains in excess of 100 species and is a prominent component of coral-reef faunas worldwide, provides an example. Its taxonomy has long been problematic due to the subtlety of diagnostic characters and apparently widespread variability within species. Here we use partial mt COI and 16S rRNA sequences and morphological characters to reconstruct relationships among 31 species in the morphologically well-defined gambarelloides species group, a putative clade of obligate sponge associates that is mostly endemic to the Caribbean and contains the only known eusocial marine animals. Analysis of the combined data produced a single tree with good support for many terminal clades and for relationships with outgroups, but poor support for branches near the base of the gambarelloides group. Most basal branches are extremely short and terminal branches are long, suggesting a relatively ancient, but rapid radiation of the gambarelloides group. This hypothesis is supported by significant departure from a null model of temporally random cladogenesis. Calibration of divergence times among gambarelloides-group species using data from three geminate pairs of Synalpheus species separated by the isthmus of Panama suggests a major radiation between ~5 and 7 Mya, a few My before final closure of the Panamanian seaway during a period of spreading

carbonate environments in the Caribbean; a second, smaller radiation occurred ~4 Mya. This molecular evidence for a rapid radiation among Caribbean marine organisms in the late Miocene/early Pliocene is strikingly similar to patterns documented from fossil data for several other Caribbean reef-associated invertebrate taxa. The similar patterns and timing of cladogenesis evidenced by molecular and fossil data for different Caribbean and East Pacific taxa suggests that the radiation involved a wide range of organisms, and strengthens the case that poor basal resolution in the gambarelloides group of *Synalpheus* reflects a real evolutionary phenomenon. The rapid radiation also helps explain the historical difficulty of diagnosing species in *Synalpheus*.

Moulton, J. K. and B. M. Wiegmann (2004). "Evolution and phylogenetic utility of CAD (rudimentary) among Mesozoic-aged Eremoneuran Diptera (Insecta)." Molecular Phylogenetics and Evolution **31**(1): 363.

<http://www.sciencedirect.com/science/article/B6WNH-49M0ND6-3/2/08e25741cdd948f7c3fc576d336dd2c7>

We sequenced nearly the entire carbamoylphosphate synthase (CPS) domain of CAD, or rudimentary, (ca. 4 kb) from 29 species of flies representing all major clades within Eremoneura, or higher flies, and several orthorrhaphous brachyceran outgroups. We compared these sequences with orthologs from *Anopheles gambiae* and *Drosophila melanogaster* to assess structure, compositional bias, and phylogenetic utility. CAD is large (6.6+ kb), complex (comprised of three major and myriad minor functional domains) and relatively free of introns, extreme nucleotide bias (except third codon positions), and large hypervariable regions. The CPS domain possesses moderate levels of nonsynonymous divergence among taxa of intermediate evolutionary age and conveys considerable phylogenetic signal. Phylogenetic analysis of CPS sequences under varying methods and assumptions resulted in well-resolved, strongly supported trees concordant with many traditional ideas about higher dipteran phylogeny and with prior inferences from 28S rDNA. The most robustly supported major eremoneuran clades were Cyclorrhapha, Platypezoidea, Eumuscomorpha, Empidoidea, Atelestidae, Empidoidea exclusive of Atelestidae, Hybotidae s.l., Microphoridae + Dolichopodidae, and Empididae s. str. Because CAD is ubiquitous, apparently single copy (at least within holometabolous insects), readily obtained from several insect orders using primers described herein, and exhibits considerable phylogenetic utility, it should have wide applicability in insect molecular systematics.

Nichols, S. A. (2005). "An evaluation of support for order-level monophyly and interrelationships within the class Demospongiae using partial data from the large subunit rDNA and cytochrome oxidase subunit I." Molecular Phylogenetics and Evolution **34**(1): 81.

<http://www.sciencedirect.com/science/article/B6WNH-4DTKC5P-4/2/312b682cd042c5f188b175463f2f0487>

Large subunit ribosomal DNA (LSU rDNA) sequence data from 120 taxa and cytochrome oxidase subunit 1(COI) sequence data from 27 taxa are analyzed separately and together to estimate the internal phylogeny of the class Demospongiae and to evaluate how consistent these data are with pre-existing hypotheses of relationship concerning order-level monophyly and relationships. The monophyly of Porifera is only slightly inconsistent with LSU data, which do not support the monophyly of the class Demospongiae regardless of the inclusion or exclusion of Homoscleromorpha (this result is likely due to the placement of a single hexactinellid taxon within the Demospongiae), however, no LSU support is found for the monophyly of Silicea (Demospongiae + Hexactinellida) unless homoscleromorphs are excluded. Neither the subclasses Ceractinomorpha and Tetractinomorpha, nor the orders Halichondrida, Hadromerida,

and Haplosclerida are supported as monophyletic under any data partition. The haplosclerid suborders Haplosclerina and Petrosina are supported as monophyletic to the exclusion of the suborder Spongillina, and the orders Dictyoceratida, Verongida, Poecilosclerida, Astrophorida, Spirophorida, Homosclerophorida, and Agelasida are largely reconstructed as monophyletic, with the exception of few anomalously placed taxa. Few inter-order relationships are strongly supported by any data partition, but there is moderate support for a verongid + chondrosid clade and a tetractinellid + halichondrid clade. Furthermore, LSU data strongly support the existence of two novel clades that do not correspond to the existing classification and that show no morphological uniformity. Finally, every data partition supports the monophyly of a clade that includes the order Agelasida, some members of the genus *Axinella*, and two taxa tentatively identified as belonging to the orders Hadromerida and Halichondrida.

Oh, S.-H. and D. Potter (2003). "Phylogenetic utility of the second intron of LEAFY in *Neillia* and *Stephanandra* (Rosaceae) and implications for the origin of *Stephanandra*." Molecular Phylogenetics and Evolution **29**(2): 203.

<http://www.sciencedirect.com/science/article/B6WNH-48GF21T-K/2/a44881d415a6bb4987ee8aa137a63be8>

A homeotic gene, LEAFY, has been suggested to be a single-copy gene in diploid angiosperms. Nucleotide sequences of the second intron of this gene, along with those of several regions of the chloroplast genome (trnL-trnF, trnD-trnY-trnE-trnT, and matK-trnK) and nuclear ribosomal ITS, were obtained from the species of *Neillia* and *Stephanandra* to examine the phylogenetic utility of the intron and to elucidate the phylogenetic relationships among species of the two genera. PCR amplification of the second intron of LEAFY using universal degenerate primers produced PCR products in sufficient quantity for successful direct sequencing. The length of the intron ranged from 591 to 622 base pairs (bp) in *Neillia* and *Stephanandra*, except in *N. thibetica* (ca. 1370 bp), and sequence analysis of this region from multiple accessions revealed low levels of infraspecific variation. Comparison of the LEAFY data with ITS and cpDNA data demonstrated that the LEAFY intron was the most variable and useful for phylogenetic analysis at the species level, providing many more phylogenetically informative characters per 100 bp (7.4) than either ITS (3.2) or cpDNA (0.7). Phylogenetic analyses of LEAFY data using both maximum parsimony and likelihood methods generated well supported and highly resolved gene trees with few homoplasies (CI=0.97). *Stephanandra* is monophyletic and is nested within *Neillia* in both LEAFY and cpDNA trees, while the relationship is poorly resolved by ITS data. LEAFY and cpDNA data, however, strongly conflicted with each other with respect to the position of *Stephanandra*: LEAFY trees placed *Stephanandra* as sister to the ((*N. affinis*, *N. gracilis*), *N. thyrsoflora*) clade whereas cpDNA data suggested *Stephanandra* is sister to *N. uekii*. Both gene trees, however, are nearly identical to each other when *Stephanandra* is excluded. A hybrid origin of *Stephanandra* is suggested as a plausible hypothesis to explain the incongruence between LEAFY and cpDNA data sets, though gene duplication/loss and lineage sorting events cannot be ruled out as possibilities.

Perdices, A., E. Bermingham, et al. (2002). "Evolutionary history of the genus *Rhamdia* (Teleostei: Pimelodidae) in Central America." Molecular Phylogenetics and Evolution **25**(1): 172.

<http://www.sciencedirect.com/science/article/B6WNH-46RD1H5-1/2/4bf7fbc07bc70e7c2c0724472c26ff56>

We constructed phylogenetic hypotheses for Mesoamerican *Rhamdia*, the only genus of primary freshwater fish represented by sympatric species across Central America. Phylogenetic relationships were inferred from analysis of 1990 base pairs (bp) of mitochondrial DNA (mtDNA),

represented by the complete nucleotide sequences of the cytochrome b (cyt b) and the ATP synthase 8 and 6 (ATPase 8/6) genes. We sequenced 120 individuals from 53 drainages to provide a comprehensive geographic picture of Central American Rhamdia systematics and phylogeography. Phylogeographic analysis distinguished multiple Rhamdia mtDNA lineages, and the geographic congruence across evolutionarily independent Rhamdia clades indicated that vicariance has played a strong role in the Mesoamerican diversification of this genus.

Phylogenetic analyses of species-level relationships provide strong support for the monophyly of a trans-Andean clade of three evolutionarily equivalent Rhamdia taxa: *R. guatemalensis*, *R. laticauda*, and *R. cinerascens*. Application of fish-based mitochondrial DNA clocks ticking at 1.3-1.5% sequence divergence per million years (Ma), suggests that the split between cis- and trans-Andean Rhamdia extends back about 8 Ma, and the three distinct trans-Andean Rhamdia clades split about 6 Ma ago. Thus the mtDNA divergence observed between cis- and trans-Andean Rhamdia species is too low to support an ancient colonization of Central America in the Late Cretaceous or Paleocene as had been hypothesized in one colonization model for Mesoamerican fishes. Rather the mtDNA data indicate that Rhamdia most likely colonized Central America in the late Miocene or Pliocene, promoting a strong role for the Isthmus of Panama in the Mesoamerican expansion of this genus. Basal polytomies suggest that both the *R. laticauda* and *R. guatemalensis* clades spread rapidly across the Central American landscape, but differences in the average mtDNA genetic distances among clades comprising the two species, indicate that the *R. laticauda* spread and diversified across Mesoamerica about 1 million years before *R. guatemalensis*.

Pinou, T., S. Vicario, et al. (2004). "Relict snakes of North America and their relationships within Caenophidia, using likelihood-based Bayesian methods on mitochondrial sequences." Molecular Phylogenetics and Evolution **32**(2): 563.

<http://www.sciencedirect.com/science/article/B6WNH-4C5HRR5-1/2/3e347039028d538cfe2b3153047698e0>

This paper focuses on the phylogenetic relationships of eight North American caenophidian snake species (*Carphophis amoena*, *Contia tenuis*, *Diadophis punctatus*, *Farancia abacura*, *Farancia erytrogramma*, *Heterodon nasicus*, *Heterodon platyrhinos*, and *Heterodon simus*) whose phylogenetic relationships remain controversial. Past studies have referred to these "relict" North American snakes either as colubrid, or as Neotropical dipsadids and/or xenodontids. Based on mitochondrial DNA ribosomal gene sequences and a likelihood-based Bayesian analysis, our study suggests that these North American snakes are not monophyletic and are nested within a group (Dipsadoidea) that contains the Dipsadidae, Xenodontidae, and Natricidae. In addition, we use the relationships proposed here to highlight putative examples of parallel evolution of hemipenial morphology among snake clades.

Plocik, A., J. Layden, et al. (2004). "Comparative analysis of NBS domain sequences of NBS-LRR disease resistance genes from sunflower, lettuce, and chicory." Molecular Phylogenetics and Evolution **31**(1): 153.

<http://www.sciencedirect.com/science/article/B6WNH-49M0ND6-1/2/87fb955d1c9b8609a6d0f53dcc32b97a>

Plant resistance to many types of pathogens and pests can be achieved by the presence of disease resistance (R) genes. The nucleotide binding site-leucine rich repeat (NBS-LRR) class of R-genes is the most commonly isolated class of R-genes and makes up a super-family, which is often arranged in the genome as large multi-gene clusters. The NBS domain of these genes can be targeted by polymerase chain reaction (PCR) amplification using degenerate primers.

Previous studies have used PCR derived NBS sequences to investigate both ancient R-gene evolution and recent evolution within specific plant families. However, comparative studies with the Asteraceae family have largely been ignored. In this study, we address recent evolution of NBS sequences within the Asteraceae and extend the comparison to the *Arabidopsis thaliana* genome. Using multiple sets of primers, NBS fragments were amplified from genomic DNA of three species from the family Asteraceae: *Helianthus annuus* (sunflower), *Lactuca sativa* (lettuce), and *Cichorium intybus* (chicory). Analysis suggests that Asteraceae species share distinct families of R-genes, composed of genes related to both coiled-coil (CC) and toll-interleukin-receptor homology (TIR) domain containing NBS-LRR R-genes. Between the most closely related species, (lettuce and chicory) a striking similarity of CC subfamily composition was identified, while sunflower showed less similarity in structure. These sequences were also compared to the *A. thaliana* genome. Asteraceae NBS gene subfamilies appear to be distinct from *Arabidopsis* gene clades. These data suggest that NBS families in the Asteraceae family are ancient, but also that gene duplication and gene loss events occur and change the composition of these gene subfamilies over time.

Quandt, D., S. Huttunen, et al. (2004). "Molecular phylogenetics of the Meteoriaceae s. str.: focusing on the genera *Meteorium* and *Papillaria*." Molecular Phylogenetics and Evolution **32**(2): 435.

<http://www.sciencedirect.com/science/article/B6WNH-4CJ46KB-1/2/c3fb43da1314a188e6d1e66fcdabafb2>

In order to delimit and understand the evolution of the Meteoriaceae, we provide phylogenetic analyses using the internal transcribed spacer 2 (ITS2) of nuclear ribosomal DNA in combination with two plastid markers, trnL-F and psbT-H. In contrast to the widely used trnL-F region, the psbT-H gene cluster, coding for proteins of photosystem II, has been rarely used to address systematic questions among the different land plant lineages. To overcome the problem of potential ambiguous alignments of non-coding DNA regions, the data were independently analyzed using direct optimization. The comparison and evaluation of the obtained results showed that the inferred cladograms based on the different phylogenetic approaches are very similar, with only minor differences. In combination with morphological characters, generic relationships as well as taxonomic and nomenclatural problems, especially regarding the key genera *Meteorium* and *Papillaria* are discussed in detail. New insights into generic relationships of the Meteoriaceae are provided, such as the exclusion of the monospecific southern South American genera *Ancistrodes* and *Cryphaeophilum*, which are subsequently transferred to the Hookeriaceae and Cryphaeaceae, respectively. Phylogenetic reconstructions using maximum likelihood as well as parsimony approaches reveal that at the familial level the Meteoriaceae s. l. are polyphyletic, if the formerly recognized "Trachypodaceae" are considered as a separate family. Based on our results we favor the synonym of the Trachypodaceae with the Meteoriaceae.

Quenouille, B., E. Bermingham, et al. (2004). "Molecular systematics of the damselfishes (Teleostei: Pomacentridae): Bayesian phylogenetic analyses of mitochondrial and nuclear DNA sequences." Molecular Phylogenetics and Evolution **31**(1): 66.

<http://www.sciencedirect.com/science/article/B6WNH-49M0ND6-2/2/16716c181922f3479428cedc5bde9ece>

Damselfishes in the family Pomacentridae represent one of the few families of reef fishes found on coral reefs irrespective of location. At a local scale, damselfishes are often the most abundant coral reef fish, and their study has provided much of our current understanding of the ecology of tropical reef animals. The study of phylogenetic relationships among the Pomacentridae has lagged ecological investigation of the group, thus limiting historical perspective on the remarkable

species richness of the family. In this study, we used 1989 bp of DNA sequence representing three mitochondrial genes and 1500 bp of the single copy nuclear RAG1 region to infer hypotheses of relationship for the group. Our analysis includes 103 Pomacentridae species in 18 genera, and three of the four named subfamilies: Amphiprioninae, Chrominae, and Pomacentrinae. The Bayesian method of phylogenetic reconstruction was applied to the data, because even with a large number of sequences it is an efficient means of analysis that provides intuitive measures of support for tree topologies and for the parameters of the nucleotide substitution model. Four Pomacentridae clades were identified with high statistical support whether the data were analyzed from a mtDNA, RAG1 or combined perspective, and in all analyses the current subfamilial classification of the Pomacentridae was rejected. At the genus level, Amphiprion, Chromis, and Chrysiptera were also rejected as natural groups. *Abudefduf*, *Amblyglyphidodon*, *Dascyllus*, *Neoglyphidodon*, *Neopomacentrus*, and *Pomacentrus* were each strongly supported as monophyletic genera but the support for monophyly is nonetheless compromised by sample size, except in the case of *Dascyllus* and *Abudefduf* for which we have sampled almost all of the described species.

Rees, D. J., M. Dioli, et al. (2003). "Molecules and morphology: evidence for cryptic hybridization in African *Hyalomma* (Acari: Ixodidae)." *Molecular Phylogenetics and Evolution* **27**(1): 131.

<http://www.sciencedirect.com/science/article/B6WNH-47HK3HT-4/2/7a02a57a6345395797585b5574c2bea8>

The role of natural hybridization and introgression as part of the evolutionary process is of increasing interest to zoologists, particularly as more examples of gene exchange among species are identified. We present mitochondrial and nuclear sequence data for *Hyalomma dromedarii*, *Hyalomma truncatum*, and *Hyalomma marginatum rufipes* (Acari: Ixodidae) collected from one-humped camels in Ethiopia. These species are well differentiated morphologically and genetically; sequence data from the mitochondrial DNA (mtDNA) cytochrome oxidase I gene indicates 10-14% divergence between the species. However, incongruence between morphology and the mtDNA phylogeny was observed, with multiple individuals of *H. dromedarii* and *H. truncatum* present on the same mtDNA lineage as *H. marginatum rufipes*. Thus, individuals with morphology of *H. dromedarii* and *H. truncatum* are indistinguishable from *H. marginatum rufipes* on the basis of mtDNA. Multiple copies of ITS-2 were subsequently cloned and sequenced for a subset of individuals from the mtDNA phylogeny, representing both 'normal' and 'putative hybrid' individuals. Very low sequence divergence (0.3%) was observed within 'normal' individuals of both *H. dromedarii* and *H. truncatum* relative to the 'putative hybrid' individuals (6 and 2.7%, respectively). The pattern of intra-individual variation in ITS-2 within 'putative hybrid' individuals, particularly in *H. dromedarii*, strongly suggests that gene flow has occurred among these *Hyalomma* species, but no indication of this is given by the morphology of the individuals.

Saux, C., B. L. Fisher, et al. (2004). "Dracula ant phylogeny as inferred by nuclear 28S rDNA sequences and implications for ant systematics (Hymenoptera: Formicidae: Amblyoponinae)." *Molecular Phylogenetics and Evolution* **33**(2): 457.

<http://www.sciencedirect.com/science/article/B6WNH-4D0Y3T4-2/2/9cff2b5e674430ff63f21a597f23128f>

Ants are one of the most ecologically and numerically dominant families of organisms in almost every terrestrial habitat throughout the world, though they include only about 1% of all described insect species. The development of eusociality is thought to have been a driving force in the striking diversification and dominance of this group, yet we know little about the evolution of the major lineages of ants and have been unable to clearly determine their primitive characteristics.

Ants within the subfamily Amblyoponinae are specialized arthropod predators, possess many anatomically and behaviorally primitive characters and have been proposed as a possible basal lineage within the ants. We investigate the phylogenetic relationships among the members of the subfamily, using nuclear 28S rDNA sequence data. Outgroups for the analysis include members of the poneromorph and leptanillomorph (Apomyrma, Leptanilla) ant subfamilies, as well as three wasp families. Parsimony, maximum likelihood, and Bayesian analyses provide strong support for the monophyly of a clade containing the two genera Apomyrma + Mystrium (100% bpp; 97% ML bs; and 97% MP bs), and moderate support for the monophyly of the Amblyoponinae as long as Apomyrma (Apomyrminae) is included (87% bpp; 57% ML bs; and 76% MP bs). Analyses did not recover evidence of monophyly of the Amblyopone genus, while the monophyly of the other genera in the subfamily is supported. Based on these results we provide a morphological diagnosis of the Amblyoponinae that includes Apomyrma. Among the outgroup taxa, Typhlomyrmex grouped consistently with Ectatomma, supporting the recent placement of Typhlomyrmex in the Ectatomminae. The results of this present study place the included ant subfamilies into roughly two clades with the basal placement of Leptanilla unclear. One clade contains all the Amblyoponinae (including Apomyrma), Ponerinae, and Proceratiinae (Poneroid clade). The other clade contains members from subfamilies Cerapachyinae, Dolichoderinae, Ectatomminae, Formicinae, Myrmeciinae, and Myrmicinae (Formicoid clade).

Siddall, M. E., S. L. Perkins, et al. (2004). "Leech mycetome endosymbionts are a new lineage of alphaproteobacteria related to the Rhizobiaceae." Molecular Phylogenetics and Evolution **30**(1): 178.

<http://www.sciencedirect.com/science/article/B6WNH-48WPS6T-1/2/3a576ccfa3363585d3fe4cee4414fec7>

Mycetomal organs attached to the esophagus of hematophagous leeches which are known to harbor endosymbiotic bacteria were removed from three species in the leech family Glossiphoniidae. Anatomical observations indicated that placobdellid mycetomes are paired and caecate, inserting into the esophagus posterior to the proboscis. Light and electron microscopy demonstrated that there is a single layer of mycetome epithelial cells harboring Gram-negative rods and that these epithelial cells are ultrastructurally distinct from neighboring esophageal epithelial cells. Fluorescent in situ hybridization with eubacterial and alphaproteobacterial probes localized the bacteria solely to the mycetomes both in adult and in unfed juvenile leeches whereas a gammaproteobacterial probe did not yield a bound fluorescent signal. DNA was isolated from these tissues and subjected to PCR amplification using bacteria-specific primers for 16S and 23S rDNA. Results from sequencing the amplification products and phylogenetic analysis with other Alphaproteobacteria revealed that the bacteria resident in these organs comprise a new genus of Alphaproteobacteria, *Reichenowia* n. gen., closely related to the nitrogen-fixing, nodule-forming Rhizobiaceae. The three bacterial strains, though different from each other were each other's closest relatives, suggesting a history of close coevolution with their leech hosts.

Steiper, M. E. and M. Ruvolo (2003). "New World monkey phylogeny based on X-linked G6PD DNA sequences." Molecular Phylogenetics and Evolution **27**(1): 121.

<http://www.sciencedirect.com/science/article/B6WNH-47HK3HT-5/2/7c38c4ea76ffaf0797640536304f7b10>

The Platyrrhini, or New World monkeys, are an infraorder of Primates comprised of 16 genera. Molecular phylogenetic analyses have consistently sorted these genera into three groups: the Pitheciidae (e.g., saki and titi monkeys), Atelidae (e.g., spider and howler monkeys), and Cebidae

(e.g., night monkeys, squirrel monkeys, and tamarins). No consensus has emerged on the relationships among the three groups or within the Cebidae. Here, ~0.8 kb of newly generated intronic DNA sequence data from the X-linked glucose-6-phosphate dehydrogenase (G6PD) locus have been collected from nine New World monkey taxa to examine these relationships. These data are added to 1.3 kb of previously generated G6PD intronic DNA sequence data [Mol. Phylogenet. Evol. 11 (1999) 459]. Using distance and parsimony-based techniques, G6PD sequences provide support for an initial bifurcation between the Pitheciidae and the remaining platyrrhines, linking Atelidae and Cebidae as sister taxa. Bayesian methods provided a conflicting phylogeny with Atelidae as outgroup. Within the Cebidae, a sister relation between Aotus and the Cebus/Saimiri clade is favored by parsimony analysis, but not by other analyses. Potential reasons for the difficulty in resolving family level New World monkey phylogenetics are discussed.

Steppan, S. J., B. L. Storz, et al. (2004). "Nuclear DNA phylogeny of the squirrels (Mammalia: Rodentia) and the evolution of arboreality from c-myc and RAG1." Molecular Phylogenetics and Evolution **30**(3): 703.

<http://www.sciencedirect.com/science/article/B6WNH-4967F2T-2/2/48f4439f6e122d16606e39f6dfd9ed0f>

Although the family Sciuridae is large and well known, phylogenetic analyses are scarce. We report on a comprehensive molecular phylogeny for the family. Two nuclear genes (c-myc and RAG1) comprising approximately 4500 bp of data (most in exons) are applied for the first time to rodent phylogenetics. Parsimony, likelihood, and Bayesian analyses of the separate gene regions and combined data reveal five major lineages and refute the conventional elevation of the flying squirrels (Pteromyinae) to subfamily status. Instead, flying squirrels are derived from one of the tree squirrel lineages. C-myc indels corroborate the sequence-based topologies. The common ancestor of extant squirrels appears to have been arboreal, confirming the fossil evidence. The results also reveal an unexpected clade of mostly terrestrial squirrels with African and Holarctic centers of diversity. We present a revised classification of squirrels. Our results demonstrate the phylogenetic utility of relatively slowly evolving nuclear exonic data even for relatively recent clades.

Stone, K. D. and J. A. Cook (2002). "Molecular evolution of Holarctic martens (genus Martes, Mammalia: Carnivora: Mustelidae)." Molecular Phylogenetics and Evolution **24**(2): 169.

<http://www.sciencedirect.com/science/article/B6WNH-46CS03K-1/2/b8ff70778e19d9cba7229387ab76c489>

Thacker, C. E. (2003). "Molecular phylogeny of the gobioid fishes (Teleostei: Perciformes: Gobioidei)." Molecular Phylogenetics and Evolution **26**(3): 354.

<http://www.sciencedirect.com/science/article/B6WNH-47S6M2C-2/2/e7535d0f07bc07da50e3c06f69281281>

The phylogeny of groups within Gobioidei is examined with molecular sequence data. Gobioidei is a speciose, morphologically diverse group of teleost fishes, most of which are small, benthic, and marine. Efforts to hypothesize relationships among the gobioid groups have been hampered by the prevalence of reductive evolution among goby species; such reduction can make identification of informative morphological characters particularly difficult. Gobies have been

variously grouped into two to nine families, several with included subfamilies, but most existing taxonomies are not phylogenetic and few cladistic hypotheses of relationships among goby groups have been advanced. In this study, representatives of eight of the nine gobioid families (Eleotridae, Odontobutidae, Xenisthmidae, Gobiidae, Kraemeriidae, Schindleriidae, Microdesmidae, and Ptereleotridae), selected to sample broadly from the range of goby diversity, were examined. Complete sequence from the mitochondrial ND1, ND2, and COI genes (3573 bp) was used in a cladistic parsimony analysis to hypothesize relationships among the gobioid groups. A single most parsimonious topology was obtained, with decay indices indicating strong support for most nodes. Major phylogenetic conclusions include that Xenisthmidae is part of Eleotridae, and Eleotridae is paraphyletic with respect to a clade composed of Gobiidae, Microdesmidae, Ptereleotridae, Kraemeriidae, and Schindleriidae. Within this five-family clade, two clades are recovered. One includes Gobionellinae, which is paraphyletic with respect to Kraemeriidae, Sicydiinae, Oxudercinae, and Amblyopinae. The other contains Gobiinae, also paraphyletic, and including Microdesmidae, Ptereleotridae, and Schindleriidae. Previous morphological evidence for goby groupings is discussed; the phylogenetic hypothesis indicates that the morphological reduction observed in many goby species has been derived several times independently.

Van Borm, S., T. Wenseleers, et al. (2003). "Cloning and sequencing of wsp encoding gene fragments reveals a diversity of co-infecting Wolbachia strains in *Acromyrmex* leafcutter ants." Molecular Phylogenetics and Evolution **26**(1): 102.

<http://www.sciencedirect.com/science/article/B6WNH-475B9D7-3/2/0bbab6a302941e820c97890b65266546>

Wall, D. P. (2002). "Use of the nuclear gene glyceraldehyde 3-phosphate dehydrogenase for phylogeny reconstruction of recently diverged lineages in *Mitthyridium* (Musci: Calymperaceae)." Molecular Phylogenetics and Evolution **25**(1): 10.

<http://www.sciencedirect.com/science/article/B6WNH-46WNYGM-3/2/daef3b413ab5e1d0d2843c69bb6296dd>

A portion of the nuclear gene glyceraldehyde 3-phosphate dehydrogenase (gpd) was sequenced in 26 representatives of the paleotropical moss, *Mitthyridium*, and a group of 20 outgroup taxa to assess its utility for phylogenetic reconstruction compared with the better understood chloroplast markers, *rps4* and *trnL*. Primers based on plant and fungal sequences were designed to amplify *gpd* in plants universally with the exclusion of fungal contaminants. The piece amplified spanned 4 introns and 3 of 9 exons, based on comparisons with complete sequence from *Arabidopsis*. Size variation in *gpd* ranged from 891 to 1007 bp, in part attributable to 6 indels of variable length found within the introns. Intron 6 contributed most of the length variation and contained a variable purine-repeat motif of possible use as a microsatellite. Phylogenetic analyses of the full *gpd* amplicon yielded well-resolved trees that were in nearly full accord with the trees derived from the cpDNA partitions for analyses of both the ingroup and ingroup + outgroup taxon sets. Pairwise nucleotide substitution rates of *gpd* were as much as 2.2 times higher than those in *rps4* and 2.8 times higher than in *trnL*. Excision of the introns left suitable numbers of parsimony informative characters and demonstrated that the full *gpd* amplicon could be compartmentalized to provide resolution for both shallow and deep phylogenetic branches. Exons of *gpd* were found to behave in a clock-like fashion for the 26 ingroup taxa and select outgroups. In general, *gpd* was found to hold great promise not only for improving resolution of chloroplast-derived phylogenies, but also for phylogenetic reconstruction of recent, diversifying lineages.

Weksler, M. (2003). "Phylogeny of Neotropical oryzomyine rodents (Muridae: Sigmodontinae) based on the nuclear IRBP exon." Molecular Phylogenetics and Evolution **29**(2): 331.

<http://www.sciencedirect.com/science/article/B6WNH-48KFF3P-5/2/d09fea423931fb9beff14d8a7536865f>

Sigmodontine rodents are the most diverse family-level mammalian clade in the Neotropical region, with about 70 genera and 320 recognized species. Partial sequences (1266 bp) from the first exon of the nuclear gene encoding the Interphotoreceptor Retinoid Binding Protein (IRBP) were used to infer the phylogenetic relationships among 44 species representing all 16 currently recognized genera of the largest sigmodontine tribe, the Oryzomyini. Monophyly of the tribe was assessed relative to 15 non-oryzomyine sigmodontine taxa representing all major sigmodontine lineages. Twelve taxa from seven muroid subfamilies were used as outgroups. The resulting matrix included 71 taxa and 386 parsimony-informative characters. Phylogenetic analysis of this matrix resulted in 16 equally parsimonious cladograms, which contained the following well-supported groups: (i) a monophyletic Oryzomyini, (ii) a clade containing all oryzomyines except *Scolomys* and *Zygodontomys*, (iii) a clade containing *Oecomys*, *Handleyomys*, and several species of forest-dwelling *Oryzomys*, and (iv) a clade containing the remaining oryzomyine taxa. The last clade is composed of two large subclades, each with lower nodal support, containing the following taxa: (i) *Microryzomys*, *Oligoryzomys*, *Neacomys*, and *Oryzomys balneator*; (ii) *Holochilus*, *Lundomys*, *Pseudoryzomys*, *Nectomys*, *Amphinectomys*, *Sigmodontomys*, and several species of open-vegetation or semiaquatic *Oryzomys*. Regarding relationships among non-oryzomyine taxa, sigmodontines, neotomines, and tylomyines do not form a monophyletic group; a clade containing *Rheomys* and *Sigmodon* is basal relative to all other sigmodontines; and the remaining sigmodontines are grouped in three clades: the first containing *Thomasomyini*, *Akodontini*, and *Reithrodon*; the second containing *Abrothrichini*, *Phyllotini*, plus *Wiedomys*, *Juliomys*, *Irenomys*, and *Delomys*; and the third containing the oryzomyines. No conflict is observed between IRBP results and previous robust hypotheses from mitochondrial data, while a single case of incongruence is present between the IRBP topology and robust hypothesis from morphological studies.

Wesson, D. M., C. H. Porter, et al. (1992). "Sequence and secondary structure comparisons of ITS rDNA in mosquitoes (Diptera: Culicidae)." Molecular Phylogenetics and Evolution **1**(4): 253.

<http://www.sciencedirect.com/science/article/B6WNH-4DXK9Y9-B/2/aa000f97baf606884d8eecb21770d436>

Sequences of the internal transcribed spacers (ITS1 and ITS2) of the mosquito *Aedes aegypti*, and the ITS2 of six related species, *A. simpsoni*, *A. albopictus*, *A. vexans*, *A. triseriatus*, *Haemagogus mesodentatus*, and *Psorophora ferox* are reported. Intraspecific variation in *A. aegypti* ITS1 is 1.07% among four clones from three individuals, and in the ITS2 is 1.17% among 15 clones from four individuals. In *A. simpsoni*, intraspecific ITS2 variation is 0.46% among 10 clones from a single individual. Alignment of the ITS2 sequence of the seven species reveals several homologous domains. Secondary structure predictions for the ITS2 region indicate that these domains base pair to form a core region central to several stem features. The sequence outside the ITS2 homologous domains tends to be GC-rich and characteristically slippage generated; these areas preserve or add to the stem length of the predicted secondary structures. These ITS2 intraspacer variable regions resemble previously described expansion segments of the 28S gene region. Evolutionary analysis of the ITS2 of these species, using both sequence and secondary structure information, leads to the prediction of divergence in the mosquito tribe Aedini that is not clearly reflected in current taxonomic designations.

Williams, S. T., D. G. Reid, et al. (2003). "A molecular phylogeny of the Littorininae (Gastropoda: Littorinidae): unequal evolutionary rates, morphological parallelism, and biogeography of the Southern Ocean." Molecular Phylogenetics and Evolution **28**(1): 60.

<http://www.sciencedirect.com/science/article/B6WNH-48FK4VN-1/2/b9140e4a7cf75e4f5c9a8d95913e23c6>

A molecular phylogeny is presented for the subfamily Littorininae (including representatives of all subgeneric taxa and all members of a group of southern-temperate species formerly classified as 'Nodilittorina'), based on sequence data from two nuclear (18S rRNA, 28S rRNA) and two mitochondrial (12S rRNA, CO1) genes. The phylogeny shows considerable disagreement with earlier hypotheses derived from morphological data. In particular, 'Nodilittorina' is polyphyletic and is here divided into four genera (Echinolittorina, Austrolittorina, Afrolittorina new genus, and the monotypic Nodilittorina s.s.). The phylogenetic relationships of 'Littorina' striata have been controversial and it is here transferred to the genus Tectarius, a surprising relationship for which there is little morphological support. The relationships of the enigmatic Mainwaringia remain poorly resolved, but it is not a basal member of the subfamily. The two living species of Mainwaringia are remarkable for a greatly elevated rate of evolution in all four genes examined; it is suggested that this may be connected with their protandrous hermaphroditism, which is unique in the family. The molecular phylogeny provides a new framework for the adaptive radiation of the Littorininae, showing more frequent shifts between habitats and climatic regimes than previously suspected, and striking parallelism of morphological characters. The fossil record of littorinids is poor, but ages of clades are estimated using a calibration based on a Lower Eocene age of the genus Littoraria. Using these estimates, the antitropical distribution of Littorina and Afrolittorina is an ancient pattern of possibly Cretaceous age. The five members of Austrolittorina show a Gondwanan distribution in Australia, New Zealand, and South America. Based on the morphological uniformity within this clade, relatively recent (Plio-Pleistocene) trans-Pacific dispersal events seemed a likely explanation, as proposed for numerous other congeneric marine taxa. However, molecular estimation of ages of divergence suggest an initial vicariance between Australian and South American lineages at 40-73 Ma, contemporary with the later stages of fragmentation of the Gondwanan supercontinent, followed by more recent (but still mid-Cenozoic) dispersal events across the Tasman Sea and the Pacific Ocean. Afrolittorina is another Cretaceous clade, now restricted to southern Africa and southern Australia, but divergence between these lineages (29-55 Ma) post-dates Gondwanan fragmentation. Within both Austrolittorina and Afrolittorina all sister-species divergences are estimated to fall in the range 10-47 Ma, so that there is no evidence for speciation events in the Plio-Pleistocene.

Winkworth, R. C. and M. J. Donoghue (2004). "Viburnum phylogeny: evidence from the duplicated nuclear gene GBSSI." Molecular Phylogenetics and Evolution **33**(1): 109.

<http://www.sciencedirect.com/science/article/B6WNH-4CT5YFS-4/2/87b46543eff7302df012b11c010371d0>

DNA sequencing studies of the granule-bound starch synthase gene (GBSSI) indicate the presence of two loci in Viburnum. Gene trees from separate and combined phylogenetic analyses of the GBSSI paralogues are generally congruent with each other and with trees from previous analyses, especially those of Donoghue et al. [Syst. Bot. 29 (2004) 188] based on nuclear ribosomal ITS and chloroplast trnK intron DNA sequences. Specifically, our GBSSI trees confirm (i) the monophyly of some and non-monophyly of other traditionally recognized taxonomic sections, (ii) the presence of three major supra-sectional lineages within Viburnum, and (iii) the resolution of many species relationships within the section-level clades. Analyses of GBSSI also provide greater resolution of relationships within the largest supra-sectional lineage. Relationships at the base of the Viburnum phylogeny remain uncertain; in particular, the position of the root, relationships among the supra-sectional clades, and the exact placement of several

smaller groups (e.g., *Viburnum clemensiae*, *Viburnum urceolatum*, and section *Pseudotinus*). In two lineages each GBSSI paralogue is represented by two distinct sequences. The presence of additional copies appears to be correlated with polyploidy in these clades. Placement of the homoeologues in our gene trees suggests the possibility of a hybrid origin for these polyploids.

Yang, Y., Y.-p. Zhang, et al. (2004). "Phylogenetic relationships of *Drosophila melanogaster* species group deduced from spacer regions of histone gene H2A-H2B." Molecular Phylogenetics and Evolution **30**(2): 336.

<http://www.sciencedirect.com/science/article/B6WNH-49H1KWC-1/2/deae7d1c950b45c0a903f2f56914abad>

Nucleotide sequences of the spacer region of the histone gene H2A-H2B from 36 species of *Drosophila melanogaster* species group were determined. The phylogenetic trees were reconstructed with maximum parsimony, maximum likelihood, and Bayesian methods by using *Drosophila pseudoobscura* as the out group. Our results show that the *melanogaster* species group clustered in three main lineages: (1) montium subgroup; (2) ananassae subgroup; and (3) the seven oriental subgroups, among which the montium subgroup diverged first. In the third main lineage, *suzukii* and *takahashii* subgroups formed a clade, while *eugracilis*, *melanogaster*, *elegans*, *ficusphila*, and *rhopalosa* subgroups formed another clade. The bootstrap values at subgroup levels are high. The phylogenetic relationships of these species subgroups derived from our data are very different from those based on some other DNA data and morphology data.

Molecular Therapy (11)

Budak-Alpdogan, T., O. Alpdogan, et al. (2004). "Methotrexate and cytarabine inhibit progression of human lymphoma in NOD/SCID mice carrying a mutant dihydrofolate reductase and cytidine deaminase fusion gene." Molecular Therapy **10**(3): 574.

<http://www.sciencedirect.com/science/article/B6WNJ-4CX0442-3/2/ad29328aed9b28823a6396b4b92a14e0>

An SFG-based retroviral bicistronic vector containing a double-mutant dihydrofolate reductase-cytidine deaminase fusion cDNA (F/S DHFR-CD) with IRES-eGFP confers resistance to both methotrexate (MTX) and cytarabine (ara-C). Two weeks after transplantation with marrow transduced with either a fusion or a control gene (eGFP-IRES-NeoR), human lymphoma (SKI-DLCL-1) cells were injected sc into the flanks of nonobese diabetic/severe combined immune deficiency mice. In mock-transplanted mice, maximal tolerated dose (MTD) of posttransplant MTX/ara-C (15/10 mg/kg/day, x 3) was unable to control tumor growth. Transfer of the fusion gene allowed doses of MTX/ara-C (25/15 mg/kg/day, x 4) twofold higher than the MTD to be tolerated. The tumor burden defined the efficiency of posttransplant chemotherapy; early treatment, 48 h after tumor inoculation, provided tumor-free survival, while starting treatment after having palpable tumor growth (7 days) delayed tumor growth a median time of 28 days. In addition, the early treated group had higher gene expression in peripheral blood and marrow cells than the late treated group ($P < 0.05$), suggesting that early treatment allowed for enrichment of transduced marrow progenitors. These results encourage clinical studies using this retroviral fusion gene construct.

Kawamoto, K., M. Yagi, et al. (2003). "Hearing and hair cells are protected by adenoviral gene therapy with TGF- β 1 and GDNF." Molecular Therapy 7(4): 484.

<http://www.sciencedirect.com/science/article/B6WNJ-484SKC8-4/2/70e25f43022bd8276359de1ab799a6f5>

Glial cell line-derived neurotrophic factor (GDNF) overexpression in the inner ear can protect hair cells against degeneration induced by aminoglycoside ototoxicity. The protective efficiency of GDNF increases when it is combined with co-factors such as transforming growth factor β 1 (TGF- β 1), a ubiquitous cytokine. The aim of this study was to determine whether TGF- β 1 receptors are expressed in the inner ear and whether a cocktail of GDNF and TGF- β 1 transgenes provides enhanced protection of the inner ear against ototoxic trauma. Using RT-PCR analysis, we determined that both TGF- β 1 receptors, type 1 and 2 are present in rat cochlea. We co-inoculated two adenoviral vectors, one encoding human TGF- β 1 gene (Ad.TGF- β 1) and the other encoding human GDNF gene (Ad.GDNF) into guinea pig cochleae 4 days prior to injecting an ototoxic dose of aminoglycosides. Inoculated ears had better hearing and fewer missing inner hair cells after exposure to the aminoglycoside ototoxicity, as compared with controls and ears treated only with Ad.GDNF. Cochleae with TGF- β 1 overexpression exhibited fibrosis in the scala tympani regardless of the presence of GDNF. Our results suggest that the adenovirus-mediated overexpression of GDNF and TGF- β 1 can be used in combination to protect cochlear hair cells and hearing from ototoxic trauma.

Koponen, J. K., A.-M. Turunen, et al. (2002). "Escherichia coli DNA Contamination in AmpliTaq Gold Polymerase Interferes with TaqMan Analysis of lacZ." Molecular Therapy 5(3): 220.

<http://www.sciencedirect.com/science/article/B6WNJ-458P5J1-5/2/5d90565fd191514ce68c084d8fcf8983>

Real-time PCR is a powerful method for the quantification of gene expression in biological samples. This method uses TaqMan chemistry based on the 5'-exonuclease activity of the AmpliTaq Gold DNA polymerase which releases fluorescence from hybridized probes during synthesis of each new PCR product. Many gene therapy studies use lacZ, encoding Escherichia coli β -galactosidase, as a marker gene. Our results demonstrate that E. coli DNA contamination in AmpliTaq Gold polymerase interferes with TaqMan analysis of lacZ gene expression and decreases sensitivity of the method below the level required for biodistribution and long-term gene expression studies. In biodistribution analyses the contamination can lead to false-negative results by masking low-level lacZ expression in target and ectopic tissues, and false-positive results if sufficient controls are not used. We conclude that, to get reliable TaqMan results with lacZ, adequate controls should be included in each run to rule out contamination from AmpliTaq Gold polymerase.

Park, F., K. Ohashi, et al. (2003). "The effect of age on hepatic gene transfer with self-inactivating lentiviral vectors in vivo." Molecular Therapy 8(2): 314.

<http://www.sciencedirect.com/science/article/B6WNJ-48S335N-3/2/914fd1b4e5e91a9aed457c3e3f924ea9>

It is known that cellular proliferation, by either compensatory regeneration or direct hyperplasia, can augment lentiviral vector transduction into hepatocytes in vivo. For this reason, the present

study was designed to determine if adolescent mice (31/2 weeks of age), which still have relatively proliferating livers, would have differential transduction compared to older (7 weeks of age) mice. Self-inactivating lentiviral vectors containing the human [alpha]1-antitrypsin (hAAT) promoter driving the expression of either the bacterial lacZ gene or the hAAT cDNA were generated for these studies. We found that adolescent mice given lentiviral vectors expressing lacZ (50 [mu]g p24/mouse) via intravenous administration had a significantly higher level of hepatocyte transduction as measured by X-gal staining of liver sections compared to the 7-week-old mice. In addition, serum hAAT levels were nearly 40-fold higher in 31/2-week-old mice administered lentiviral vectors expressing hAAT (50 [mu]g p24/mouse) compared to the 7-week-old mice. Moreover, the incorporation of a matrix attachment region from immunoglobulin [kappa] significantly increased transduction of hepatocytes in vivo. Although there was a small reduction in the circulating levels of hAAT, likely due to an immune response against the transgene product, gene expression was sustained for the duration of the study (30 weeks in total). In conclusion, the present study strongly demonstrates that lentiviral vector transduction efficiency and transgene expression were significantly enhanced in adolescent compared to older mice.

Recchia, A., L. Perani, et al. (2004). "Site-Specific Integration of Functional Transgenes into the Human Genome by Adeno/AAV Hybrid Vectors." Molecular Therapy **10**(4): 660.

<http://www.sciencedirect.com/science/article/B6WNJ-4D2MWN3-1/2/5b188e67d5ba1ad7fb85d946763844d5>

Uncontrolled insertion of gene transfer vectors into the human genome is raising significant safety concerns for their clinical use. The wild-type adeno-associated virus (AAV) can insert its genome at a specific site in human chromosome 19 (AAVS1) through the activity of a specific replicase/integrase protein (Rep) binding both the AAVS1 and the viral inverted terminal repeats (ITRs). AAV-derived vectors, however, do not carry the rep gene and cannot maintain site-specific integration properties. We describe a novel hybrid vector carrying an integration cassette flanked by AAV ITRs and a tightly regulated, drug-inducible Rep expression cassette in the framework of a high-capacity, helper-dependent adenoviral (Ad) vector. Rep-dependent integration of ITR-flanked cassettes of intact size and function was obtained in human primary cells and cell lines in the absence of selection. The majority of integrations were site specific and occurred within a 1000-bp region of the AAVS1. Genome-wide sequencing of integration junctions indicates that nonspecific integrations occurred predominantly in intergenic regions. Site-specific integration was obtained also in vivo, in an AAVS1 transgenic mouse model: upon a single tail vein administration of a nontoxic dose of Ad/AAV vectors, AAVS1-specific integrations were detected and sequenced in DNA obtained from the liver of all animals in which Rep expression was induced by drug treatment. Nonrandom integration of double-stranded DNA can therefore be obtained ex vivo and in vivo by the use of hybrid Ad/AAV vectors, in the absence of toxicity and with efficiency compatible with gene therapy applications.

Sagawa, T., M. Takahashi, et al. (2004). "Prolonged Survival of Mice with Multiple Liver Metastases of Human Colon Cancer by Intravenous Administration of Replicable E1B-55K-Deleted Adenovirus with E1A Expressed by CEA Promoter." Molecular Therapy **10**(6): 1043.

<http://www.sciencedirect.com/science/article/B6WNJ-4DFT351-1/2/69b5f7574f8b07893fcda0ba03a97c7b>

Liver is the most preferential site for metastasis of colon cancer. We, in the present study, constructed a self-replicable adenovirus in which E1A is driven by a CEA promoter and E1B-55K is deleted from the E1B region (AdCEAp/Rep) and examined its effects on multiple metastases of a human colon cancer cell in a mouse xenograft model. We first showed effective replication of

the virus in various CEA-producing human colon cancer cells (M7609, HT-29) and subsequent lysis of the infected cells in vitro. We then demonstrated that a single intratumoral injection of the virus (1×10^8 PFU/100 [μl]) induced a complete regression of subcutaneous tumors (M7609) inoculated into nude mice. Further, we demonstrated that systemic administration of the virus (1×10^8 PFU/100 [μl]) through the tail vein to nude mice, which 1 week prior had been inoculated with tumor cells (colon carcinoma cell line HT-29) via the spleen and showed apparent multiple metastases in the liver, effectively suppressed the metastasis formation. The mean survival time of the treated mice was significantly longer than that of the controls. Thus, the systemic administration of AdCEAp/Rep was considered to be effective on multiple liver metastases of CEA-positive colon cancer in a xenograft model.

Sakurai, F., H. Mizuguchi, et al. (2003). "Characterization of in vitro and in vivo gene transfer properties of adenovirus serotype 35 vector." *Molecular Therapy* 8(5): 813.

<http://www.sciencedirect.com/science/article/B6WNJ-49BY1MY-2/2/e8800324eaaef73456c064e756654352>

We have recently developed a replication-defective, recombinant adenovirus (Ad) vector composed of the whole Ad serotype 35 (Ad35), a member of subgroup B. We describe herein the in vitro and in vivo gene transfer properties of Ad35 vector in comparison with Ad serotype 5 (Ad5) and the Ad5F35 vector, which is a fiber-substituted Ad5 vector containing Ad35 fiber proteins. In vitro, Ad35 vector efficiently transduced not only human CAR-positive cells but also CAR-negative cells. Following intravenous administration into mice, both Ad5 and Ad35 vectors were rapidly cleared from the bloodstream with a half-life of approximately 3 min. Ad5 vector-mediated transgene expression predominantly occurred in liver parenchymal cells, although the Ad5 vector was delivered to both liver parenchymal and nonparenchymal cells. In contrast, Ad35 vector was efficiently taken up by liver nonparenchymal cells and mediated transduction efficiency in the liver on a level 4 log orders lower than the Ad5 vector. These findings demonstrate that Ad35 vector is an attractive vehicle for gene transfer into human cells, while the biodistribution profile of Ad35 vector in mice is much different from that of the Ad5 vector.

Sastry, L., Y. Xu, et al. (2003). "Certification assays for HIV-1-based vectors: frequent passage of gag sequences without evidence of replication-competent viruses." *Molecular Therapy* 8(5): 830.

<http://www.sciencedirect.com/science/article/B6WNJ-49M6PD7-3/2/393a4735cc15bb3bc4b6f8c8104ad6e5>

A principal concern regarding the safety of HIV-1-based vectors is replication-competent lentivirus (RCL). We have developed two PCR assays for detecting RCL; the first detects recombination between gag regions in the transfer vector and the packaging construct (sensitivity of detection ~10-100 copies of target sequence). The second assay uses real-time PCR to detect vesicular stomatitis virus glycoprotein (VSVG) envelope DNA (sensitivity ~5-50 VSVG sequences). In an attempt to amplify any RCL, test vectors were used to transduce C8166 and 293 cells, which were then screened weekly for 3 weeks. Psi-gag recombinants were routinely detected (20 of 21 analyses) in four transductions using the RRL-CMV-GFP vector. In contrast, VSVG sequences were detected only once in 21 analyses. Interestingly, p24 levels (as measured by ELISA) were occasionally detectable after 3 weeks of culture. To determine if a true RCL was present, 21-day cell-free medium was used to transduce naive cells. No evidence of psi-gag or VSVG transfer was detected, indicating that the recombination events were insufficient to reconstitute a true RCL. These findings have important implications for the design and safety of HIV-1-based vectors intended for clinical applications.

Tani, K., M. Azuma, et al. (2004). "Phase I Study of Autologous Tumor Vaccines Transduced with the GM-CSF Gene in Four Patients with Stage IV Renal Cell Cancer in Japan: Clinical and Immunological Findings." Molecular Therapy **10**(4): 799.

<http://www.sciencedirect.com/science/article/B6W NJ-4D4D25C-1/2/bb85481b77b0592b3330fa583af4f890>

We produced lethally irradiated retrovirally GM-CSF-transduced autologous renal tumor cell vaccines (GVAX) from six Japanese patients with stage IV renal cell cancer (RCC). Four patients received GVAX ranging from 1.4×10^8 to 3.7×10^8 cells on 6-17 occasions. Throughout a total of 48 vaccinations, there were no severe adverse events. After vaccination, DTH skin tests became positive to autologous RCC (auto-RCC) in all patients. The vaccination sites showed significant infiltration by CD4+ T cells, eosinophils, and HLA-DR-positive cells. The kinetic analyses of cellular immune responses using peripheral blood lymphocytes revealed an enhanced proliferative response against auto-RCC in four patients, and cytotoxicity against auto-RCC was augmented in three patients. T cell receptor [beta]-chain analysis revealed oligoclonal expansion of T cells in the peripheral blood, skin biopsy specimens from DTH sites, and tumors. Western blot analysis demonstrated the induction of a humoral immune response against auto-RCC. Two of the four patients are currently alive 58 and 40 months after the initial vaccination with low-dose interleukin-2. Our results suggest that GVAX substantially enhanced the antitumor cellular and humoral immune responses, which might have contributed to the relatively long survival times of our patients in the present study.

Yamada, K., A. Ramezani, et al. (2003). "Phenotype correction of fanconi anemia group a hematopoietic stem cells using lentiviral vector." Molecular Therapy **8**(4): 600.

<http://www.sciencedirect.com/science/article/B6W NJ-4967F4P-2/2/b8015fc7290ec41a9c75bce0990aa49e>

Fanconi anemia (FA) is an autosomal recessive disease characterized by progressive bone marrow failure due to defective stem cell function. FA patients' cells are hypersensitive to DNA cross-linking agents such as mitomycin C (MMC), exposure to which results in cytogenetic aberrations and cell death. To date Moloney murine leukemia virus vectors have been used in clinical gene therapy. Recently, third-generation lentiviral vectors based on the HIV-1 genome have been developed for efficient gene transfer to hematopoietic stem cells. We generated a self-inactivating lentiviral vector expressing the FA group A cDNA driven by the murine stem cell virus U3 LTR promoter and used the vector to transduce side-population (SP) cells isolated from bone marrow of Fanconi anemia group A (Fanca) knockout mice. One thousand transduced SP cells reconstituted the bone marrow of sublethally irradiated Fanca recipient mice. Phenotype correction was demonstrated by stable hematopoiesis following MMC challenge. Using real-time PCR, one proviral vector DNA copy per cell was detected in all lineage-committed cells in the peripheral blood of both primary and secondary recipients. Our results suggest that the lentiviral vector transduces stem cells capable of self-renewal and long-term hematopoiesis in vivo and is potentially useful for clinical gene therapy of FA hematopoietic cells.

Zhao, S., M. A. Weinreich, et al. (2004). "In Vivo Selection of Genetically Modified Erythroid Cells Using a Jak2-Based Cell Growth Switch." Molecular Therapy **10**(3): 456.

<http://www.sciencedirect.com/science/article/B6W NJ-4CVX3HM->

1/2/22b6bc4aa85b9cbc08e047206d7cf951

Cell-based therapies have potential widespread applications in clinical medicine, and methods for controlling the fate of transplanted cells are needed. We have previously described a means for directing the growth of genetically modified cells in vivo using a derivative of the thrombopoietin receptor, mpl, that is reversibly activated by a drug called a chemical inducer of dimerization (CID). Since Jak2 participates in signaling from a number of different cytokine receptors (including mpl), we tested whether direct activation of the JH1 domain of Jak2 would broaden the repertoire of hematopoietic lineages responsive to the CID. While the engineered Jak2 induced a significant rise in genetically modified red cells, as we have observed previously with mpl, it lacked mpl's ability to expand genetically modified platelets and failed to expand genetically modified granulocytes, B cells, or T cells. These findings identify a signaling molecule other than mpl that can function as a cell growth switch in vivo and demonstrate that signaling molecules used for in vivo selection need not be confined to receptors. The erythroid-restricted growth response suggests that CID-activated Jak2 may be well suited to gene therapy applications in sickle cell anemia or [beta]-thalassemia.

Mutagenesis (5)

Breton, J., F. Sichel, et al. (2003). "Simultaneous use of DGGE and DHPLC to screen TP53 mutations in cancers of the esophagus and cardia from a European high incidence area (Lower Normandy, France)." *Mutagenesis* **18**(3): 299-306.

<http://mutage.oupjournals.org/cgi/content/abstract/18/3/299>

We investigated TP53 mutation patterns in cancers of the esophagus and cardia of patients coming from Lower Normandy, a region situated in the highest incidence area in Europe. To screen tumor samples, we first used denaturing gradient gel electrophoresis (DGGE), a well-characterized technique which constituted our reference method. Then the results were compared with those obtained by denaturing high performance liquid chromatography (DHPLC), a recent and automatic screening technology. Analysis of the TP53 mutations profile showed that the detected alterations were mainly point mutations. Ninety-seven percent (33/34) of esophageal squamous cell carcinoma samples presented at least one mutation or polymorphism. The proportion of somatic, non-silent and sequence-confirmed mutations was 76% (26/34). The most common substitutions were G->A transitions, which could be related to nitrosamines, acetaldehyde or factors prone to producing mucosal irritation, like hot beverages. G->T transversions, which were also frequently detected, could originate from benzo[a]pyrene in tobacco smoke. A->T transversions were not revealed in our series, which constitutes a discordance with mutational spectra already performed in north-western France. Concerning adenocarcinoma of the esophagus and cardia, the alteration frequency was 69% (11/16), with a majority of G->A transitions at CpG dinucleotides. They are probably related to endogenous process mediated by inflammatory diseases like gastro-esophageal reflux and Barrett's esophagus. The main advantage provided by DHPLC was its ease of application. However, the optimization steps turned out to be quite critical, especially for sequences with high melting temperatures embedded in lower melting temperature fragments. Considering only the common sequences analyzed by the two techniques, four of the 46 positive samples detected by DGGE were not revealed by DHPLC. This result stresses the limited sensitivity of DHPLC compared with DGGE under the conditions described in this study.

Chen, T., K. Harrington-Brock, et al. (2002). "Mutant frequencies and loss of heterozygosity induced by N-ethyl-N-nitrosourea in the thymidine kinase gene of L5178Y/Tk+/-3.7.2C mouse lymphoma cells." Mutagenesis **17**(2): 105-109.

<http://mutage.oupjournals.org/cgi/content/abstract/17/2/105>

N-ethyl-N-nitrosourea (ENU) is a potent monofunctional ethylating agent that has been found to be mutagenic in a wide variety of organisms from viruses to mammalian germ cells. To elucidate the mutagenicity of ENU at the Tk+/- locus of mouse lymphoma cells and to confirm the ability of the mouse lymphoma assay (MLA) to detect both point mutations and large DNA alterations, Tk+/- L5178Y cells were exposed to different doses of ENU. Treatment of the cells with ENU resulted in a linear dose response with mutant frequencies of up to 16-fold over control. Evaluation of mutant clone size showed that 36% of the 100 {micro}g/ml ENU-induced clones (66% in control) were small colony mutants and 64% (34% in control) were large colony mutants. DNA isolated from mutants in the control culture and the 100 {micro}g/ml ENU treatment group was analyzed for loss of heterozygosity (LOH) using allele-specific PCR. The majority of the small colony mutants, both ENU-treated (97%) and spontaneous (91%), lost the Tk1b allele. The percentage of allele loss in ENU-induced large colony mutants was distinctly different from that of the control. Twenty-three percent of ENU-induced large colony mutants lost their Tk1b alleles, whereas 73% of the large colony mutants from the control culture lost the allele ($P < 0.001$). Overall, 50% of the Tk mutants from the 100 {micro}g/ml ENU-treated cultures (86% in control) showed LOH. Our data indicate that ENU is a potent mutagen in mouse lymphoma cells and that 100 {micro}g/ml ENU induces equal numbers of point mutations and chromosomal mutations. This study serves to verify that the MLA detects both point mutations and chromosomal mutations.

Liu, S. X., J. Cao, et al. (2003). "Molecular analysis of Tripterygium hypoglaucum (level) Hutch-induced mutations at the HPRT locus in human promyelocytic leukemia cells by multiplex polymerase chain reaction." Mutagenesis **18**(1): 77-80.

<http://mutage.oupjournals.org/cgi/content/abstract/18/1/77>

The genotoxicity and cytotoxicity of a Chinese medicinal herb, Tripterygium hypoglaucum (level) Hutch (THH), was investigated in human promyelocytic leukemia (HL-60) cells using the hypoxanthine-guanine phosphoribosyltransferase mutation assay. THH showed clear cytotoxicity and mutagenicity in HL-60 cells at concentrations between 6.7 and 20.0 mg/ml. When the mutants were characterized by techniques based on multiplex PCR, 46.6% of induced mutants were found to have deletions, whereas only 7.7% of spontaneous mutants showed deletions. The rest were not characterized, but were assumed to be mainly point mutations. Mapping of all intragenic deletion breakpoints showed a random distribution of breakpoints in nine exons. Deletion of exon 1 appeared as the only whole gene deletion, while deletions of exon 7/8 and 9 often occurred concomitantly (71.4%). It is concluded that THH is mutagenic in HL-60 cells, predominantly inducing deletions. Since this herb is widely used as a traditional medicine, its genotoxicity should be assessed in vivo in treated humans.

Recio, L., M. Donner, et al. (2004). "In vivo mutagenicity and mutation spectrum in the bone marrow and testes of B6C3F1 lacI transgenic mice following inhalation exposure to ethylene oxide." Mutagenesis **19**(3): 215-222.

<http://mutage.oupjournals.org/cgi/content/abstract/19/3/215>

The lacI mutant frequency and mutation spectrum were determined in the bone marrow and testes of B6C3F1 lacI transgenic mice exposed by inhalation to ethylene oxide (EO). Groups of male transgenic lacI B6C3F1 mice were exposed to 0, 25, 50, 100 or 200 p.p.m. EO for up to 48 weeks (6 h/day, 5 days/week) and were killed at 12, 24 or 48 weeks of EO exposure for determination of lacI mutant frequency. In the bone marrow, the lacI mutant frequency was significantly increased at the two highest exposure levels (100 and 200 p.p.m.) and at the 48 week exposure time point. The shape of the exposure-response curve for lacI mutant frequency in the bone marrow was non-linear. DNA sequence analysis of the bone marrow mutation spectrum revealed that only AT[->]TA transversions occurred at an increased frequency in EO-exposed mice: 25.4% in EO-exposed mice for 48 weeks (200 p.p.m.) compared with 1.4% in air controls. In testes, the lacI mutant frequency was increased at a single exposure level of 200 p.p.m. for 24 weeks. At 48 weeks, the lacI mutant frequency in testes was significantly increased to an equal degree at 25, 50 and 100 p.p.m. EO but not at 200 p.p.m. Analysis of the testes mutation spectrum in air control mice and in mice exposed to 200 p.p.m. EO for 48 weeks revealed that no single mutational type occurred at an increased frequency. In the testes, there was a small increase across all mutational types that was sufficient to increase the overall lacI mutation frequency although not significant individually. The mutation spectrum in testes of EO-exposed mice also revealed that the increased lacI mutant frequency observed at 25 or 50 p.p.m. EO was not due to an increase in mutant siblings (clonality). These data demonstrate that inhalation exposure to EO for up to 48 weeks produces distinct mutagenic responses in bone marrow and testes.

Singh, K. P., J. A. Lopez-Guerrero, et al. (2004). "Age, sex and co-exposure to N-ethyl-N-nitrosourea influence mutations in the Alu repeat sequences in diethylstilbestrol-induced kidney tumors in Syrian hamsters." *Mutagenesis* **19**(1): 67-73.

<http://mutage.oupjournals.org/cgi/content/abstract/19/1/67>

We report, for the first time, mutations in the Alu repeat regions in the genome of kidney tumors induced by diethylstilbestrol in Syrian hamsters. Among the 66 loci amplified by 11 random primers, 28 loci exhibited insertions, deletions or losses or gains in intensity in the genome of kidney tumor tissues compared with normal kidney tissues from age-matched hamsters. Higher numbers of mutated Alu loci were observed in the tumors of old hamsters compared with young hamsters. In N-ethyl-N-nitrosourea- and diethylstilbestrol-treated hamsters deletion of a 0.59 kb locus amplified with primer OPC03 was observed in most of the female hamsters, but not in male hamsters. An insertion mutation of a 0.498 kb locus amplified with primer OPC03 was observed in 12 of 36 diethylstilbestrol-induced kidney tumors. The cloning and sequencing of the 0.498 kb locus amplified with primer OPC03 revealed that it had significant sequence similarity to the mouse RIKEN cDNA clone. These findings indicate that age, sex and co-exposure to N-ethyl-N-nitrosourea influence mutations in the Alu repeat sequences in the genome of diethylstilbestrol-induced kidney tumors in Syrian hamsters. Structural alterations in Alu repeats in critical target genes may be involved in diethylstilbestrol-induced carcinogenesis.

An, J. and A. W. Hsie (1993). "Polymerase chain reaction-based deletion screening of bleomycin induced 6-thioguanine-resistant mutants in Chinese hamster ovary cells: The effects of an inhibitor and a mimic of superoxide dismutase." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **289**(2): 215.

<http://www.sciencedirect.com/science/article/B6T2C-47PCN7M-41/2/3157a48d39359d02b082784374179db8>

Bleomycin-induced 6-thioguanine-resistant mutants pretreated with or without TRIEN (triethylene-tetramine), a superoxide dismutase (SOD) inhibitor, or TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl), an SOD mimic, were analyzed by polymerase chain reaction (PCR)-based deletion screening in a Chinese hamster ovary (CHO) clone K1-BH4 and its derivative AS52 cells. As we proposed earlier, TRIEN would decrease and TEMPOL would increase the intracellular level of hydroxyl radical leading to a higher and lower recovery of deletion mutants. We found that the proportion of the deletion mutants induced by bleomycin at the hypoxanthine-guanine phosphoribosyltransferase (hprt) locus in K1-BH4 cells was 45.5%. The proportion of deletion HPRT- mutants induced by bleomycin pretreated with TRIEN was 31.0% and with TEMPOL was 50.0%. The proportion of deletion mutants induced by bleomycin on the xanthine-guanine phosphoribosyltransferase (gpt) gene in AS52 cells was 61.0%. The proportion of deletion GPT- mutants induced by bleomycin pretreated with TRIEN was 56.8% and with TEMPOL was 61.4%. The trend of the change of the proportion of bleomycin-induced deletion mutants as affected by TRIEN and by TEMPOL provides molecular evidence for the involvement of reactive oxygen species (ROS) in bleomycin mutagenesis in mammalian cells, in which deletion is a major type of induced mutation.

Andreassen, A., L. Mollersen, et al. (2002). "One dose of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) or 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) induces tumours in Min/+ mice by truncation mutations or LOH in the Apc gene." Mutation Research/Genetic Toxicology and Environmental Mutagenesis **517**(1-2): 157.

<http://www.sciencedirect.com/science/article/B6T2D-45KT0KY-3/2/8c513c577d359ce72d07305926844f43>

The C57BL/6J-Min/+ (multiple intestinal neoplasia) mouse has a heterozygous nonsense ApcMin (adenomatous polyposis coli) mutation, and numerous adenomas spontaneously develop in the intestine. Neonatal exposure of Min/+ mice to the food carcinogens 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) or 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) (one injection of 50 mg/kg) increased the number of small intestinal tumours about three- and two-fold, respectively. The number of colonic tumours was only increased in males. We examined whether the wild-type Apc allele was affected in intestinal tumours induced by either PhIP or IQ. In spontaneously formed and in IQ-induced small intestinal and colonic tumours from these mice, the main mechanism for tumour induction was loss of wild-type Apc allele, i.e. loss of heterozygosity (LOH). In contrast to the IQ-induced (84% LOH) and spontaneously (88% LOH) formed tumours, only 55% of the PhIP-induced small intestinal tumours from males showed LOH. Tumours that apparently had retained the wild-type Apc allele were further analysed for the presence of truncated Apc proteins by the in vitro synthesised protein (IVSP) assay. Truncated Apc proteins, indicating truncation mutations in exon 15 of the Apc gene, were detected in two of the 12 PhIP-induced tumours in segment 2 (codons 686-1217), and two of five IQ-induced tumours, one in segment 2 and the other in segment 3 (codons 1099-1693). Three of these four mutations, all in segment 2 of the Apc gene, were confirmed by sequencing. The PhIP-induced mutations were detected at codon 1125 (C deletion) and 1130 (G-T transversion), and the IQ-induced mutation was at codon 956 (C-T transition). Importantly, no truncated proteins were detected in tumours from unexposed mice with apparently retained wild-type Apc allele. These results show that one injection of either PhIP or IQ induces intestinal tumours in the Min/+ mice

by inactivation of the wild-type Apc allele either by causing LOH or truncation mutations.

Bell, D. A., J. G. Levine, et al. (1991). "DNA sequence analysis of revertants of the hisD3052 allele of *Salmonella typhimurium* TA98 using the polymerase chain reaction and direct sequencing: Application to 1-nitropyrene-induced revertants." Mutation Research/Environmental Mutagenesis and Related Subjects **252**(1): 35.

<http://www.sciencedirect.com/science/article/B73H5-47RNW7X-4Y/2/b29592180fa2fd00c8e8df584ca00592>

We have used the polymerase chain reaction (PCR) to speed the DNA sequence analysis of revertants of *Salmonella typhimurium* TA98. Briefly, a crude DNA extract from a single colony was prepared and used in an asymmetric PCR to amplify a 328-bp fragment containing the hisD3052 mutation approximately in the center. Following ultrafiltration, the ssDNA was sequenced using an end-labeled probe and dideoxy sequencing. The most frequent mutation among the revertants was a -2 deletion of GC or CG within the sequence CGCGCGCG, which is upstream of the hisD3052 mutation. This deletion occurred in 38% (6/16) of the spontaneous (-S9) revertants and in 94% (15/16) of a set of 1-nitropyrene-induced revertants. Other mutations, mostly deletions but also some complex mutations (i.e., single mutational events involving a combination of insertions, deletions, and substitutions), occurred within quasipalindromic regions of DNA. Possible mutational mechanisms are discussed, and the results with 1-NP are compared to those obtained in other systems.

Bellanne-Chantelot, C., S. Beaufils, et al. (1997). "Search for DNA sequence variations using a MutS-based technology." Mutation Research/Mutation Research Genomics **382**(1-2): 35.

<http://www.sciencedirect.com/science/article/B6T2F-3SY9RHW-5/2/e07fdfe956294f9c0abaa386b2beb8ac>

The search for DNA sequence variations (DSV) is emphasized with genetic studies of a large number of multifactorial diseases. Saturation of regions of interest with diallelic polymorphisms will be an essential step to pinpoint, through association studies, predisposing genes. We have developed a solid-phase method based on the ability of mismatch binding protein MutS to recognize single nucleotide mismatches. This approach was applied to the study of 83 sequence-tagged sites (STSs) extracted from an eight centimorgans (cM) chromosome 21 region. One-third of tested STSs were found to be polymorphic leading to a frequency of one DSV every 822 base pairs (bp). Sequencing of analyzed STSs showed the high reliability of the MutS-based technology for mismatches up to 2 bp in DNA fragments ranging in size from 200 bp to 1 kilobase (kb). The entire assay which is performed in a solid-phase format without the need of electrophoresis or sequencing, will provide an efficient tool for new polymorphism detection.

Bigbee, W. L., J. C. Fuscoe, et al. (1998). "Human in vivo somatic mutation measured at two loci: individuals with stably elevated background erythrocyte glycophorin A (gpa) variant frequencies exhibit normal T-lymphocyte hprt mutant frequencies." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **397**(2): 119.

<http://www.sciencedirect.com/science/article/B6T2C-3SY8DSV-1/2/dceccc39de58bdd5e7786012edb9cb14>

A survey of glycophorin A (gpa) in vivo somatic cell mutation in a population of 394 healthy people from 8 to 77 years of age (mean age $\pm$ SD 41 $\pm$ 15 years) revealed a subset of 37 individuals with stably elevated allele-loss and/or allele-loss with duplication variant erythrocyte frequencies (Vf) exceeding 30×10^{-6} . These 37 individuals with gpa outlier Vf are significantly older (phprt mutant frequencies (Mf) in the peripheral blood T-lymphocytes of 27 of these individuals, together with 15 matched control individuals with unremarkable gpa Vf, was undertaken to determine if these subjects also displayed elevated mutation frequencies at this independent locus indicative of globally elevated somatic mutation. The hpert Mf in these 27 subjects (geometric mean 11.5×10^{-6} (dispersion interval 5.8×10^{-6} to 22.8×10^{-6})) was not significantly different from that observed in the 15 controls (geometric mean 12.1×10^{-6} (dispersion interval 5.7×10^{-6} to 25.5×10^{-6})). These Mf are higher than typically reported values reflecting the older age distribution of these individuals (arithmetic mean age $\pm$ SD 53 $\pm$ 12 and 50 $\pm$ 16 years for the subjects and controls, respectively). Taken together, these data suggest that several genetic mechanisms may be responsible for producing the gpa outlier Vf observed in these subjects. The observation that hpert Mf were not increased indicates that the majority did not arise by a genome-wide increased rate of somatic mutation detectable at both loci. The fixation and subsequent expansion of 'jackpot' mutations at the gpa locus occurring early in embryonic/fetal development also does not appear to be a predominant mechanism. Some cases may result from a stable over-representation of gpa variant cells, perhaps associated with a marked age-dependent decrease in the number of contributing erythroid stem cells in the bone marrow. The subset that displays elevated allele-loss with duplication Vf involving both gpa alleles may represent individuals with increased rates of somatic recombination. Elevations arising by this mechanism are not detected in the hpert assay, but could be confirmed using an autosomal locus in vivo somatic cell mutation endpoint such as the hla-a assay. Of primary biological significance, these results demonstrate that genetic/stochastic processes leading to the loss of heterozygosity of somatic cells occur ubiquitously in humans and in some individuals this level of somatic mosaicism can approach a frequency of 10^{-3} at the gpa locus in erythroid lineage cells.

Boei, J. J. W. A., S. Vermeulen, et al. (1996). "Detection of chromosomal aberrations by fluorescence in situ hybridization in the first three postirradiation divisions of human lymphocytes." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **349**(1): 127.

<http://www.sciencedirect.com/science/article/B6T2C-40PRW3G-F/2/cad6907bf4b24fae3fb31320e5a520c7>

Chromosomal aberrations in human lymphocytes were analyzed by fluorescence in situ hybridization (FISH) in the first 3 postirradiation (0 and 2 Gy) divisions. Cells were grown in the presence of BrdU, collected at different sampling times (47, 70 and 91 h) and analyzed using an alphoid centromeric probe and PCR amplified DNA libraries for chromosomes 2 and 8. Following differential staining of sister chromatids, the analyzed cells were identified to be either in the first, second or third mitosis after irradiation. The frequencies of both dicentric and fragments showed a reduction of about 50% after each cell generation, whereas translocations were more persistent. Cells within the same postirradiation division showed higher aberration frequencies when derived from later sampling times, indicating a delay in progression of aberrant cells. As a result, the frequencies for dicentric and fragments remained rather constant at different sampling times if the cell cycle parameter was not taken into account. Thus, the average generation time of the lymphocytes had a clear effect on the obtained aberration frequencies. The described method allows the study of the persistence of chromosome damage using the FISH technique during 3 subsequent cell divisions in vitro.

Branda, R. F., A. R. Lafayette, et al. (1999). "The effect of folate deficiency on the hpert mutational

spectrum in Chinese hamster ovary cells treated with monofunctional alkylating agents." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **427**(2): 79.

<http://www.sciencedirect.com/science/article/B6T2C-3WV9HMS-2/2/d568691d665e64e6793932c04b52aaf2>

Folic acid deficiency acts synergistically with alkylating agents to increase DNA strand breaks and mutant frequency at the hprt locus in Chinese hamster ovary (CHO) cells. To elucidate the mechanism of this synergy, molecular analyses of hprt mutants were performed. Recently, our laboratory showed that folate deficiency increased the percentage of clones with intragenic deletions after exposure to ethyl methanesulfonate (EMS) but not N-nitroso-N-ethylurea (ENU) compared to clones recovered from folate replete medium. This report describes molecular analyses of the 37 hprt mutant clones obtained that did not contain deletions. Folate deficient cells treated with EMS had a high frequency of G>A transitions at non-CpG sites on the non-transcribed strand, particularly when these bases were flanked on both sides by G:C base pairs. Thirty-three percent of these mutations were in the run of six G's in exon 3. EMS-treated folate replete cells had a slightly (but not significantly) lower percentage of G>A transitions, and the same sequence specificity. Treatment of folate deficient CHO cells with ENU resulted in predominantly T>A transversions and C>T transitions relative to the non-transcribed strand. These findings suggest a model to explain the synergy between folate deficiency and alkylating agents: (1) folate deficiency causes extensive uracil incorporation into DNA; (2) greatly increased utilization of base excision repair to remove uracil and to correct alkylator damage leads to error-prone DNA repair. In the case of EMS, this results in more intragenic deletions and G:C to A:T mutations due to impaired ligation of single-strand breaks generated during base excision repair and a decreased capacity to remove O6-ethylguanine. In the case of ENU additional T>A transversions and C>T transitions are seen, perhaps due to mis-pairing of O2-ethylpyrimidines. Correction of folate deficiency may reduce the frequency of these types of genetic damage during alkylator therapy.

Branda, R. F., J. P. O'Neill, et al. (2001). "The effect of folate deficiency on the cytotoxic and mutagenic responses to ethyl methanesulfonate in human lymphoblastoid cell lines that differ in p53 status." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **473**(1): 51.

<http://www.sciencedirect.com/science/article/B6T2C-428FM50-6/2/0413baa0368923fdb3f01c5926ccffcc>

Folic acid deficiency acts synergistically with alkylating agents to increase genetic damage at the HPRT locus in Chinese hamster ovary cells in vitro and in rat splenocytes in vivo. The present studies extend these observations to human cells and, in addition, investigate the role of p53 activity on mutation induction. The human lymphoblastoid cell lines TK6 and WTK1 are derived from the same parental cell line (WI-L2), but WTK1 expresses mutant p53. Treatment of folate-replete or deficient WTK1 and TK6 cells with increasing concentrations (0-50 [μg/ml] of ethyl methanesulfonate (EMS) resulted in significantly different HPRT mutation dose-response relationships (G>A transition on the non-transcribed strand. These transitions were mainly at non-CpG sites, particularly when these bases were flanked 3' by a purine or on both sides by G:C base pairs. A smaller number of G>A transitions occurred on the transcribed strand (C>T=14%), resulting in 79% total G:C>A:T transitions. There were more genomic deletions in folate-deficient (15%) as compared to replete cells (4%) of both cell types. Mutations that altered RNA splicing were common in both cell types and under both folate conditions, representing 33% of the total mutations. These studies indicate that cells expressing p53 activity exhibit a higher rate of mutation induction but are more sensitive to the toxic effects of alkylating agents than those lacking p53 activity. Folate deficiency tends to reduce toxicity but increase mutation induction after EMS treatment. The p53 gene product did not have a major influence on the molecular spectrum after treatment with EMS, while folate deficiency increased the frequency of deletions in

both cell types.

Brisebois, J. J. and M. S. DuBow (1993). "Selection for spontaneous null mutations in a chromosomally-integrated HSV-1 thymidine kinase gene yields deletions and a mutation caused by intragenic illegitimate recombination." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **287**(2): 191.

<http://www.sciencedirect.com/science/article/B6T2C-47PCN50-2R/2/557d85d561bc6f79e3c1ce737a92e2bb>

Spontaneous null mutations represent low frequency events that irreversibly and completely inactivate a gene, and can often consist of major gene alterations. To study the molecular mechanisms leading to recessive spontaneous null mutations in the human genome, we designed and tested a selection procedure in cell culture to enrich for this rare class of spontaneous mutations. The KT cell line contains the herpes simplex virus type 1 (HSV-1) thymidine kinase (tk) gene and the neomycin-resistance gene (neo), from plasmid pSV2neoKT, integrated as a single-copy in the human tk- cell line 143B. The HSV-1 tk gene was the target for spontaneous gene inactivation, and antiviral drugs (acyclovir, trifluorothymidine and ganciclovir) were used, in combination, to provide a selective enrichment for null mutations over the background of more frequent and revertible point mutations. The tk- mutations obtained with this multiple drug selection assay appeared at a very low frequency, rarely reverted to wild-type (tk+), and the TK protein was observed only in 4.8% of these null mutants. Deletions of the entire tk gene, or its 3' region, constituted the major class of DNA rearrangements seen in the null mutations. Additionally, one of the null mutants contained an intragenic 106-bp duplication within a 43-bp deleted region of the tk gene. We propose this mutation to be the outcome of an intragenic gene conversion event which may have been facilitated by short regions of junctional homology.

Brooks, E. M., R. F. Branda, et al. (2001). "Molecular description of three macro-deletions and an Alu-Alu recombination-mediated duplication in the HPRT gene in four patients with Lesch-Nyhan disease." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **476**(1-2): 43.

<http://www.sciencedirect.com/science/article/B6T2C-42YW37B-5/2/1486006039ca23f1f5b58112cd3868a3>

Mutations in the HPRT gene cause a spectrum of diseases that ranges from hyperuricemia alone to hyperuricemia with profound neurological and behavioral dysfunction. The extreme phenotype is termed Lesch-Nyhan syndrome. In 271 cases in which the germinal HPRT mutation has been characterized, 218 different mutations have been found. Of these, 34 (13%) are large- (macro-) deletions of one exon or greater and four (2%) are partial gene duplications. The deletion breakpoint junctions have been defined for only three of the 34 macro-deletions. The molecular basis of two of the four duplications has been defined. We report here the breakpoint junctions for three new deletion mutations, encompassing exons 4-8 (20 033 bp), exons 4 and 5 (13 307 bp) and exons 5 and 6 (9454 bp), respectively. The deletion breakpoints were defined by a combination of long polymerase chain reaction (PCR) amplifications, and conventional PCR and DNA sequencing. All three deletions are the result of non-homologous recombinations. A fourth mutation, a duplication of exons 2 and 3, is the result of an Alu-mediated homologous recombination between identical 19 bp sequences in introns 3 and 1. In toto, two of three germinal HPRT duplication mutations appear to have been caused by Alu-mediated homologous recombination, while only one of six deletion mutations appears to have resulted from this type of recombination mechanism. The other five deletion mutations resulted from non-homologous

recombination. With this admittedly limited number of characterized macro-mutations, Alu-mediated unequal homologous recombinations account for at least 8% (3 of 38) of the macro-alterations and 1% (3 of 271) of the total HPRT germinal mutations.

Burvall, K., M. Ridanpaa, et al. (1993). "Separation of transforming N-ras mutations in codons 12, 13 and 61 by denaturing gradient gel electrophoresis: investigation based on a set of phagemid constructs." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **285**(2): 287.

<http://www.sciencedirect.com/science/article/B6T2C-47PCNK6-8F2/c0a5dc38c987023d4c455a109a7fce9c>

In the present study we have introduced 19 activating base pair substitutions into N-ras cDNA by use of an in vitro site-directed mutagenesis system. Six mutants were constructed for N-ras codon 12 (exon 1), six for codon 13 (exon 1), and seven for codon 61 (exon 2). Fifteen out of 19 PCR-amplified mutation sequences showed a clear separation from the wild type of denaturing gradient gel electrophoresis runs as homoduplex band, and the rest could be separated after heteroduplex formation with wild-type DNA. These constructs can be used as controls in many screening systems for analyzing activating point mutations of the N-ras gene.

Cattaneo, M., M. Zollo, et al. (2001). "Allelic polymorphisms in the transcriptional regulatory region of human SEL1L." Mutation Research/Mutation Research Genomics **458**(3-4): 71.

<http://www.sciencedirect.com/science/article/B6T2F-453BS2D-2/2/6a0ce9b51263125ba3168099e89b3fef>

In this work, we explored the existence of genetic variants within the SEL1L transcriptional regulatory region by direct sequencing of the basal promoter. SEL1L is the human ortholog of the *Caenorhabditis elegans* gene *sel-1*, a negative regulator of LIN-12/NOTCH receptor proteins. To understand the relation in SEL1L transcription pattern observed in different epithelial cells, we analysed its promoter activity. We found it to be considerably higher only in pancreatic cells. We then looked for the presence of genetic variability within this region by sequencing the minimal promoter of 63 individuals (126 alleles); two new and associated polymorphic variants were found only in few lung carcinoma bearing patients. The functional effects of this polymorphism was analysed by transient transfection assay which resulted in a significant increase in the transcriptional activity of the gene.

Chakravarti, D., P. C. Mailander, et al. (2000). "Evidence that error-prone DNA repair converts dibenzo[a,l]pyrene-induced depurinating lesions into mutations: formation, clonal proliferation and regression of initiated cells carrying H-ras oncogene mutations in early preneoplasia." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **456**(1-2): 17.

<http://www.sciencedirect.com/science/article/B6T2C-41P18S3-2/2/f724e51e501aa29c3bd92e563fee9eeb>

Initiation of skin tumors in mice is associated with the formation of oncogenic mutations in the H-ras gene. Mice treated on the dorsal skin with the potent polycyclic aromatic hydrocarbon (PAH) carcinogen dibenzo[a,l]pyrene (DB[a,l]P) form papillomas carrying the H-ras codon 61 (CAA to CTA) mutations. These mutations are induced in early preneoplastic skin within 1 day after

DB[a,l]P treatment (Oncogene 16 (1998) 3203-3210) and appear to be related to DB[a,l]P-Ade-depurinating adducts (Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 10422-10426). The rapid kinetics of mutation induction suggests that abasic sites generated from base depurination may undergo error-prone excision repair in pre-S-phase cells to induce these mutations. Analysis of mutations in the H-ras exon 1 and 2 region in DB[a,l]P-treated early preneoplastic skin indicated great changes in mutation spectra in the preneoplastic period. The initial spectra contained abundant A->G mutations, which frequently occurred 3' to a putative conserved sequence (TGN-doublet). These mutations appeared to be induced initially as mismatched (G.T) heteroduplexes and then converted into double-stranded mutations by one round of replication. Unlike the A->G mutations found in DB[a,l]P-treated skin (which forms 99% depurinating adducts), A->G mutations found in anti-DB[a,l]P-diol epoxide-treated skin (forms 97% stable adducts) did not appear to be G.T heteroduplexes. These results, therefore, suggest that under these conditions, the repair errors occurred only from abasic sites but not from stable adducts. Initiated cells carrying specific oncogenic mutations, formed presumably by misrepair, underwent rapid clonal expansion and regression (transient clonoplasia). The multiplication of initiated stem cells during transient clonoplasia may be a factor determining the tumor-initiating potential of some PAH carcinogens.

Chen, J. B., V. N. Dobrovolsky, et al. (1999). "Development of a mouse cell line containing the [Phi]X174 am3 allele as a target for detecting mutation." Mutation Research/Genetic Toxicology and Environmental Mutagenesis **444**(2): 347.

<http://www.sciencedirect.com/science/article/B6T2D-3XNJV81-8/2/2d3846166dcb3891bb56e76c9d1be2db>

Transgenic mice containing multiple copies of the [Phi]X174 am3 allele are being developed as a model for detecting tissue-specific in vivo mutation. In order to derive an analogous system for measuring am3 mutation in vitro, cells were cultured from 15-day-old C57Bl/6J mouse embryos that were homozygous for the transgene and these cells were transfected with a plasmid expressing the SV40 large T-antigen. Two G418-resistant colonies were isolated from this culture and expanded to continuously proliferating cell lines (PX-1 and PX-2). Line PX-2 was treated with up to 1.0 mg/ml of N-ethyl-N-nitrosourea (ENU), assayed for survival by cloning efficiency after overnight culture, and assayed for am3 mutations after 5 days of culture. Survival decreased to 31% at the highest dose of ENU, while mutant frequency increased with dose from approximately 2×10^{-7} in the untreated cells to 13×10^{-7} in cultures treated with 0.6 mg/ml of ENU. PX-2 cells also were treated with 0 and 0.6 mg/ml of ENU and mutant frequency assays were performed after 5, 24, 48 and 72 h of growth. The mutant frequency in the treated culture increased to 20×10^{-7} at 48 h and remained approximately the same at 72 h. These results indicate that PX-2 cells should be a useful resource for developing the in vivo am3 mutant assay and for evaluating the sensitivity of the am3 allele to various classes of mutagens.

Colussi, N., X. van Leeuwen, et al. (1998). "Similar mutational spectra in the HPRT gene of human and hamster cell lines after exposure to either low dose rate or high dose rate X-rays." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **401**(1-2): 89.

<http://www.sciencedirect.com/science/article/B6T2C-3SY9PXB-N/2/e2534d8253f442c590663d195b58c787>

The dose rate at which cells are exposed to X-rays may influence the nature of induced mutations. To investigate this, the molecular spectra were determined at the HPRT gene in a hamster (V79) and a human (WI-L2-NS) cell line after the same total dose of X-rays has been administered at either a low dose rate (LDR; 3.33 mGy/min) or a high dose rate (HDR; 1.24-1.4 Gy/min) X-irradiation. Mutational spectra appeared similar, the fraction of mutants carrying

deletions ranging between 59%-66% for the V79 strain and between 64%-75% for the WI-L2-NS strain, and independent of the irradiation conditions. The data indicate no effect of ongoing repair processes under LDR conditions on the kind of induced mutations in mammalian cells.

Conforti-Froes, N., R. El-Zein, et al. (1997). "Predisposing genes and increased chromosome aberrations in lung cancer cigarette smokers." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **379**(1): 53.

<http://www.sciencedirect.com/science/article/B6T2C-3RC53GM-6/2/75c4af4aeb5ad57d13ea56b696c100c4>

Genotoxic effects linking cigarette smoking with lung cancer have not been consistently demonstrated, therefore claims for the cause-effect relationships are vigorously contested. Using matched populations of 22 lung cancer patients who have been cigarette smokers (LCP), 22 non-cancerous cigarette smokers (SC) and 13 non-smokers (NSC), we have applied the fluorescence in situ hybridization (FISH) tandem probe assay to elucidate the frequency of chromosome breakage among the participants. Two probes were used, a classical satellite probe which hybridizes to the large heterochromatin region of chromosome 1, and an [alpha]-satellite probe which targets a small region adjacent to the heterochromatin probe. The highest frequency of structural aberrations was observed in LCP (1.4+/-0.1) followed by SC (1.25+/-0.1) and NSC (0.4+/-0.1). Aberration frequencies were not significantly different between LCP and SC ($p>0.05$), however, a statistically significant difference was detected between the smoker populations combined (LCP and SC) and the NSC ($p=0.5$; $p0.05$). In addition, the aberration frequencies were influenced by the inheritance of polymorphic glutathione S-transferase (GST) genes. LCPs missing one or the other GST (GSTM1 or GSTT1) genes were found to have significantly higher chromosome breaks compared to LCPs with both genes present ($p<0.05$). Our data indicate that genetic predisposition and chromosome aberrations may be mechanistically related to the initiation of lung carcinogenesis; therefore, they may be useful biomarkers for lung cancer among cigarette smokers.

Dabholkar, M., F. Bostick-Bruton, et al. (1993). "Expression of excision repair genes in non-malignant bone marrow from cancer patients." Mutation Research/DNA Repair **293**(2): 151.

<http://www.sciencedirect.com/science/article/B6T2B-47GGK7W-1Y/2/11e50c161110bdb69633a06e19ba9be2>

The patterns of expression of 3 human DNA-repair genes (ERCC1, ERCC2, ERCC6) were assessed in 52 bone-marrow specimens obtained from cancer patients prepared for autologous bone-marrow transplantation. Marrow was collected prior to the initiation of treatment in patients with sarcoma or testicular cancer; marrow was collected after initial cytoreductive therapy for patients with non-Hodgkin's lymphoma, Hodgkin's disease, and other tumors. Slot-blot analysis of marrow RNA showed a bimodal pattern of ERCC1, ERCC2 and ERCC6 gene expression with relative expression values ranging more than 200-fold. This pattern was seen in all patient groups and appeared to be independent of whether or not patients had received prior chemotherapy. In all patient groups, when expression was low for ERCC1, expression was also low for ERCC2 and ERCC6, suggesting that expression of these genes may be coordinated within an individual although they are located on two different chromosomes. Southern blot analyses of Pst I digest of DNA from 6 bone-marrow samples indicate no differences in ERCC1 gene copy number between high expressors and low expressors. There is absence of restriction fragment length polymorphism for ERCC1 suggesting that the different levels of expression in high and low expressors were not due to major deletions or rearrangements of the ERCC1 gene. We conclude that expression of these ERCC genes may vary widely between individuals, and that within an

individual, their expression may be linked and coordinated by a common regulatory mechanism.

de Boer, J. G., J. D. Curry, et al. (1993). "A fast and simple method to determine the clonal relationship among human T-cell lymphocytes." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **288**(1): 173.

<http://www.sciencedirect.com/science/article/B6T2C-47PG610-2R/2/516aef1bb0a56a15e22da65a05c1ed56>

For the analysis of mutation in human T-cell lymphocytes, it is crucial to determine the clonal relationship between isolated mutants, particularly if they harbour identical mutations. Here we report an efficient method to determine the clonal relationships between these cells. The method is based on the analysis of restriction fragment length polymorphisms of a polymerase chain reaction amplified, rearranged T-cell receptor [gamma]-gene. As few as 600 cells are sufficient, regular agarose gels can be used for the separation of the restriction fragments, no radioactive label is required, and results can be obtained in 2 days.

Dempsey, J. L., Y. Odagiri, et al. (1993). "In vivo mutations at the H-2 locus in mouse lymphocytes." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **285**(1): 45.

<http://www.sciencedirect.com/science/article/B6T2C-47PCNHS-7H/2/5099fc5b1fcd2a2c543ea452d628c8fb>

An experimental system has been developed to study in vivo autosomal mutations in murine splenic lymphocytes. Mutant lymphocytes were isolated by immunocytotoxicity using monoclonal antibodies directed against the k and d alleles of the K and D H-2 histocompatibility loci and were enumerated using limiting-dilution cloning. Genomic allele loss in mutant clones was detected using allele-specific primers in a polymerase chain reaction. Mutant clones were classified on the basis of phenotypic and genotypic criteria into "no change", deletion or recombination mutants. The geometric mean mutation frequency in 102 mice was 2.42×10^{-4} . Detailed phenotypic and genotypic study of 87 mutant clones from 4 mice revealed "no change" mutants in 83%, mutants due to deletion in 7% and mutants due to recombination in 7%. Anomalous results were obtained in 3% of mutant clones. The development of an animal model for study of in vivo mutations at an autosomal locus will further advance study of mutations, particularly those involving chromosomal changes such as mitotic recombination.

Denissenko, M. F., J. Cahill, et al. (1999). "Quantitation and mapping of aflatoxin B1-induced DNA damage in genomic DNA using aflatoxin B1-8,9-epoxide and microsomal activation systems." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **425**(2): 205.

<http://www.sciencedirect.com/science/article/B6T2C-3W9D0FD-4/2/1534373a823c488dbeec1e5f85d64788>

Aflatoxin B1 (AFB1) is a mutagenic and carcinogenic mycotoxin which may play a role in the etiology of human liver cancer. In vitro studies have shown that AFB1 adducts form primarily at the N7 position of guanine. Using quantitative PCR (QPCR) and ligation-mediated PCR (LMPCR), we have mapped total AFB1 adducts in genomic DNA treated with AFB1-8,9-epoxide and in hepatocytes exposed to AFB1 activated by rat liver microsomes or human liver and enterocyte microsomal preparations. The p53 gene-specific adduct frequencies in DNA, modified

in cells with 40-400 [μM] AFB1, were 0.07-0.74 adducts per kilobase (kb). In vitro modification with 0.1-4 ng AFB1-8,9-epoxide per microgram DNA produced 0.03-0.58 lesions per kb. The adduct patterns obtained with the epoxide and the different microsomal systems were virtually identical indicating that adducts form with a similar sequence-specificity in vitro and in vivo. The lesions were detected exclusively at guanines with a preference towards GpG and methylated CpG sequences. The methods utilizing QPCR and LMPCR thus provide means to assess gene-specific and sequence-specific AFB1 damage. The results also prove that microsomally-mediated damage is a suitable method for avoiding manipulations with very unstable DNA-reactive metabolites and that this damage can be detected by QPCR and LMPCR.

Denslow, N. D., J. Kocerha, et al. (2004). "Gene expression fingerprints of largemouth bass (*Micropterus salmoides*) exposed to pulp and paper mill effluents." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **552**(1-2): 19.

<http://www.sciencedirect.com/science/article/B6T2C-4CTTR00-1/2/7f4aabca57d9173ae993e5d9f331204c>

Effluents from pulp and paper mills that historically have used elemental chlorine in the bleaching process have been implicated in inhibiting reproduction in fish. Compounds with estrogenic and androgenic binding affinities have been found in these effluents, suggesting that the impairment of reproduction is through an endocrine-related mode of action. To date, a great deal of attention has been paid to phytoestrogens and resin acids that are present in mill process streams as a result of pulping trees. Estrogen and estrogen mimics interact directly with the estrogen receptor and have near immediate effects on gene transcription by turning on the expression of a unique set of genes. Using differential display (DD) RT-PCR, we examined changes in gene expression induced by exposure to paper mill effluents. Largemouth bass were exposed to 0, 10, 20, 40, and 80% paper mill effluent concentrations in large flow-through tanks for varied periods of time including 7, 28 or 56 days. Plasma hormone levels in males and females and plasma vitellogenin (Vtg) in females decreased with dose and time. Measurements of changes in gene expression using DD RT-PCR suggest that the gene expression patterns of male fish do not change much with exposure, except for the induction of a few genes including CYP 1A, a protein that is induced through the action of the Ah receptor in response to dioxin and similar polycyclic aromatic hydrocarbons. However, in the case of females, exposure to these effluents resulted in an up-regulation of CYP 1A that was accompanied by a generalized down-regulation of genes normally expressed during the reproductive season. These antiestrogenic changes are in agreement with previous studies in bass exposed to these effluents, and could result in decreased reproductive success in affected populations.

Ding, B., O. A. Ryder, et al. (2000). "Molecular basis of albinism in the rhesus monkey." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **449**(1-2): 1.

<http://www.sciencedirect.com/science/article/B6T2C-3YYMKKC-15/2/301ea52a5cae420814cc5afe84e64d0c>

Sequence analysis of the tyrosinase (TYR) coding region from one albino rhesus monkey (*Macaca mulatta*) family revealed that the two monkeys with phenotype similar to human TYR-negative oculocutaneous albinism (OCA) were homozygous for a missense mutation (S184TER) in exon 1 at codon 184. The offspring of one of the albino monkey ("Kangkang") are all heterozygous for the S184TER mutation, but the S184TER mutation was not observed in 93 control individuals. We conclude that the point mutation is responsible and sufficient to generate the albino rhesus monkey phenotype. The rough age of the S184TER nonsense mutation may be about 0.8 million years using a rate of 0.16% per million years.

Douglas, M. P. and S. O. Rogers (1998). "DNA damage caused by common cytological fixatives." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **401**(1-2): 77.

<http://www.sciencedirect.com/science/article/B6T2C-3SY9PXB-M/2/46098cfd7bc8353bb62157461a93c55f>

Tissues from nine species of plants and fungi were treated separately with eight solutions, including seven cytological fixatives (3.7% formaldehyde at pH 3.0 and 7.0, FAA at pH 3.0 and 7.0, 1% glutaraldehyde at pH 3.0 and 7.0, and Lavdowsky's fluid at pH 3.0) and one storage buffer (SED=NaCl-EDTA-DMSO, pH 7.0). DNA from untreated tissue and SED-treated tissue was of high molecular weight (>50 kb). DNA from glutaraldehyde-treated tissues averaged 20 kb in length, while DNA from all other treatments averaged less than 8 kb in length. Each DNA was subjected to amplification using the polymerase chain reaction, followed by sequencing of 250 bp near the 3' end of the nuclear rRNA small subunit gene. Glutaraldehyde treatments (at pH 3.0 and 7.0) produced damaged bases at rates of 0.0% to less than 0.1%. Treatments with Lavdowsky's fluid (containing mercuric chloride), FAA at pH 7.0, and SED produced rates of 0.0% to 3.6%. FAA at pH 3.0 produced rates of 7.6% to 15.6%. Nearly 100 attempts to amplify from specimens treated with 3.7% formaldehyde (at pH 3.0 and 7.0) failed, indicating extreme damage to the DNA.

Englander, E. W. and B. H. Howard (1997). "Alu-mediated detection of DNA damage in the human genome." Mutation Research/DNA Repair **385**(1): 31.

<http://www.sciencedirect.com/science/article/B6T2B-3RD0T5D-4/2/34cc67fe387930f444ad065ba938d1c9>

A new approach to monitoring UV damage and repair in the human genome has been developed. The proposed approach is based on a combination of features unique to interspersed repetitive Alu elements, and the ability of certain DNA lesions to block Taq polymerase-mediated DNA synthesis: namely, the extraordinary abundance of Alu repeats throughout the human genome in conjunction with distinct sequence motifs comprising long runs of T residues which are likely targets for formation of UV lesions. Hence, Taq polymerase-mediated extension synthesis with Alu specific primers was employed to visualize formation of discrete predicted adducts within the element. Several variations of the Alu-primer driven amplification protocol were developed to monitor the following aspects of damage: (i) induction of UV-photoproducts at predicted sites within the Alu sequence, (ii) modification of extension synthesis profiles, (iii) UV dose dependent, quantitative inhibition of Alu-primer driven amplification. The assays reveal sites of predicted Taq polymerase blockage within the Alu sequence, a global decrease in the mean length of extension products, and a measurable reduction in the quantity of extension products that is inversely proportional to UV dose. Thus, the exceptional abundance of Alu repeats and their primary sequence features, in combination with the ability of UV lesions to block elongation by Taq polymerase, provide a novel and sensitive system for detecting UV damage in the human genome. The system detects UV damage at levels that are compatible with cellular DNA repair, and provides a unique amplification-based protocol for probing the overall integrity of human DNA.

Fowler, M. L., R. E. Sigler, et al. (1995). "Absence of Ki-ras mutations in exocrine pancreatic tumors from male rats chronically exposed to gabapentin." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **327**(1-2): 151.

<http://www.sciencedirect.com/science/article/B6T2C-3YKM47Y-26/2/20df78dd8d0e0fc228287446c06bed3e>

Human pancreatic malignancies originating from duct cells most frequently demonstrate activation of Ki-ras gene by G-to-A transition at codons 12 and 13. Rat pancreatic exocrine tumors more frequently and almost exclusively derive from acinar cells and thus differ morphologically from human pancreatic neoplasms. Male Wistar rats fed with 2% gabapentin (1-(aminomethyl)cyclohexane acetic acid) in diet for 2 years developed pancreatic exocrine adenomas and adenocarcinomas. To study the mutations in Ki-ras gene, rat pancreatic proliferative lesions induced by gabapentin were retrospectively analyzed by PCR amplification of DNA isolated from paraffin sections of formalin-fixed rat pancreatic adenomas and adenocarcinomas, using specific primers for regions encoding exon 1 (codon 12/13) and exon 2 (codon 61). The amplified 110-bp fragments of exon 1 and exon 2 were analyzed for mutations at codon 12/13 and 61. The results showed Ki-ras mutations at codon 12 in human pancreatic carcinomas. Novel mutations GGT-to-TGT and GGT-to-CGT were detected at codon 12 in 1/5 and 2/5 human pancreatic tumors. Rat adenomas or carcinomas induced by gabapentin expressed wild type sequences at codons 12, 13 and 61. These findings were confirmed by allele-specific oligonucleotide hybridization, single-strand confirmation polymorphism of exon 1 and direct sequencing of exon 1 and exon 2. The absence of mutations in these rat pancreatic tumors suggests that these tumors do not correspond to the human tumors, and that the pathogenesis of this rodent tumor formation may follow different molecular mechanisms.

Furitsu, K., H. Ryo, et al. (2005). "Microsatellite mutations show no increases in the children of the Chernobyl liquidators." Mutation Research/Genetic Toxicology and Environmental Mutagenesis **581**(1-2): 69.

<http://www.sciencedirect.com/science/article/B6T2D-4F3FF7P-2/2/a20da8250e65071616364173fde26981>

We performed a study on Belarusian "liquidators", exploring whether increase in the frequencies of germline mutations at microsatellite loci could be found in their progeny. The liquidators, mostly young males, were those involved (during 1986 and 1987) in clean-up operations in the radioactively contaminated area following the Chernobyl nuclear power plant accident in 1986. Many liquidators fathered children during the clean-up period and after the work had been terminated. The numbers of families studied were 64 (liquidators) and 66 (controls). A total of 72 loci (31 autosomal, one X-linked and 40 Y-linked) were used. DNA was isolated from peripheral blood lymphocytes and the microsatellite loci were amplified by the polymerase chain reaction with fluorescence-labelled primers. Mutations were detected as variations in the length of the loci. At the Y-linked loci, the mutation rates (expressed as number of mutations among the total number of loci for the individuals included) are 2.9×10^{-3} (4/1392) and 2.1×10^{-3} (3/1458) in the children of exposed and control parents, respectively. This difference is not statistically significant. At the autosomal loci, the corresponding estimates are 5.9×10^{-3} (11/1862; exposed group) and 8.5×10^{-3} (18/2108; control). Again, the difference is not significant. The possibility that the Belarusian population might have been unexpectedly exposed to some chemical contaminants in the environment appears unlikely in view of the finding that the spontaneous mutation rates at the same set of loci in several non-Belarusian populations were similar to those in Belarus. The estimated mean radiation dose to the liquidators was small, being about 39 mSv, and this might be one reason why no increases in mutation rates due to radiation could be found.

Fusco, J. C., G. W. Knapp, et al. (1998). "The frequency of illegitimate V(D)J recombinase-mediated mutations in children treated with etoposide-containing antileukemic therapy." Mutation Research/Genetic Toxicology and Environmental Mutagenesis **419**(1-3): 107.

<http://www.sciencedirect.com/science/article/B6T2D-3V4J6JF-C/2/3b0c7dc9c94c477b26dd88c6a3a7dac2>

Etoposide is among the most widely used anti-cancer drugs. Its use, however, has been associated with increased risk of secondary acute myeloid leukemia (AML) which is characterized by chromosomal translocations suggesting involvement of recombination-associated motifs at the breakpoints. A PCR-based assay was developed to quantitate the frequency of two illegitimate V(D)J recombinase-mediated genomic rearrangements--a 20-kb deletion in the hprt gene and the bcl2/IgH translocation (t(14;18)) found in non-Hodgkin's lymphoma. We examined both lymphocyte and non-lymphocyte blood cell DNA of children with acute lymphoblastic leukemia (ALL) for changes in the frequencies of these biomarkers during etoposide therapy to determine the level of illegitimate V(D)J recombination changes during therapy. A low level of t(14;18) was found in the lymphocytes before etoposide treatment, which was significantly reduced during etoposide therapy. In before-etoposide samples, no t(14;18) were found among 7.72×10^7 non-lymphocytes; during treatment none were found among 1.87×10^8 non-lymphocytes. Deletions were not found before etoposide treatment in either the lymphocytes (6.67×10^7) or non-lymphocytes (5.43×10^7) and were non-significantly elevated during etoposide therapy (1 in 1.4×10^8 lymphocytes and 1 in 1.39×10^8 non-lymphocytes). It is interesting to note the one patient with an hprt deletion mutation in non-lymphocytes; V(D)J recombination is not normally found in this cell type, but is the cell type from which AML derives. Several patients had clones of t(14;18)-bearing cells as determined by DNA sequence analysis. These results suggest that this etoposide-based chemotherapy was ineffective in producing genomic rearrangements mediated by illegitimate V(D)J recombination in these patients.

Fuscoe, J. C., L. J. Zimmerman, et al. (1992). "Analysis of X-ray-induced HPRT mutations in CHO cells: Insertion and deletions." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **269**(2): 171.

<http://www.sciencedirect.com/science/article/B6T2C-4864J8P-3R/2/c0f18dc78dd6a730ec3d07965a4c8913>

Molecular alterations were examined in the hypoxanthine guanine phosphoribosyltransferase (hprt) gene of 41 independent X-ray-induced thioguanine-resistant (TGR) Chinese hamster ovary (CHO) cell clones. Rapid screening of the clones by multiplex polymerase chain reaction (PCR) for the presence or absence of exons revealed that the causes of the mutant phenotype were total gene deletion (26/41), partial gene deletion (4/41), and an insertion (1/41). No alterations of exon number or sizes were apparent in 10 of the mutants. Southern blot analysis confirmed the deletion data and revealed an additional class of mutants that had a gene disruption but retained all hprt exons (2/41). Therefore, at least 80% of the ionizing radiation-induced mutations were due to mechanisms involving DNA breakage and rejoining. The distribution of deletion sizes that the two DNA breaks required for a deletion are not independent events. A possible mechanism is presented. In addition, the DNA sequence of the insertion mutation was determined. The insertion (229 bp) is coupled with a deletion (31 bp). An imperfect inverted repeat with flanking hprt DNA was identified and may be involved in the insertion event.

Garcia, V., J. Silva, et al. (2004). "Overexpression of p16INK4a correlates with high expression of p73 in breast carcinomas." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **554**(1-2): 215.

<http://www.sciencedirect.com/science/article/B6T2C-4CNGT3D-2/2/4b2ae19decc7430beb1affcd3f44cf98>

The p16-cyclin D-Cdk4(6)-pRB-E2F and p73 pathways are involved in the control of cell-cycle progression, and genetic lesions in both pathways frequently occur in breast carcinomas and other human cancers. The p16INK4a gene is involved in regulation of the G1/S transition, and when overexpressed, the p73 gene activates transcription of p53-responsive genes and promotes apoptosis. These pathways are related, for instance, p73 is also downstream of E2F-1, since E2F-1 induces p73-mediated apoptosis in the absence of p53. We studied 93 breast cancer patients to identify alterations in the expression of p16INK4a and p73 by semiquantitative RT-PCR analysis and possible interactions between them and correlations with clinicopathological parameters. p73 was overexpressed in 24 cases. Overexpression of p16INK4a was detected in 17 cases and underexpression in 32 cases. A significant correlation was observed between the overexpression of both genes ($P = 0.05$). Concurrent overexpression of p73 and p16INK4a was significantly correlated with metastases in three or more lymph nodes ($P = 0.0007$), positive immunohistochemistry for p53 ($P = 0.014$), vascular invasion ($P = 0.048$) and negative progesterone receptors ($P = 0.004$). These results indicate that concomitant overexpression of p16INK4a and p73 may be involved in breast cancer and associated with poor tumor characteristics.

Gruz, P., K. Matsui, et al. (1996). "Construction of a new system for separate expression of mutagenesis proteins: the abilities to promote UV mutagenesis and interchangeability of MucA', MucB, SamA' and SamB proteins in Salmonella typhimurium." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **354**(2): 157.

<http://www.sciencedirect.com/science/article/B6T2C-3WF7M86-2/2/d8615633f131d8db01097f8fd7923a88>

The two distinct mucAB and samAB operons originally isolated from the plasmids of Salmonella typhimurium encode proteins engaged in induced mutagenesis. They represent two extreme cases among the so far characterized members of the enterobacterial umuDC family in respect to both the strength and the specificity of their effect. It is suggested that the MucA and SamA proteins are post-translationally processed to MucA' and SamA', respectively, which lack the N-terminal 25 amino acids and are the active species in mutagenesis. For the purpose of characterizing the individual activities of these proteins, we developed a new system for their SOS-independent separate and controllable expression in enterobacteria. Besides the matured forms of MucA', SamA' as well as MucB and SamB proteins we also expressed hybrid HisTag-MucA' and HisTag-SamA' proteins in which a synthetic 24 amino acid HisTag region replaces the natural 25 amino acid N-terminal leader present in the MucA and SamA precursors. In this study, we analyzed the effect of the mutagenesis proteins on the UV mutability of *S. typhimurium* YG5144. None of the proteins, if expressed alone, promoted UV mutagenesis. Different combinations of the proteins promoted mutagenesis to different extents in the order MucA' + MucB > SamA' + SamB >= HisTag-MucA' + MucB >= SamA' + MucB > MucA' + SamB > HisTag-SamA' + SamB. The mutagenesis enhancing potential of the combinations with MucB protein decreased as the expression of the proteins increased while the mutagenesis enhancing potential of the combinations with SamB protein increased together with the increase in the expression. The artificially expressed MucA' + MucB proteins were as active as their MucAB counterparts expressed from the plasmid pKM101 in promoting UV mutagenesis, but they were remarkably more efficient than their pKM101-born counterparts in promoting spontaneous mutagenesis. We conclude that the MucA'B and SamA'B proteins are partly interchangeable and the functionality of the resulting A' + B complex is largely dependent on the appropriate B-protein.

Harrington-Brock, K., D. D. Collard, et al. (2003). "Bromate induces loss of heterozygosity in the Thymidine kinase gene of L5178Y/Tk+/-3.7.2C mouse lymphoma cells." Mutation Research/Genetic Toxicology and Environmental Mutagenesis **537**(1): 21.

<http://www.sciencedirect.com/science/article/B6T2D-48BM66N-1/2/c5423a539d8917faa72bdf37549e8e5e>

Potassium bromate (KBrO₃) induces DNA damage and tumors in mice and rats, but is a relatively weak mutagen in microbial assays and the in vitro mammalian Hprt assay. Concern that there may be a human health risk associated with bromate, a disinfectant by-product of ozonation, has accompanied the increasing use of ozonation as an alternative to chlorination for treatment of drinking water. In this study, we have evaluated the mutagenicity of KBrO₃ and sodium bromate (NaBrO₃) in the Tk gene of mouse lymphoma cells. In contrast to the weak mutagenic activity seen in the previous studies, bromate induced a mutant frequency of over 100×10^{-6} at 0.6 mM with minimal cytotoxicity (70-80% survival) and over 1300×10^{-6} at 3 mM (~10% survival). The increase in the Tk mutant frequency was primarily due to the induction of small colony of Tk mutants. Loss of heterozygosity (LOH) analysis of 384 mutants from control and 2.7 mM KBrO₃-treated cells showed that almost all (99%) bromate-induced mutants resulted from LOH, whereas in the control cultures 77% of the Tk mutants were LOH. Our results suggest that bromate is a potent mutagen in the Tk gene of mouse lymphoma cells, and the mechanism of action primarily involves LOH. The ability of the mouse lymphoma assay to detect a wider array of mutational events than the microbial or V79 Hprt assays may account for the potent mutagenic response.

Honda, T., N. Sadamori, et al. (1996). "Spontaneous immortalization of cultured skin fibroblasts obtained from a high-dose atomic bomb survivor." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **354**(1): 15.

<http://www.sciencedirect.com/science/article/B6T2C-3W0FDH0-3/2/1024eb01232b72a4588fa063626eae4b>

Two immortal fibroblastic cell strains (substrains) were established by culturing healthy skin cells obtained from a high-dose atomic bomb survivor (female, age 76 years, 5.14 Gy) for more than 4 years. Designated FM-U and FM-M, the two substrains share the same marker chromosome, t(5q-;6p+), but are karyotypically different, possessing hypodiploid chromosome numbers (39-43) in the former and hypertriploid (69-76) in the latter. Thus far, the two strains have passed through 117 and 156 subcultures or more than 230 and 310 cumulative population doublings, respectively, each passage requiring 4-6 days in the former and 3-4 days in the latter. In the process of immortalization, sequential rearrangement among various chromosomes presumably due to telomeric and interstitial telomeric fusions took place following the telomere shortening, particularly in the senescence and postsenescence phase cells. Of particular interest is the fact that loss of heterozygosity (LOH) of the p53 gene was demonstrated in these immortalized cell populations. In addition, the allelic patterns of the LOH of p53 differed. Further evidence indicative of infinite proliferation was demonstrated in both strains, such as the telomere elongation and the significantly low frequency of cells possessing dicentric chromosomes.

Jacobi, F. K., J. Meyer, et al. (2001). "Quantitation of heteroplasmy in mitochondrial DNA mutations by primer extension using VentR(R)(exo-) DNA polymerase and RFLP analysis." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **478**(1-2): 141.

<http://www.sciencedirect.com/science/article/B6T2C-4378X0V-F/2/9742cd25f3c74cbd4321540005f1c061>

In this report we describe a simple and rapid protocol for reliable quantitation of mitochondrial DNA (mtDNA) mutations, which is basically a modification of the traditional polymerase chain reaction (PCR)/restriction fragment length polymorphism (RFLP) analysis technique. Up to now,

the PCR/RFLP method has been of limited use for the accurate determination of ratios of mutant and wild type molecules, largely owing to the formation of heteroduplex molecules by PCR and incompleteness of restriction digestion. In order to overcome this problem, we have introduced a single-step primer extension reaction using VentR(R)(exo-) DNA polymerase and a fluorescence-labeled primer to the standard assay. The labeled homoduplex molecules are then digested with a restriction endonuclease, and the nucleic acids fractionated on an automated DNA sequencer equipped with GENESCAN(TM) analysis software. The amount of mutant mtDNA is readily estimated from fluorescence intensities of the wild-type and mutant mtDNA fragments corrected for incomplete digestion as monitored by a homologous control fragment. The accuracy of the improved protocol was determined by constructing standard curves obtained from defined mixtures of genomic DNA containing homoplasmic wild-type and mutant mtDNA. The expected values were obtained, with an observed correlation coefficient of 0.997 and a typical variability of +/-5% between repeated measurements. Further validation of the protocol is provided by the screening of five patients and unaffected subjects carrying the guanine to adenine transition at the nucleotide 3460 of the mitochondrial genome responsible for the mitochondrial disorder of Leber's hereditary optic neuropathy.

Jansen, J. G., H. Vrieling, et al. (1992). "The gene encoding hypoxanthine-guanine phosphoribosyltransferase as target for mutational analysis: PCR cloning and sequencing of the cDNA from the rat." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **266**(2): 105.

<http://www.sciencedirect.com/science/article/B6T2C-4864J4D-2D/2/608dd291595aba97e09f1f5a7494fb27>

In this paper, the cloning and nucleotide sequence of the cDNA of the rat gene coding for hypoxanthine-guanine phosphoribosyltransferase (hprt) is reported. Knowledge of the cDNA sequence is needed, among other reasons, for the molecular analysis of hprt mutations occurring in rat cells, such as skin fibroblasts isolated according to the granuloma pouch assay. The rat hprt cDNA was synthesized and used as a template for in vitro amplification by PCR. For this purpose, oligonucleotide primers were used, the nucleotide sequences of which were based on mouse and hamster hprt cDNA sequences. Sequence analysis of 1146 bp of the amplified rat hprt cDNA showed a single open reading frame of 654 bp, encoding a protein of 218 amino acids. In the predicted rat hprt amino acid sequence, the proposed functional domains for 5'-phosphoribosyl-1-pyrophosphate (PRPP) and nucleotide binding in phosphoribosylating enzymes as well as a region near the carboxyl terminal part were highly conserved when compared with amino acid sequences of other mammalian hprt proteins. Analysis of hprt amino acid sequences of 727 independent hprt mutants from human, mouse, hamster and rat cells bearing single amino acid substitutions revealed that a large variety of amino acid changes were located in these highly conserved regions, suggesting that all 3 domains are important for proper catalytic activity. The suitability of the hprt gene as target for mutational analysis is demonstrated by the fact that amino acid changes in at least 151 of the 218 amino acid residues of the hprt protein result in a 6-thioguanine-resistant phenotype.

Johnson, B. L., B. Thyagarajan, et al. (1996). "Elevated levels of recombinational DNA repair in human somatic cells expressing the *Saccharomyces cerevisiae* RAD52 gene." Mutation Research/DNA Repair **363**(3): 179.

<http://www.sciencedirect.com/science/article/B6T2B-3WF7M8X-4/2/397304a310fed992018ffb4d4a22ce43>

The *Saccharomyces cerevisiae* RAD52 gene was introduced into the human fibrosarcoma-

derived cell line HT1080. Transfected cell lines that expressed the yeast transgene catalyzed inter-plasmid homologous DNA recombination at frequencies approx. 12-fold higher than did control cells. Additional experiments revealed that yeast RAD52 gene expression increased the level of resistance to the DNA damaging agents diepoxybutane, and methyl methanesulfonate, but did not alter sensitivity to ultraviolet radiation. These results indicate that the *S. cerevisiae* Rad52 protein can function in a human somatic cell background and provide support for the idea that a homologous recombination-based DNA repair process functions in mammalian somatic cells.

Khaidakov, M., M. G. Manjanatha, et al. (2002). "Molecular analysis of mitochondrial DNA mutations from bleomycin-treated rats." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **500**(1-2): 1.

<http://www.sciencedirect.com/science/article/B6T2C-44J3V88-5/2/732aec0fd7730e34ad7c3ab27703d9fb>

In our previous studies, we have shown the mutagenicity of bleomycin (BLM) at the nuclear hprt locus. In the present study we have analyzed mutagenic effects of BLM in mitochondrial DNA (mtDNA) using short extension-PCR (SE-PCR) method for detection of low-copy deletions. Fisher 344 rats were treated with a single dose of BLM and total DNA preparations from splenic lymphocytes were processed in SE-PCR assay. Spontaneous deletions were typically flanked by direct repeats (78.5%), while the in BLM-treated group, direct repeats were found in only 46.6% of breakpoints. The ratio between deletions based on direct repeats and random sequence deletions changed from 3.67 in control group to 0.87 in BLM-treated animals, which corresponds to an approximate 1.7-fold increase in the deletion mutation frequency. Furthermore, 62.5% of deletions not flanked by direct repeats in the treated group contained cleavage sites for BLM. The localization of breakpoints was not entirely random. We have found four clusters containing deletions from both groups indicative of deletion hot spots. The results indicate that BLM exposure may be associated with the induction of mtDNA mutations, and suggest the utility of SE-PCR method for evaluating drug-induced genotoxicity.

Kinashi, Y., H. Nagasawa, et al. (1995). "Molecular structural analysis of 417 HPRT mutations induced by restriction endonucleases in Chinese hamster ovary (CHO) cells." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **326**(1): 83.

<http://www.sciencedirect.com/science/article/B6T2C-3YKM495-2T/2/18fbd8eb65f3db85cc2000697fa37f9d>

CHO cells were exposed to 11 different restriction endonucleases by electroporation and their mutagenicity was measured. Nine of them have one or more recognition sites within exons of the HPRT gene, whereas the remaining two cut in introns only. The mutagenic efficiency of the various enzymes varied markedly; mutagenicity of Sau3AI was considerably higher than that of the other enzymes. Neither cytotoxicity nor mutagenicity could be related to the number or location of recognition sites within the cDNA. A total of 417 independent restriction enzyme induced mutant clones were isolated from 20 separate experiments for molecular analysis; all nine exons of the HPRT gene were analyzed by a modified multiplex deletion screening method with polymerase chain reaction (PCR) amplification. Among spontaneously arising mutants, 70.8% showed no change in PCR pattern, indicating a small scale change (point mutation), whereas partial deletions were observed in 24.7%, and total deletions in 4.5% of mutant clones. In contrast, approximately 70% of restriction enzyme induced mutants showed partial or total deletions. There was no obvious relationship between type of break (blunt versus staggered ends), and the DNA structure of the mutations induced. For partial deletions, the distribution of

breakpoints within introns appeared to occur at random, and did not correlate with the mutagenicity of a given enzyme. Thus, though DNA double-strand breaks appear to be important mutagenic lesions that can induce a high frequency of deletion mutants, no specific relationship of mutagenic potential to the type of breaks, their sites within the HPRT gene or the molecular structure of the mutations induced could be identified.

Klein, C. B., L. Su, et al. (1994). "Transgenic gpt⁺ V79 cell lines differ in their mutagenic response to clastogens." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **304**(2): 217.

<http://www.sciencedirect.com/science/article/B6T2C-47P87B6-56/2/0e27ea3325a1b931d2b921632c01d697>

Several gpt⁺ transgenic cell lines were derived from hprt⁻ V79 cells to study mutagenesis mechanisms in mammalian cells. The G12 cell line was previously shown to be hypermutable by X-rays and UV at the gpt locus compared to the endogenous hprt gene of the parental V79 cells (Klein and Rossman, 1990), and is now shown to be highly mutable by the clastogenic anti-tumor agent bleomycin sulfate. A second transgenic cell line G10, which has a different gpt insertion site, was studied in comparison with G12. Both G12 and G10 cell lines carry the stable gpt locus at a single integration site in the Chinese hamster genome, and neither spontaneously deletes the integrated gpt sequence at a high frequency. Although spontaneous mutation to 6-thioguanine resistance in G10 cells is 3-4 times higher than in G12 cells, the cell lines differ to a much greater extent when mutated by clastogens. In comparison to G12 cells, the gpt locus in G10 cells is up to 13 times more sensitive to bleomycin mutagenesis and 5 times more responsive to X-ray mutagenesis. In contrast, there is much less difference in UV-induced mutagenesis and no differences in mutagenesis induced by alkylating agents such as N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). The dose-dependent decrease in survival of the transgenic cells is the same for all mutagens tested, and does not differ from that of V79 cells. Neither transgenic cell line is generally hypermutable, since mutagenesis at an endogenous gene, Na⁺K⁺/ATPase, is similar to that of the parental V79 cell line. Although both cell lines can be induced to delete the transgene following clastogen exposure, deletions are not the only recovered mutations, and the cell lines can also be used to study mutations within the PCR recoverable gpt gene. The utility of these transgenic cells to investigate genome position effects related to mammalian mutagenesis mechanisms is discussed.

Koch, W. H., A. R. Fernandez de Henestrosa, et al. (2000). "Identification of mucAB-like homologs on two IncT plasmids, R394 and Rts-1." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **457**(1-2): 1.

<http://www.sciencedirect.com/science/article/B6T2C-41TMPSB-1/2/c2e563316c5998c6ef91e3852004b8cb>

Recent phylogenetic analysis of the superfamily of lesion-replicating DNA polymerases suggest that they can be broadly divided into four sub-groups comprised of UmuC-like, DinB-like, Rev1-like and Rad30-like proteins. The UmuC-like sub-family is best characterized at the genetic level and sequence analysis of eleven umu orthologs, residing on bacterial chromosomes or on self-transmissible R-plasmids allows further subdivision into five sub-groups (UmuDC, MucAB, ImpAB, RumAB and RulAB) based on amino acid sequence conservation. Some of these orthologs are apparently inactive in situ, but may promote increased mutagenesis and survival when subcloned and expressed from high-copy number plasmids. We were, therefore, interested in devising an assay that would identify umuC-like genes in situ in the absence of a functional assay. To this end, degenerate primers directed towards conserved amino acid regions within the

UmuC-like sub-family of DNA polymerases were designed and used to identify mucAB-like operons on the IncT plasmids, R394 and Rts-1. Interestingly, DNA sequence analysis of an ~7 kb region of R394 identified two LexA-regulated genes immediately downstream of mucAB(R394) that are similar to the chromosomally-encoded *Escherichia coli* *tus* gene and the IncI plasmid-encoded *impC* gene, respectively. Analysis of the R394 and Rts-1 *mucB* genes revealed that both contain insertions which result in the expression of a truncated inactive MucB protein. While R394 was unable to restore mutagenesis functions to a [Delta]umuDC *E. coli* strain, Rts-1 surprisingly promoted significant levels of MMS-induced SOS mutagenesis, raising the possibility that Rts-1 encodes another, yet unidentified, umu-like homolog.

Kovalchuk, O., P. Burke, et al. (2003). "Genome hypermethylation in *Pinus silvestris* of Chernobyl--a mechanism for radiation adaptation?" Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **529**(1-2): 13.

<http://www.sciencedirect.com/science/article/B6T2C-490RJBG-1/2/69817d8631e91f775c86e51b2020965b>

Adaptation is a complex process by which populations of organisms respond to long-term environmental stresses by permanent genetic change. Here we present data from the natural "open-field" radiation adaptation experiment after the Chernobyl accident and provide the first evidence of the involvement of epigenetic changes in adaptation of a eukaryote-Scots pine (*Pinus silvestris*), to chronic radiation exposure. We have evaluated global genome methylation of control and radiation-exposed pine trees using a method based on cleavage by a methylation-sensitive HpaII restriction endonuclease that leaves a 5' guanine overhang and subsequent single nucleotide extension with labeled [3H] dCTP. We have found that genomic DNA of exposed pine trees was considerably hypermethylated. Moreover, hypermethylation appeared to be dependent upon the radiation dose absorbed by the trees. Such hypermethylation may be viewed as a defense strategy of plants that prevents genome instability and reshuffling of the hereditary material, allowing survival in an extreme environment. Further studies are clearly needed to analyze in detail the involvement of DNA methylation and other epigenetic mechanisms in the complex process of radiation stress and adaptive response.

Kovalchuk, O., P. Burke, et al. (2004). "Methylation changes in muscle and liver tissues of male and female mice exposed to acute and chronic low-dose X-ray-irradiation." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **548**(1-2): 75.

<http://www.sciencedirect.com/science/article/B6T2C-4BYC4M8-3/2/00d73c092cb48b51b50548c52e91420a>

The biological and genetic effects of chronic low-dose radiation (LDR) exposure and its relationship to carcinogenesis have received a lot of attention in the recent years. For example, radiation-induced genome instability, which is thought to be a precursor of tumorigenesis, was shown to have a transgenerational nature. This indicates a possible involvement of epigenetic mechanisms in LDR-induced genome instability. Genomic DNA methylation is one of the most important epigenetic mechanisms. Existing data on radiation effects on DNA methylation patterns is limited, and no one has specifically studied the effects of the LDR. We report the first study of the effects of whole-body LDR exposure on global genome methylation in muscle and liver tissues of male and female mice. In parallel, we evaluated changes in promoter methylation and expression of the tumor suppressor gene p16INKa and DNA repair gene O6-methylguanine-DNA methyltransferase (MGMT). We observed different patterns of radiation-induced global genome DNA methylation in the liver and muscle of exposed males and females. We also found sex and tissue-specific differences in p16INKa promoter methylation upon LDR exposure. In male liver

tissue, p16INKa promoter methylation was more pronounced than in female tissue. In contrast, no significant radiation-induced changes in p16INKa promoter methylation were noted in the muscle tissue of exposed males and females. Radiation also did not significantly affect methylation status of MGMT promoter. We also observed substantial sex differences in acute and chronic radiation-induced expression of p16INKa and MGMT genes. Another important outcome of our study was the fact that chronic low-dose radiation exposure proved to be a more potent inducer of epigenetic effects than the acute exposure. This supports previous findings that chronic exposure leads to greater genome destabilization than acute exposure.

Kulaeva, O. I., E. V. Koonin, et al. (1996). "Identification of a DinB/UmuC homolog in the archeon *Sulfolobus solfataricus*." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **357**(1-2): 245.

<http://www.sciencedirect.com/science/article/B6T2C-3W258J8-Y/2/e3ee92e432039f07f56b9fc1d38c5f41>

To date, eight closely related homologs of the *Escherichia coli* UmuC protein have been identified. All of these homologs appear to play critical roles in damage-inducible mutagenesis in enterobacteriaceae. Recently, a distantly related UmuC-homolog, DinB, has also been identified in *E. coli*. Using the polymerase chain reaction together with degenerate primers designed against conserved regions found in UmuC-like proteins, we have identified a new member of the UmuC-superfamily in the archeon *Sulfolobus solfataricus*. This new homolog shows high sequence similarity to DinB and a lower level of similarity to UmuC. As a consequence, we have called this new gene *dbh* (dinB homolog). Analysis of approximately 2.7 kb DNA encompassing the *dbh* region revealed several open reading frames (orfs). One, encoding a putative ribokinase, was located immediately upstream of *dbh*. This orf overlaps the *dbh* gene by 4 bp suggesting that both proteins might be coordinately expressed. Further upstream of the ribokinase-*dbh* locus was another orf encoding a potential ATPase homologous to two uncharacterized *S. cerevisiae* proteins (YD9346.02c and SC38KCXVI_20) and another *E. coli* DNA repair protein, RuvB. While this is the first report of a UmuC-like homolog in an archeon, we detected additional homologs using protein sequence comparisons in Gram-positive bacteria, cyanobacteria, and among potential human EST products, indicating that UmuC-related proteins comprise a ubiquitous superfamily of proteins probably involved in DNA repair and mutagenesis.

Kusewitt, D. F., J. Dyle, et al. (1998). "Altered UV resistance and UV mutational spectrum in repair-proficient murine fibroblasts expressing endonuclease V." Mutation Research/DNA Repair **407**(2): 157.

<http://www.sciencedirect.com/science/article/B6T2B-3TC13P0-8/2/e5d6486f80e4b0be5a4cbca280992857>

In previously reported studies, we transfected repair-proficient murine fibroblasts with the denV gene of bacteriophage T4 and showed that expression of encoded endonuclease V markedly enhanced cyclobutane pyrimidine dimer (CPD) repair and reduced the frequency of ultraviolet radiation (UV)-induced mutations. In the present studies, we compared the spectra of UV-induced mutations at the *hprt* locus in denV-transfected and control cells. A significant difference in mutation types was observed. While multiple base deletions and single base insertions were found in denV-transfected but not control cells, multiple tandem and non-tandem point mutations identified in control cells were absent in denV-transfected cells. When we compared colony survival following UV exposure in the two cell lines, it appeared that endonuclease V expression did not enhance UV resistance, instead denV-transfected cells had increased susceptibility to low fluences of UV. The effects of endonuclease V expression on UV resistance and on UV

mutational spectrum are likely to be due both to the removal of CPDs and to the novel enzymatic activity of endonuclease V.

Li, B. H., S. Larsen, et al. (1991). "Diverse backmutations at an ochre defect in the *tyrA* gene sequence of *E. coli* B/r." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **246**(1): 139.

<http://www.sciencedirect.com/science/article/B6T2C-47P87R7-94/2/0820b78a542e569ba5938eafec4bcb22>

A DNA fragment including most of the *tyrA* gene from *E. coli* B/r strain WU (Tyr⁻, Leu⁻) was amplified in vitro by polymerase chain reaction. The sequence was determined, first, for essentially all of the fragment to locate an ochre nonsense defect, and second, repeatedly for a region of the fragment from several independent isolates containing backmutations at the ochre codon (spontaneous and UV-induced). There were 20 single base differences in the *tyrA* gene region from the analogous wild-type *E. coli* K12 sequence: an ochre codon at amino acid position 161, 18 silent changes (1 at the first codon base and 17 at the third) and one replacement of valine by alanine. Different backmutations at the ochre codon encoded lysine, glutamine, glutamic acid, leucine, cysteine, phenylalanine, serine or tyrosine. The diversities of base substitutions at the ochre codon after UV mutagenesis or after mutagenesis where targeting by dimers was reduced or eliminated (after photoreversal of irradiated cells treated with nalidixic acid to induce SOS functions or after UV mutagenesis of cells containing amplified DNA photolyase) were similar (with two notable exceptions). The overall differences between the gene sequences for *E. coli* K12 or B/r seemed consistent with the neutral theory of molecular evolution.

Liang, L., C. Shao, et al. (2002). "Radiation-induced genetic instability in vivo depends on p53 status." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **502**(1-2): 69.

<http://www.sciencedirect.com/science/article/B6T2C-45CW865-2/2/132c7bd724c1d23729c0e34b129c7513>

In response to ionizing radiation and other agents that damage DNA, the p53 tumor suppressor protein activates multiple cellular processes including cell cycle checkpoints and programmed cell death. Although loss of p53 function is associated with radiation-induced genetic instability in cell lines, it is not clear if this relationship exists in vivo. To study the role of p53 in maintenance of genetic stability in normal tissues following irradiation, we have measured mutant frequencies at the adenine phosphoribosyltransferase (*Aprt*) and hypoxanthine-guanine phosphoribosyltransferase (*Hprt*) loci and examined mechanisms of loss of heterozygosity (LOH) in normal T cells of p53-deficient, *Aprt* heterozygous mice that were subjected to whole-body irradiation with a single dose of 4 Gy X-rays. The radiation-induced mutant frequency at both the *Aprt* and *Hprt* loci was elevated in cells from mice with different p53 genotypes. The radiation-induced elevation of p53^{-/-} mice was significantly greater than that of p53^{+/-} or p53^{+/+} mice and was caused by several different kinds of mutational events at the both chromosomal and intragenic levels. Most significantly, interstitial deletion, which occurs rarely in unirradiated mice, became the most common mechanism leading to LOH in irradiated p53 null mice. These observations support the idea that absence or reduction of p53 expression enhances radiation-induced tumorigenesis by increasing genetic instability at various loci, such as those for tumor suppressor genes.

Lippert, M. J., Q. Chen, et al. (1998). "Increased transcription decreases the spontaneous mutation rate at the thymidine kinase locus in human cells." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **401**(1-2): 1.

<http://www.sciencedirect.com/science/article/B6T2C-3SY9PXB-C/2/368f60f1c5931336047cce53ec9fcb89>

Transcription increases DNA repair efficiency and modulates the distribution of certain types of DNA damage. Furthermore, increased transcription level stimulates spontaneous mutation rate in yeast. We explored whether transcription level affects spontaneous mutation rate in human cells. We first developed two thymidine kinase (tk) inducible human cell lines using the Gal4-Estrogen receptor system. In our TK6i-G3 and G9 tk heterozygous cell lines, the active tk allele is linked to an inducible promoter element. Tk mRNA is induced following treatment with estrogen. Spontaneous mutation rate was significantly decreased in human cell lines after induction in contrast to the report in yeast. Thus, humans may have evolved different or additional mechanisms to deal with transcription related spontaneous mutagenesis.

Liu, P. K., J. M. Trujillo, et al. (1993). "Spectrum of spontaneous mutation in animal cells containing an aphidicolin-resistant DNA polymerase [alpha]." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **288**(2): 229.

<http://www.sciencedirect.com/science/article/B6T2C-47PCNN3-8T/2/8b1d4fc54fdab5b5adef3696b91ab34>

We have used a polymerase chain reaction (PCR)-based exon screening assay to determine the spectrum of spontaneous hypoxanthine phosphoribosyltransferase (hprt) gene mutations occurring in an aphidicolin-resistant V79 Chinese hamster cell line (designated Aphr-4-2) that contains a mutant DNA polymerase-[alpha] and displays a spontaneous mutator phenotype. PCR analyses of 71 independent, 6-thioguanine (TG)-resistant sublines isolated from Aphr-4-2 or parental V79-743X cells using hprt exon 3- and exon 9-specific oligonucleotide primer pairs revealed the loss of exon 3 or 9 from 6 of 60 Aphr-4-2 derived-, and from 1 of 11 parental V79-derived, TG-resistant mutants. Exons 3 and 9 were both lost from 5 of 60 Aphr-4-2-derived mutants, while none of the 11 V79-derived mutants had lost both exons. The results of these PCR-screening assays were further corroborated by Southern and Northern blot hybridization analyses of 28 mutants: 22 of 28 mutants contained an intact hprt gene by Southern analysis; of these 22 mutants 6 of 11 Aphr-4-2-derived mutants contained either reduced or undetectable steady state mRNA levels in contrast to all 11 V79-derived mutants that contained normal amounts of a normal-sized hprt mRNA. The results of our PCR and blot hybridization analyses indicate that the rates of base substitution and deletion mutagenesis are elevated in Aphr-4-2 cells, and suggest that DNA polymerase-[alpha] may play a role in determining the rate of different molecular types of spontaneous mutations in vivo.

Livak, K. J., W. A. Little, et al. (1998). "Polymorphisms in the human DNA ligase I gene (LIG1) including a complex GT repeat." Mutation Research/Mutation Research Genomics **406**(1): 1.

<http://www.sciencedirect.com/science/article/B6T2F-3V4J6S2-1/2/553b33ca561d521b0e225d038468c26b>

Sequencing of a human DNA ligase I cDNA clone derived from HeLa cells revealed two unreported differences with the published sequence: a single base change and a three-base deletion. Both differences are in exon 6, and were analyzed by amplifying a segment containing

exon 5, intron 6, and exon 6. The first finding was that intron 6 is approximately 2.6 kb in size, not the 1 kb reported in the literature. By sequence analysis of amplified segments, the single-base difference in exon 6 was shown to be polymorphic, with HeLa cells heterozygous for the A/C difference. Analysis of 60 unrelated individuals found a frequency of 0.5 for each allele. Primer extension reactions across the exon 5/exon 6 boundary were performed on cDNA obtained from HeLa cells and human thymus. The results show that the three-base deletion is due to a variation in splicing. For both HeLa and thymus, two-thirds of the transcripts are like the published cDNA sequence and one-third have the three-base deletion. Finally, sequencing of part of intron 6 revealed the presence of a complex GT repeat consisting of a 48-50 nucleotide polypurine tract followed by a variable number of GT residues. This entire unit of polypurine tract plus GTs is repeated three times. Detection of the repeated sequences required the development of specialized cloning and PCR conditions. Analysis of a pedigree showed that this complex repeat is polymorphic.

Ludwig, D. L., M. A. MacInnes, et al. (1998). "A murine AP-endonuclease gene-targeted deficiency with post-implantation embryonic progression and ionizing radiation sensitivity." Mutation Research/DNA Repair **409**(1): 17.

<http://www.sciencedirect.com/science/article/B6T2B-3TXKBPW-3/2/0c205c6100fa571e7bd065f96f9635e6>

Apurinic/apurimidine endonuclease (here designated APE/REF) carries out repair incision at abasic or single-strand break damages in mammals. This multifunctional protein also has putative role(s) as a cysteine 'reducing factor' (REF) in cell-stress transcriptional responses. To assess the significance of APE/REF for embryonic teratogenesis we constructed a more precisely targeted Ape/Ref-deficient genotype in mice. Ape/Ref gene replacement in ES cells eliminated the potential of APE/REF protein synthesis while retaining the Ape/Ref bi-directional promoter that avoided potential inactivation of an upstream gene. Chimeric animals crossed into Tac:N:NIH3T3 produced germline transmission. Homozygous null Ape/Ref-embryos exhibited successful implantation and nearly normal developmental progression until embryonic day 7.5 followed by morphogenetic failure and adsorption of embryos by day 9.5. We characterized the cellular events proceeding to embryonic lethality and examined ionizing radiation sensitivity of pre-implantation Ape/Ref-null embryos. After intermating of heterozygotes, Mendelian numbers of putative Ape/Ref-null progeny embryos at day 6.5 displayed a several-fold elevation of pycnotic, fragmenting cell nuclei within the embryo proper--the epiblast. Increased cell-nucleus degeneration occurred within epiblast cells while mitosis continued and before obvious morphogenetic disruption. Mitogenic response to epiblast cell death, if any, was ineffective for replacement of lost cells. Extra-embryonic yolk sac, a trophectoderm derived lineage retained normal appearance to day 9. Explanted homozygous Ape/Ref-null blastocysts displayed increased sensitivity to [gamma]-irradiation, most likely a manifestation of APE/REF incision defect. Our study establishes that this new Ape/Ref deficiency genotype is definitely capable of post-implantation developmental progression to the onset of gastrulation. Function(s) of APE/REF in base damage incision and also conceivably in mitogenic responses towards epiblast cell death are critical for transit through the gastrulation stage of embryonic growth and development.

Malfatti, M. A., R. W. Wu, et al. (2005). "The effect of UDP-glucuronosyltransferase 1A1 expression on the mutagenicity and metabolism of the cooked-food carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine in CHO cells." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **570**(2): 205.

<http://www.sciencedirect.com/science/article/B6T2C-4F8TKJM-1/2/4456db7629b8bfbe40b54a8b3d5d6022>

UDP-glucuronosyltransferase proteins (UGT) catalyze the glucuronidation of both endogenous and xenobiotic compounds. In previous studies, UGT1A1 has been implicated in the detoxification of certain food-borne carcinogenic-heterocyclic amines. To determine the importance of UDP-glucuronosyltransferase 1A1 (UGT1A1) in the biotransformation of the cooked-food carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), genetically modified CHO cells that are nucleotide excision repair-deficient, and express cytochrome P4501A2 (UV5P3 cell line) were transfected with a cDNA plasmid of human UGT1A1 to establish the UDP-glucuronosyltransferase 1A1 expressing 5P3hUGT1A1 cell line. Expression of the UGT1A1 gene was verified by screening neo gene expressing clonal isolates (G-418 resistant) for their sensitivity to cell killing from PhIP exposure. Five of 11 clones were chosen for further analysis due to their resistance to cell killing. Western blot analysis was used to confirm the presence of the UGT1A1 and CYP1A2 proteins. All five clones displayed a 52-kDa protein band, which corresponded to a UGT1A1 control protein. Only four of the clones had a protein band that corresponded to the CYP1A2 control protein. Correct fragment size of the cDNAs in the remaining four clones was confirmed by RT-PCR and quantification of the mRNA product was accomplished by real-time RT-PCR. Expression of UGT1A1 in the transfected cells was 104-105-fold higher relative to the UV5P3 parental cells. One clone (#14) had a 10-fold higher increase in expression at 1.47×10^5 over the other three clones. This clone was also the most active in converting N-hydroxy-PhIP to N-hydroxy-PhIP glucuronide conjugates in microsomal metabolism assays. Based on the D50 values, the cytotoxic effect of PhIP was decreased [not, vert, similar]350-fold in the 5P3hUGT1A1 cells compared to the UV5P3 control cells. In addition, no significant increase in mutation frequency was observed in the transfected cells. These results clearly indicate that UGT1A1 plays a critical role in PhIP biotransformation, providing protection against PhIP-mediated cytotoxicity and mutagenicity.

Mazur-Melnyk, M., G. R. Stuart, et al. (1996). "Benzo[a]pyrenediol-epoxide induces loss of heterozygosity in Chinese hamster ovary cells heterozygous at the *aprt* locus." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **358**(1): 89.

<http://www.sciencedirect.com/science/article/B6T2C-3W2TBF1-9/2/9e04a1a315d32d55e3bcacfb2b101be5>

Benzo[a]pyrenediol-epoxide (BPDE), a metabolite of the ubiquitous environmental carcinogen benzo[a]pyrene (B[a]P), has been implicated as a point mutagen. However, as mutational events other than point mutations are also often associated with cancer, we have investigated whether BPDE can induce other classes of mutation. This was done by analyzing mutation at the *aprt* and *hprt* loci, both in hemizygous (D422) and heterozygous (D423) Chinese hamster ovary (CHO) cell strains. Southern blotting analysis indicated that BPDE is not an effective producer of either deletions or insertions in the hemizygous environment. The analysis of mutation in the *aprt* heterozygote was done to investigate the frequency of loss of heterozygosity (LOH) events following BPDE treatment. Using PCR to produce an artificial restriction fragment length polymorphism in the functional *aprt* allele, BPDE was found to induce LOH in about one-quarter of the mutants recovered. While the precise mechanism of this phenomenon remains obscure, it is likely to have important implications, since similar events involving homologous recombination in somatic cells may have an impact in tumorigenesis.

McKinzie, P. B. and B. L. Parsons (2002). "Detection of rare K-ras codon 12 mutations using allele-specific competitive blocker PCR." Mutation Research/Genetic Toxicology and Environmental Mutagenesis **517**(1-2): 209.

<http://www.sciencedirect.com/science/article/B6T2D-45NGSX6-1/2/72c28f66445465d05bc80de191c6bac6>

Allele-specific competitive blocker PCR (ACB-PCR) is a sensitive allele-specific amplification method in which preferential amplification of the mutant allele occurs by using a primer that has more mismatches to the wild-type allele than to the mutant allele (mutant-specific primer, MSP). Additionally, a non-extendable primer with more mismatches to the mutant allele than to the wild-type allele (blocker primer, BP) competes with the MSP for binding to the wild-type allele, thereby reducing background amplification from the wild-type allele. ACB-PCR primer design is largely dependent upon the basepair substitution being measured, making it unclear if this method is broadly applicable. In an earlier study, an H-ras codon 61 CAA->AAA mutation had been detected by ACB-PCR at a sensitivity of 10⁻⁵. In this study, ACB-PCR was applied to two human K-ras codon 12 mutations: GGT->GTT and GGT->GAT. The method was optimized by systematically altering the concentrations of Perfect Match PCR Enhancer, MSP, BP, and dNTPs. For each mutation, mutant fractions as low as 10⁻⁵ were detected, indicating that this assay can be used on a variety of base substitution mutations. In addition, the results suggest that the 3'-terminal mismatches between the MSP and wild-type allele may be used to predict the ACB-PCR conditions that will be appropriate for the detection of other base substitution mutations. The range of concentrations for each of these components is narrow, making this method relatively easy to apply to additional mutational targets.

Meng, Q., R. F. Henderson, et al. (1999). "Mutagenicity of 1,3-butadiene at the Hprt locus of T-lymphocytes following inhalation exposures of female mice and rats." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **429**(1): 107.

<http://www.sciencedirect.com/science/article/B6T2C-3X3KGXB-8/2/8d885d8245209e8e478f647d794ff218>

The species specific response to 1,3-butadiene (BD), an important industrial chemical, was investigated by determining the influence of exposure duration and exposure concentration on the mutagenicity of BD in mice and rats and by defining the spectra of mutations in the Hprt gene T-cell mutants from control and BD-exposed mice. Female B6C3F1 mice and F344 rats (4-5 weeks old) were exposed by inhalation to 0, 20, 62.5, or 625 ppm of BD for up to 4 weeks (6 h/day, 5 days/week). Groups of control and exposed animals (n=4-12/group) were necropsied at multiple time points after exposure and the T-cell cloning assay was used to measure Hprt mutant frequencies in lymphocytes isolated from spleen. Mutant clones collected from control and BD-exposed mice were propagated and analyzed by RT-PCR to produce Hprt cDNA for sequencing. In animals necropsied 4 weeks after 2 or 4 weeks of BD exposure (0 or 625 ppm), the rate of accumulation of mutations was greater in mice than in rats. Supra-linear dose-response curves were observed in BD-exposed mice, indicating a higher efficiency of mutant induction at lower concentrations of BD. The mutagenic potency estimates (represented by the differences in the areas under the mutant T-cell 'manifestation' curves of treated vs. control animals) in mice were 11 and 61 following 4 weeks of exposures to 62.5 and 625 ppm of BD, respectively, while mutant frequencies (Mfs) in rats were significantly increased only at 625 ppm BD (mutagenic potency of 7). Molecular analysis of Hprt cDNA from expanded T-cell clones from control and BD-exposed mice demonstrated an increased frequency of mutants in exposed animals that likely contain large deletions in the Hprt gene (P=0.016). These data indicate that both exposure duration and exposure concentration are important in determining the magnitude of mutagenic response to BD, and that mutagenic and carcinogenic properties of BD in mice may be related more to the ability of its metabolites to cause chromosomal deletions than to produce point mutations.

Mironov, N. M., F. Bleicher, et al. (1993). "Nonrandom distribution of O6-methylguanine in H-ras gene sequence from DNA modified with N-methyl-N-nitrosourea." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **288**(2): 197.

<http://www.sciencedirect.com/science/article/B6T2C-47PCNN3-8N/2/596f779ee8fd98d9c9dbc2c10a0cee52>

The distribution of O6-meG in the rat H-ras gene sequence was studied using PCR by transition of O6-meG to adenine during the reaction. In order to study the transition mutations the PCR product was cloned in a replicative form of phage M13mp18 and sequenced. The use of PCR for detection of O6-meG was validated by using oligonucleotides (61 bases) containing one O6-meG residue at a defined site. After treatment of rat liver DNA by N-methyl-N-nitrosourea in vitro, a striking nonrandom sequence distribution of O6-meG was observed. Sixty-eight per cent of O6-methylated Gs were found in the middle G of the sequences GGT and GGA in the H-ras gene whereas no methylation was found in the middle G of the sequences AGG, GGG, TGT, TGC, CGA and CGC. No O6-meG adduct was found in the 12th codon of H-ras (sequence GGA). The frequency of O6-meG formation as a function of two flanking nucleotides on each side of the target guanine was calculated as an approach to understanding more distant sequence effects. It was found that in the DNA sequence studied the formation of O6-meG was highest if the G was flanked by PyPu or PuPu on the 5' side (Py, pyrimidine and Pu, purine) whereas PuPu on the 3' side showed maximal inhibition of O6-meG formation.

Mollersen, L., R. Vikse, et al. (2004). "Adenomatous polyposis coli truncation mutations in 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-induced intestinal tumours of multiple intestinal neoplasia mice." Mutation Research/Genetic Toxicology and Environmental Mutagenesis **557**(1): 29.

<http://www.sciencedirect.com/science/article/B6T2D-4B7YPB6-1/2/4dbb27ff0716869ef6f5221f1f4c8b3f>

The heterocyclic amine 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) induces intestinal tumours in C57BL/6J-multiple intestinal neoplasia (Min)/+ mice. The main mechanism for PhIP-induced tumour induction in Min/+ mice is loss of the wild-type adenomatous polyposis coli (Apc) allele, i.e. loss of heterozygosity (LOH). In this study, single injections of either 10, 17.5 or 25 mg/kg PhIP on days 3-6 after birth all increased the mean number of small intestinal tumours two to three-fold, from 37.7 in controls to 124.8 in the PhIP-treated Min/+ mice. In total, we analysed 292 small intestinal tumours and 253 of these had LOH. The frequency of LOH in the Apc gene was 88, 93, 83 and 84% in tumours of 0, 10, 17.5 and 25 mg/kg PhIP-treated mice, respectively. Therefore, these lower doses of PhIP did not reduce the frequency of LOH, as found in our previous study with a single injection of 50 mg/kg PhIP (Mutat. Res. 1-2 (2002) 157). In the second part of this study, we wanted to characterise Apc truncation mutations from tumour samples apparently retaining the Apc wild-type allele from this and two previous experiments with PhIP-exposed Min/+ mice. In the first half of exon 15 in Apc, we verified 25 mutations from 804 tumour samples of PhIP-treated mice. Of these were 60% G->T transversions, and 16% G deletions, indicating that these are the predominant types of PhIP-induced truncation mutations in the Apc gene in Min/+ mice. Most of the mutations were located between codon 989 and 1156 corresponding to the first part of the [beta]-catenin binding region. We also identified two Apc truncation mutations from 606 spontaneously formed intestinal tumours from untreated Min/+ mice, one C->T transition and one T insertion, which were different from those induced by PhIP.

Monroe, J. J., M. G. Manjanatha, et al. (2001). "Extent of CpG methylation is not proportional to the in vivo spontaneous mutation frequency at transgenic loci in Big Blue(TM) rodents." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **476**(1-2): 1.

<http://www.sciencedirect.com/science/article/B6T2C-42YW37B-1/2/1db32d0f59afce3dd23e3bf9b9b3d2be>

The lacI transgene used in the Big Blue(TM) (BB) mouse and rat mutation assays typically displays spontaneous mutation frequencies in the 5×10^{-5} range. Recently, the bone marrow and bladder of the Big Blue(TM) rat were reported to have, by an order of magnitude, the lowest spontaneous mutation frequencies ever observed for lacI in a transgenic animal, approaching the value for endogenous targets such as hprt ($\sim 10^{-6}$). Since spontaneous mutations in transgenes have been attributed in part to deamination of 5-methylcytosine in CpG sequences, we have investigated the methylation status of the lacI transgene in bone marrow of BB rats and compared it to that present in other tissues including liver, spleen, and breast. The first 400 bases of the lacI gene were investigated using bisulfite genomic sequencing since this region contains the majority of both spontaneous and induced mutations. Surprisingly, all the CpG cytosines in the lacI sequence were fully methylated in all the tissues examined from both 2- and 14-week-old rats. Thus, there is no correlation between 5-methylcytosine content at CpG sites in lacI and the frequency of spontaneous mutation at this marker. We also investigated the methylation status of another widely used transgenic mutation target, the cII gene. The CpG sites in cII in BB rats were fully methylated while those in BB mice were partially methylated (each site approximately 50% methylated). Since spontaneous mutation frequency at cII is comparable in rat and mouse, the methylation status of CpG sequences in this gene also does not correlate with spontaneous frequency. We conclude that other mechanisms besides spontaneous deamination of 5-methylcytosine at CpG sites are driving spontaneous mutation at BB transgenic loci.

Moulds, B. A. and J. I. Goodman (1994). "Spontaneous mutation at codon 61 of the Ha-ras gene in the nascent liver of B6C3F1, C3H/He and C57BL/6 mice." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **311**(1): 1.

<http://www.sciencedirect.com/science/article/B6T2C-47PG5TS-2/2/d84bc50795489e01e624363fe5355e08>

DNA was isolated from the liver of young B6C3F1, C3H/He and C57BL/6 mice, 6-9 weeks old. A portion of exon 2 of Ha-ras was amplified by PCR allele-specific amplification. The PCR product was identified by (a) size, (b) presence of a diagnostic restriction site, and (c) direct sequencing. Our results indicate that nascent mouse liver bears a subpopulation of cells which contain a mutation in codon 61 of Ha-ras-, specifically an A to G transition at position 2. Therefore, the detection of this mutation in chemically induced mouse liver tumors does not demonstrate that the chemical in question acts as a mutagen. It might act by a nongenotoxic mechanisms, i.e., by facilitating a clonal expansion of cells bearing this spontaneous mutation.

Okinaka, R. T., A. V. Perez-Castro, et al. (1997). "Heritable genetic alterations in a xeroderma pigmentosum group G/Cockayne syndrome pedigree." Mutation Research/DNA Repair **385**(2): 107.

<http://www.sciencedirect.com/science/article/B6T2B-3RWW7NX-3/2/f3c154ae994d846d2624b5c912904ecd>

A search for genetic alterations within the XPG gene has been conducted on skin and blood cells cultured from a newly characterized xeroderma pigmentosum (XP) patient (XP20BE). This patient is the ninth known case that falls into the extremely rare XP complementation group G. Four genetic markers within the XPG gene (including two polymorphisms) demonstrated the Mendelian distribution of this gene from the parents to the patient and to an unaffected sibling. The patient (XP20BE) inherited a G to T transversion from his father in exon 1 of the XPG gene that resulted in the conversion of a glutamic acid at codon 11 to a termination codon. The patient also inherited an XP-G allele from his mother that produces an unstable or poorly expressed message. The cause of the latter defect is still uncertain. In addition to these alterations, XP20BE cDNA

contained an mRNA species with a large splicing defect that encompassed a deletion from exon 1 to exon 14. This splicing defect, however, appears to be a naturally occurring low-frequency event that results from abnormal splicing that occurs between certain conserved non-consensus splicing signals within the human XPG gene.

Orimo, H., M. Ikejima, et al. (1998). "A novel missense mutation and frameshift mutations in the type II receptor of transforming growth factor-[beta] gene in sporadic colon cancer with microsatellite instability." Mutation Research/Mutation Research Genomics **382**(3-4): 115.

<http://www.sciencedirect.com/science/article/B6T2F-3T888C3-7/2/d4fd8660014d77bbbbc6fc75a328df16>

Microsatellite instability of DNA samples of 79 sporadic colon cancer patients were analyzed. These samples were also screened to search mutations in the repeat sequences in the gene for the type II receptor of transforming growth factor-[beta] (TGF-[beta] RII) using polymerase chain reaction (PCR), electrophoresis with urea gel, and PCR-single strand conformation polymorphism (PCR-SSCP) method. The incidence of microsatellite instability, defined as severe replication error phenotype (RER) with microsatellite alterations in more than three loci, was 6%. Deletion and insertion of an A residue in the (A)₁₀ region, which cause frameshift mutation, were found in four samples and their incidence in the samples with microsatellite instability was 80%. A novel nucleotide substitution of T for G at 1918, which causes missense mutation of arginine to leucine at codon 528, was found in a sample with microsatellite instability. The mutation at 1918 was in highly conservative amino acid residue.

Otsuka, C., K. Kobayashi, et al. (2002). "Use of yeast transformation by oligonucleotides to study DNA lesion bypass in vivo." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **502**(1-2): 53.

<http://www.sciencedirect.com/science/article/B6T2C-45C06N4-1/2/2ea0ca607248cf9aa331d90de3e6f0d2>

We have studied mutagenic specificities of DNA lesions in vivo in yeast CYC1 oligonucleotide transformation assay. We introduced two lesions into oligonucleotides. One was a nucleoside analog, 3,4-dihydro-6H,8H-pyrimido[4,5-c][1,2]oxazin-7-one 2'-deoxyriboside (dP), which is highly mutagenic to bacteria. It is supposed to be a miscoding, but otherwise good template for DNA polymerases. The other lesion was the TT pyrimidine(6-4)pyrimidone photoproduct, one of the typical UV lesions, which blocks DNA replication. These oligonucleotides were used to transform yeast cyc1 mutants with ochre nonsense mutation to Cyc1⁺. As expected from its templating properties in vitro, the transforming activity of dP-containing oligonucleotides was similar to those of unmodified oligonucleotides. Results indicated that dP may direct incorporation of guanine and adenine at a ratio of 1:20 or more in vivo. An oligonucleotide containing the photoproduct showed the transforming activity of as low as 3-5% of that of the corresponding unmodified oligonucleotide. This bypass absolutely required REV1 gene. The sequence analysis of the transformants has shown that the lesion was read as TT and TC at a ratio of 3:7, indicating its high mutagenic potential.

Pogribny, I. P., S. J. James, et al. (2004). "Genomic hypomethylation is specific for preneoplastic liver in folate/methyl deficient rats and does not occur in non-target tissues." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **548**(1-2): 53.

<http://www.sciencedirect.com/science/article/B6T2C-4BNW2W0-1/2/1185e5a81031da4725d5c2644be79d36>

Chronic dietary insufficiency of the lipotropic nutrients choline and methionine is hepatocarcinogenic in male rats and certain mouse strains. Despite the fact that DNA hypomethylation is a hallmark of most cancer genomes, the tissue-specific consequences of this alternation with respect to tumorigenesis remain to be determined. In the present study, the folate/methyl deficient model of multistage hepatocarcinogenesis was used to evaluate in vivo alterations in DNA methylation in the liver, the carcinogenesis target tissue, and in non-target tissues, including pancreas, spleen, kidney, and thymus, of male F344 rats. By utilizing the HpaII/MspI-based cytosine extension assay, we demonstrated that the percent of CpG sites that lost methyl groups on both strands progressively increased in liver tissue after 9, 18, and 36 weeks of folate/methyl deficiency. The endogenous activity of DNA methyltransferase in liver of rats fed with folate/methyl deficient diet for the 36-week period gradually increased with time. In contrast, non-target tissues displayed no changes in DNA methylation level or activity of DNA methyltransferase. The failure of DNA methyltransferase to restore and maintain DNA methylation patterns in preneoplastic liver tissue may lead to the establishment of tumor-specific DNA methylation and DNA methyltransferase profiles that are not expressed in normal liver. These results provide additional information about alterations in DNA methylation during early preneoplastic stages of carcinogenesis. They also demonstrate that DNA hypomethylation is localized to tissue that undergoes carcinogenesis, and is not altered in non-target tissues.

Ponten, F., C. Williams, et al. (1997). "Genomic analysis of single cells from human basal cell cancer using laser-assisted capture microscopy." Mutation Research/Mutation Research Genomics **382**(1-2): 45.

<http://www.sciencedirect.com/science/article/B6T2F-3SY9RHW-6/2/6bbe1d386bbec9ca9645064499f98b6e>

In this study, we show that direct mutational analysis of genomic DNA can be performed on single somatic cells extracted from a frozen, immunohistochemically stained tissue section using laser-assisted capture microscopy. Eighty-nine single tumor cells were separately dissected from one case of human basal cell cancer (BCC) and p53 mutations were analyzed by direct semi-automated sequencing of PCR fragments. Amplification was obtained for at least one of the two analyzed exons from approximately 50% of the single tumor cells. Identical p53 mutations were found in widely spread areas of the tumor, suggesting a clonal proliferation originating from one cell. Interestingly, comparison between results of immunohistochemistry and genetic analysis of the single cells revealed the same p53 mutations irrespective of the p53 immunoreactivity. We propose that this approach has a great potential to allow investigation of genotypic differences in single cells and more specifically to resolve important and fundamental questions determining cancer heterogeneity.

Powell, E., L. Shi, et al. (2001). "Frequency and distribution in three ethnic populations of single nucleotide polymorphisms in the MICA gene." Mutation Research/Mutation Research Genomics **432**(3-4): 47.

<http://www.sciencedirect.com/science/article/B6T2F-4292MHS-1/2/4928e4a17e7dd3ddea3f929299350212>

The Homo sapiens major histocompatibility complex (MHC) class 1 chain related gene A (MICA) was scanned for novel single nucleotide polymorphisms (SNPs) using a panel of DNA samples

from African-, Japanese- and Mexican-Americans. Overlapping primer-pairs were used to amplify products in the size range of 300 to 400 bp that were sequenced and scanned for SNPs using Phred, Phrap, Polyphred and Consed sequence analysis programs. A total of 16 SNPs were detected, six of which represent new variant nucleotides in the Homo sapiens MICA gene. Three of the variants also represent amino acid changes in the MICA protein. Differences among the three ethnic panels in the frequency of the variant nucleotides observed were inconsistent, but significant for seven of the SNPs detected. Though a small sample size, this study represents the first multi-population based analysis of the frequency and distribution of SNPs in the MICA gene, a locus that may be essential in the antigenic recognition by $\gamma\delta$ T cells.

Purohit, S. and P. Arenaz (1999). "Molecular cloning, sequence and structure analysis of hamster apurinic/aprimidinic endonuclease (chAPE1) gene." Mutation Research/DNA Repair **435**(3): 215.

<http://www.sciencedirect.com/science/article/B6T2B-3Y8W992-2/2/f2e26411506fc21d396e71431645045a>

We have cloned a 13 kb genomic DNA fragment from the Chinese hamster ovary cell line, CHO-K1, and determined the nucleotide sequence of a 4 kb stretch of DNA which encompasses the complete sequence (2.277 kb) of the hamster apurinic/aprimidinic endonuclease (chAPE1) gene. The intron/exon boundaries, identified by RT-PCR, follow GT/AG rule. The structure of the chAPE1 gene is similar to other mammalian apurinic/aprimidinic (AP) endonuclease (hAPE1, BAP1, rAPEN and mAPE1) genes in that it has five exons and four introns with the first exon unexpressed. This structure, however, differs from one of the two structures that have been proposed for mAPE1 gene. Three transcription start sites (TSS) for the chAPE1 gene were identified by primer extension analysis at +1, +14 and +18 positions. The sequence also includes 1.72 kb of the upstream region of the chAPE1 gene. In this region, a CCAAT box but no TATA box that could initiate the transcription at the initiation sites was identified. The upstream region also includes the binding sites for a variety of other transcription factors. A polyadenylation site, 13 nucleotides downstream to the polyadenylation signal, was identified by 3'-RACE analysis. The observed 1.28 kb transcript of the chAPE1 gene is smaller than the 1.5 kb transcript of the human AP endonuclease gene. The translation of chAPE1 gene starts within the second exon with ATG and terminates in the fifth exon with UGA codons, 318 and 2121 nucleotides downstream to the first TSS, respectively. The encoded peptide of 317 amino acid residues is similar in size and is highly homologous in its amino acid sequence to mouse, rat, human, and bovine AP endonucleases.

Ray, L. S., S. Chatterjee, et al. (1996). "Catalytic activity of poly(ADP-ribose) polymerase is necessary for repair of N-methylpurines in nontranscribed, but not in transcribed, nuclear DNA sequences." Mutation Research/DNA Repair **363**(2): 105.

<http://www.sciencedirect.com/science/article/B6T2B-3VYTVB6-4/2/3221a26e3eada48db2cdfdbb9c16a986>

The role of poly(ADP-ribose) polymerase (PADPRP) in nuclear DNA repair and other nuclear processes has been intensely studied and debated for decades. Recent studies have begun to shed new light on these arguments with firm experimental data for its role, primarily, as a remodeler of chromatin structure. Those studies imply that PADPRP plays an indirect role in DNA repair, serving to expose DNA to repair enzymes through chromatin remodeling. Only DNA that is tightly packaged would require PADPRP activity for its repair; while DNA in an open conformation would be accessible to DNA repair enzymes and not require PADPRP activity. The purpose of the current studies was to address the above hypothesis directly. Using quantitative Southern blot

analysis, we studied repair in transcribed and nontranscribed nuclear DNA sequences in ADPRT 351 cells 95% deficient in PADPRP activity. Cells were exposed to methylnitrosourea (MNU) for 1 h and allowed to repair for 8 or 24 h. Densitometric scans of autoradiographs revealed that, when compared to their parental V79 cell line, ADPRT 351 cells 95% deficient in PADPRP activity were equally as efficient in repair of N-methylpurines in the transcribed sequence containing the dihydrofolate reductase gene. However, the ADPRT 351 cells were deficient in the ability to repair these lesions in the nontranscribed sequence containing the IgE gene compared to repair of the same sequence in the parental V79 cells. Nucleoid sedimentation assays demonstrated that the ADPRT 351 cells are deficient in repair across the entire genome when compared to the parental V79 cells. These studies indicate that PADPRP activity is not required for repair of N-methylpurines in transcribed nuclear DNA sequences but is necessary for the repair of these lesions in nontranscribed nuclear DNA sequences as well as across the entire genome since the DNA in a given cell is predominantly nontranscribed.

Reddy, G., E. I. Golub, et al. (1997). "Human Rad52 protein promotes single-strand DNA annealing followed by branch migration." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **377**(1): 53.

<http://www.sciencedirect.com/science/article/B6T2C-3PFBHBJ-8/2/df9cf025bb1d74e2d1df3c07f3bad52b>

In the yeast, *Saccharomyces cerevisiae*, the Rad52 gene is important for both mitotic and meiotic recombination. Homologs of the Rad52 gene have been identified in several eukaryotic organisms, ranging from yeast to man. As reported here, human Rad52 protein binds to both single- and double-stranded DNA; and acting on a pair of single-stranded and partially duplex substrates it promotes annealing of complementary strands of DNA, which is followed by branch migration.

Ruven, H. J. T., C. M. J. Seelen, et al. (1994). "Efficient synthesis of 32P-labeled single-stranded DNA probes using linear PCR; application of the method for analysis of strand-specific DNA repair." Mutation Research/DNA Repair **315**(2): 189.

<http://www.sciencedirect.com/science/article/B6T2B-47G3B79-2C/2/ffb9b861ca4a9432c33466300d399873>

We have developed a rapid method to synthesize radioactively labeled single-stranded DNA probes suitable for strand-specific analysis of single copy genes on Southern blot. Linear PCR with 10 [mu]Ci [alpha]32P-aATP (3000 Ci/mmol) as the only dATP source enabled us to generate strand-specific DNA probes with high specific activity. The probes synthesized by this method have higher specific activities and the same strand specificity compared to the end-labeled single-stranded DNA probes obtained from single-stranded M13mp18/19 vectors. Application of the method for strand-specific analysis of ultraviolet-induced DNA lesions in defined DNA sequences significantly improved the hybridization signal.

Ryu, J.-C., J.-Y. Youn, et al. (1999). "Mutation spectrum of 4-nitroquinoline N-oxide in the lacI transgenic Big Blue(R) Rat2 cell line." Mutation Research/Genetic Toxicology and Environmental Mutagenesis **445**(1): 127.

<http://www.sciencedirect.com/science/article/B6T2D-3XMGP4W->

D/2/cbfa31dae84ebe5df75801eb78f059b1

This paper describes the spectrum of mutations induced by 4-nitroquinoline N-oxide (4-NQO) in the lacI target gene of the transgenic Big Blue(R) Rat2 cell line. There are only a few report for the mutational spectrum of 4-NQO in a mammalian system although its biological and genetic effects have been well studied. Big Blue(R) Rat2 cells were treated with 0.03125, 0.0625 or 0.125 [mu]g/ml of 4-NQO, the highest concentration giving 85% survival. Our results indicated that the mutant frequency (MF) induced by 4-NQO was dose-dependent with increases from three- to seven-fold. The DNA sequence analysis of lacI mutants from the control and 4-NQO treatment groups revealed an obvious difference in the spectra of mutations. In spontaneous mutants, transition (60%) mutations, especially G:C->A:T transition (45%), were most frequent. However, the major type of base substitution after treatment of 4-NQO was transversions (68.8%), especially G:C->T:A (43.8%), while only 25% of mutants were transitions. These results are consistent with those produced by 4-NQO in other systems and the transgenic assay system will be a powerful tool to postulate more accurately the mechanism of chemical carcinogenesis involved.

Saenko, V., T. Rogounovitch, et al. (2003). "Novel tumorigenic rearrangement, [Delta]rfp/ret, in a papillary thyroid carcinoma from externally irradiated patient." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **527**(1-2): 81.

<http://www.sciencedirect.com/science/article/B6T2C-48GPC6B-1/2/c6cddc68b491c40e683aa4f6af3c4da3>

Molecular analysis of cDNA derived from a papillary thyroid carcinoma (PTC) (follicular variant of papillary thyroid carcinoma on histology) which developed in an externally irradiated patient 4 years after exposure identified a portion of the 5' region, exons 1-3, of the rfp gene juxtaposed upstream of the fragment encoding the tyrosine kinase (TK) domain of the ret gene. The fusion gene, termed [Delta]rfp/ret, was the result of a balanced chromosomal translocation t(6;10) (p21.3;q11.2) confirmed by interphase FISH painting, with breakpoints occurring in introns 3 and 11 of the rfp and ret genes, respectively. Both [Delta]rfp/ret and reciprocal ret/rfp chimeric introns had small deletions around breakpoints consistent with presumed misrepair of a radiation-induced double-strand DNA break underlying the rearrangement. No extensive sequence homology was found between the fragments flanking the breakpoints. The fusion protein retained the propensity to form oligomers likely to be mediated by a coiled-coil of the RFP polypeptide as assessed by a yeast two-hybrid system. NIH 3T3 fibroblasts stably transfected with a mammalian expression vector encoding full-length [Delta]RFP/RET readily gave rise to the tumors in athymic mice suggestive of high transforming potential of the fusion protein. Thus, the [Delta]rfp/ret rearrangement may be causatively involved in cancerogenesis and provides additional evidence of the role of activated ret oncogene in the development of a subset of papillary thyroid carcinoma.

Schmidt, A. L. and V. Mitter (2004). "Microsatellite mutation directed by an external stimulus." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **568**(2): 233.

<http://www.sciencedirect.com/science/article/B6T2C-4DM2RNY-2/2/b55f51b221d9977808d6c3bfea3d9ebe>

Microsatellites are regions of DNA containing tandem repeats of a core 2-6 bp nucleotide sequence. To test the hypothesis that microsatellite mutation can be directed by exposure to specific external cues, control and treatment groups of resistant and susceptible wheat varieties

were grown under controlled conditions and genotyped at a number of microsatellite loci that map to chromosomes known to contain *Fusarium* head blight (FHB) resistance/susceptibility loci. Genotyping was undertaken both prior to and following exposure to *Fusarium graminearum*, the FHB pathogen. Within a month of inoculation of inflorescences, 58% of experimental plants, and no control plants, had acquired a novel allele at the locus Xgwm112.1. This allele was detected only in head blight affected tissue. Uninoculated control plants, and leaf samples from inoculated plants, showed no mutation. Cloning and sequencing of PCR products indicates that the new allele was generated by contraction of the (CT)_n repeat motif. Observation of the same deletion-based mutation in all varieties, its absence in control plants not exposed to the head blight pathogen, and the detection of no similar mutational events in a control panel of loci not expected to show mutation, indicates that this example of microsatellite mutation is induced and/or caused by FHB infection.

Schwartz, J. L., R. C. Porter, et al. (1996). "The molecular nature of spontaneous mutations at the *hprt* locus in the radiosensitive CHO mutant *xrs-5*." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **351**(1): 53.

<http://www.sciencedirect.com/science/article/B6T2C-464P5VX-4/2/f8a39d9413211c9a5d56a839e621b322>

The radiosensitive mutant *xrs-5*, a derivative of the Chinese hamster ovary (CHO) K1 cell, is defective in DNA double-strand break rejoining ability and in V(D)J recombination. The radiosensitivity and defective repair phenotype are complemented by the 80-kDa subunit of the Ku protein. We determined the nature of the mutations that develop spontaneously at the *hprt* locus in this cell line using both multiplex PCR deletion screening and DNA sequencing. Ninety-two independent spontaneous mutants were analyzed and the results were compared to the mutation spectrum of 64 previously analyzed *hprt* spontaneous mutants isolated from the parental CHO-K1 cell line. More than 50% of the spontaneous *xrs-5* mutants had lost one or more exons while less than 25% of spontaneous CHO-K1 mutants had lost one or more exons. Most of the deletions in *xrs-5* cells involved the loss of multiple exons while single exon deletions predominated in CHO-K1. There was also a nonrandom distribution of breakpoints in both CHO-K1 and *xrs-5*. Most of the deletion breakpoints were 3' to exon 9, around exons 4-6, or near exon 1. Although the frequency of base substitutions was lower in *xrs-5*, the spectrum of base substitutions was qualitatively similar to that of CHO-K1. There was no significant difference in the spontaneous mutant frequency in *xrs-5* and CHO-K1. The results suggest that in certain regions of the *hprt* gene, base alterations can be converted to large deletions, and that alterations in the Ku protein complex can influence this process.

Smith-Sorensen, B., E. Hovig, et al. (1992). "Screening for mutations in human HPRT cDNA using the polymerase chain reaction (PCR) in combination with constant denaturant gel electrophoresis (CDGE)." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **269**(1): 41.

<http://www.sciencedirect.com/science/article/B6T2C-4864JF7-5F/2/e7ad8636964b42049c7fcb1147824103>

Previously, we reported the modification of denaturing gradient gel electrophoresis called constant denaturant gel electrophoresis (CDGE). CDGE separates mutant fragments in specific melting domains. CDGE seems to be a useful tool in mutation detection. Since the hypoxanthine phosphoribosyltransferase (HPRT) gene is widely used as target locus for mutation studies in vitro and in vivo, we have examined the approach of analyzing human HPRT cDNA by polymerase chain reaction (PCR) and CDGE. All nine HPRT exons are included in a 716-bp c

DNA fragment obtained by PCR using HPRT cDNA as template. When the full-length cDNA fragment was examined by CDGE, it was possible to detect mutations only in the last part of exon 8 and exon 9. However, digestion of the cDNA fragment with the restriction enzyme Aval prior to CDGE enabled us to detect point mutations in most of exon 2, the beginning of exon 3, the last part of exon 8 and exon 9. With the use of two internal primer sets, including a GC-rich clamp on one of the primers in each pair, a region containing most of the exon 3 through exon 6 was amplified and we were able to resolve fragments with point mutations in this region from wild-type DNA. The approach described here allows for rapid screening of point mutations in about two thirds of the human HPRT cDNA sequence. In a test of this approach, we were able to resolve 12 of 13 known mutants. The mutant panel included one single-base deletion, one two-base deletion and 11 single-base substitutions.

Song, L. H., V. Q. Binh, et al. (2003). "Mannose-binding lectin gene polymorphisms and hepatitis B virus infection in Vietnamese patients." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **522**(1-2): 119.

<http://www.sciencedirect.com/science/article/B6T2C-47GJ2V7-1/2/fa46efb6cf1d189ccf4a902f81286faf>

Mannose-binding lectin (MBL) is a constituent of the human innate immune system which may play an important role in combating a variety of infectious diseases. We investigated the distribution of MBL gene mutations in a Vietnamese population, using polymerase chain reaction and DNA sequence analysis, and sought associations with the outcome of hepatitis B virus (HBV) infection. For this purpose we used samples from a total of 123 patients with confirmed, well-defined HBV infections, representing a full spectrum of clinical presentation from acute to chronic to malignant states, as well as from 112 healthy controls. The only MBL gene mutation found in this population, that at codon 54 of exon 1, was present at an overall frequency of 0.12, with a trend towards a higher frequency in the HBV-infected group compared with controls (0.15 versus 0.08, $P=0.079$). Within the HBV-infected group there was a non-significant trend towards higher viral loads in those with this mutation, accompanied by significantly higher serum transaminase levels in the same individuals. Segregation according to clinical presentation showed that the mutation was present at a significantly higher frequency in the group with acute hepatitis B (AHB) compared with the healthy control group (0.25 versus 0.08, $P=0.01$), and was associated with higher serum transaminase levels. Our results indicate that a mutation of the MBL gene might influence the clinical outcome of HBV infection in Vietnamese patients.

Staedtler, F., W. Suter, et al. (2004). "Induction of A:T to G:C transition mutations by 5-(2-chloroethyl)-2'-deoxyuridine (CEDU), an antiviral pyrimidine nucleoside analogue, in the bone marrow of Muta[trademark]Mouse." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **568**(2): 211.

<http://www.sciencedirect.com/science/article/B6T2C-4DK6CXX-2/2/5ccffa94e65a97772c005aabdd0fe02>

5-(2-Chloroethyl)-2'-deoxyuridine (CEDU) is a pyrimidine nucleoside analogue formerly in development for the treatment of herpes simplex virus infections. The compound proved clearly mutagenic in the mouse spot test and exhibited weak activity in the Salmonella reverse mutation test, which led to the termination of the compound's development. In another study, CEDU, administered orally to beta-galactosidase (lacZ) transgenic mice (Muta[trademark]Mouse) for five days, induced a clear increase in lacZ mutant frequencies in spleen, lung, and bone marrow [1]. In the present follow-up study, we analyzed 32 of those lacZ mutants isolated from the bone marrow of the Muta[trademark]Mouse animals of the experiments mentioned above, in order to

obtain further information on the type of mutations induced by CEDU. CEDU induced a pronounced increase in A:T to G:C transitions. The distribution of A:T to G:C transitions was clearly non-random, showing a bias towards T to C substitutions in the coding DNA strand and a preference to occur in the sequence motif 5'-(G or C)-T-G-3'. Our data support the hypothesis that CEDU, after being phosphorylated, is incorporated into cellular DNA in place of thymidine, which leads to mispairing with guanosine during subsequent DNA replication. As a result, the compound is thought to exert its mutagenicity by inducing mismatches leading to T to C transitions. Our findings point towards a mode of mutagenic action of CEDU that differs fundamentally from that of other antiviral antinucleosides whose clastogenic and recombinogenic activities prevail.

States, J. C. and S. P. Myrand (1996). "Splice site mutations in a xeroderma pigmentosum group A patient with delayed onset of neurological disease." Mutation Research/DNA Repair **363**(3): 171.

<http://www.sciencedirect.com/science/article/B6T2B-3WF7M8X-3/2/c2a0ddc4caf5235480e1c448c5f59fb4>

XP12BE is a commonly studied XP-A cell line that exhibits slightly increased resistance to UV compared with the majority of XP-A cell lines. The elevated UV survival is common to a subset of XP-A cell lines and correlates with delayed onset of the neurological disease in patients. We identified the XPA mutations in XP12BE by single strand conformation polymorphism (SSCP) analyses and nucleotide sequencing. XP12BE is a compound heterozygote and both mutations affect mRNA splicing. One mutation is a G to C transversion within the splice donor site of intron 4 that is common to several cell lines from XP-A patients with delayed onset of neurological disease. The other mutation is a G to T transversion at the same position as a G to C transversion in the splice acceptor site of intron 3 that is common in Japanese XP-A patients. We also demonstrated the persistence of the XP12BE mutations in cell line 2-O-A2 which has been shown to express XPA protein. These results suggest that the intron 4 splice donor mutation likely produces some, at least partially functional, XPA protein that accounts for the increased UV survival of XP-A cell lines derived from patients with delayed onset of neurological disease.

Teixeira, J. P., J. Gaspar, et al. (2002). "Aromatic DNA adduct levels in coke oven workers: correlation with polymorphisms in genes GSTP1, GSTM1, GSTT1 and CYP1A1." Mutation Research/Genetic Toxicology and Environmental Mutagenesis **517**(1-2): 147.

<http://www.sciencedirect.com/science/article/B6T2D-45JYJTV-2/2/b66717cb9b7e22b72f08dbff71080293>

The aim of this study was to use DNA adducts levels, detected by ³²P-postlabelling, as a biomarker to assess human exposure to polycyclic aromatic hydrocarbons (PAHs) from a coke oven plant and explore the possible association between CYP1A1 MspI, GSTP1, GSTM1 and GSTT1 genotypes, and smoking status on bulky DNA adduct formation. A large amount of inter-individual variation in adduct level was observed among workers with the same job and the same smoking habits. No significant differences were observed in DNA adduct levels between the coke oven workers and control group. Smokers in the control group had significantly higher DNA adducts than the non-smokers in the same group (35.13±21.11 versus 11.18±8.00, per 108 nucleotides, P=0.003). In this group, the correlation between the level of DNA adducts and the cigarettes smoked was strongly significant (r=0.70, P8 nucleotides, P=0.03). These results suggests that tobacco smoke could behave as a confounding factor for evaluation of DNA adducts arising from occupational exposure. The levels of DNA adducts in smokers not occupationally exposed to PAHs is dependent on the polymorphisms CYP1A1 MspI in the 3' non-coding region (49.04±22.18 versus 25.85±15.87, per 108 nucleotides, PGST genotypes

studied.

- Ushijima, T., Y. Hosoya, et al. (1995). "A rapid method for detection of mutations in the *lacI* gene using PCR-single strand conformation polymorphism analysis: demonstration of its high sensitivity." Mutation Research/Environmental Mutagenesis and Related Subjects **334**(3): 283.

<http://www.sciencedirect.com/science/article/B73H5-4BGYP09-2/2/662569fab04dd8b445da9cfec4cff96e>

The *lacI* gene has been used as a target gene in various mutation assays. We modified single strand conformation polymorphism (SSCP) analysis by introducing restriction digestion to detect mutations in the gene rapidly, and determined the sensitivity of the method. The entire coding sequence and partial promoter region of the *lacI* gene were amplified by the polymerase chain reaction with [α]-³²PdCTP in a 1247 base pair fragment, digested into eight restriction fragments, and analyzed by SSCP. The sensitivity of the method was assessed using 160 phages with *lacI* mutations, which were selected by assay of expression of [β]-galactosidase after their infection into *E. coli*. Of the 160 mutants, 146 (91.3%) showed shifted bands in the first condition of SSCP analysis (without glycerol, 20[deg]C). The remaining 14 mutants were analyzed in a second condition (with 5% glycerol, 20[deg]C), and eight of them showed shifted bands (cumulatively 96.3% of the 160 mutants). The remaining six mutants were analyzed in a third condition (with 5% glycerol, 10[deg]C), and all of them showed shifted bands (cumulatively 100%). Sequencing of the restriction fragments with mobility shifts in the 160 mutants revealed 108 kinds of mutations, 100 (92.6%) being detected in the first condition, seven (cumulatively 99.1%) in the second condition, and one (cumulatively 100%) in the third condition. This method greatly reduced the time to identify *lacI* mutations, and allowed the detection of multiple mutations in one *lacI* mutant. The results also show that in general PCR-SSCP analysis is very sensitive when test fragments are shorter than about 250 base pairs and electrophoresis is performed under at least two conditions.

- van Diemen, P. C. M., D. Maasdam, et al. (1997). "X-ray-sensitivity of lymphocytes of aplastic- and Diamond-Blackfan-anemia patients as detected by conventional cytogenetic and chromosome painting techniques." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **373**(2): 225.

<http://www.sciencedirect.com/science/article/B6T2C-3R3PRN6-9/2/792baf05326045c34557ea226bdd3e9a>

Frequencies of spontaneously occurring and X-ray induced, stable and unstable types of chromosome aberrations in peripheral blood lymphocytes from two groups of radiosensitive patients, i.e., aplastic anemia (AA) and Diamond-Blackfan anemia (DBA), were determined. Two types of staining methods, i.e., chromosome painting with two cocktails of chromosome-specific DNA libraries (Nos. 1, 3, X and Nos. 2, 4 and 8), as well as conventional Giemsa staining, were employed. Chromosome painting was done with single and multicolor fluorescence in situ hybridization (FISH). The frequencies of spontaneously occurring chromosome aberrations in AA and DBA patients were not significantly different from healthy individuals. Hypersensitivity to X-rays was seen both in G0 as well as in G2 phase of the cell cycle in lymphocytes from AA and DBA patients, confirming our earlier findings using micronucleus (DBA) and G2 radiosensitivity (AA) assays.

Van Sloun, P. P. H., R. J. Romeijn, et al. (1999). "Molecular cloning, expression and chromosomal localisation of the mouse Rev3l gene, encoding the catalytic subunit of polymerase [zeta]." Mutation Research/DNA Repair **433**(2): 109.

<http://www.sciencedirect.com/science/article/B6T2B-3VXYRNF-4/2/74d7dec1b7157c4f199ca06b0074c24c>

The REV3 gene of *Saccharomyces cerevisiae* encodes the catalytic subunit of DNA polymerase [zeta] which is involved in translesion synthesis. The mouse homolog of this gene, Rev3l, was cloned and sequenced. The gene encodes a putative protein of 3122 amino acids. The sequence conservation to its yeast counterpart is restricted to several regions. In the carboxy-terminal part of the protein all six domains are present that are characteristic for [alpha]-type DNA polymerases. In the amino-terminal part of the protein two regions can be identified with considerable similarity to the NT boxes of mouse polymerase [delta]. In addition, a region of 60 residues unique for the REV3 homologs can be found in the middle part of the protein. The mouse REV3L protein shows strong sequence conservation with the recently cloned human REV3L protein (86% identity overall). Northern blot analysis of various tissues of the mouse revealed that transcription of the Rev3l gene was highest in brain, ovaries and testis. The human REV3L gene was localised to the long arm of chromosome 6, region 21-22. The mouse equivalent maps to chromosome 10, distal to the c-myc gene, close to the Macs gene.

Vega, F. M. D. L., K. D. Lazaruk, et al. "Assessment of two flexible and compatible SNP genotyping platforms: TaqMan[trademark] SNP Genotyping Assays and the SNPLex[trademark] Genotyping System." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis In Press, Corrected Proof <http://www.sciencedirect.com/science/article/B6T2C-4FGX5DN-2/2/d8aa9a95d474b2befa60f556c66c75d3>

In this review we describe the principles, protocols, and applications of two commercially available SNP genotyping platforms, the TaqMan[trademark] SNP Genotyping Assays and the SNPLex[trademark] Genotyping System. Combined, these two technologies meet the requirements of multiple SNP applications in genetics research and pharmacogenetics. We also describe a set of SNP selection tools and validated assay resources which we developed to accelerate the cycle of experimentation on these platforms. Criteria for selecting the more appropriate of these two genotyping technologies are presented: the genetic architecture of the trait of interest, the throughput required, and the number of SNPs and samples needed for a successful study. Overall, the TaqMan assay format is suitable for low- to mid-throughput applications in which a high assay conversion rate, simple assay workflow, and low cost of automation are desirable. The SNPLex Genotyping System, on the other hand, is well suited for SNP applications in which throughput and cost-efficiency are essential, e.g., applications requiring either the testing of large numbers of SNPs and samples, or the flexibility to select various SNP subsets.

von Wurmb, N., M. Oehmichen, et al. (1998). "Demonstration of the 4977 bp deletion in human mitochondrial DNA from intravital and postmortem blood." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **422**(2): 247.

<http://www.sciencedirect.com/science/article/B6T2C-3V8TSPF-7/2/3719f51c046bf859df864251c3db2984>

The 4977 bp deletion in mitochondrial DNA (mtDNA) is known to accumulate with age in various human tissues. Findings regarding its accumulation in blood, however, have so far been

contradictory. We investigated the levels of the 4977 bp deletion in mtDNA from 100 intravital and postmortem blood samples. Applying an improved version of a PCR plus silver staining of polyacrylamide gels, we could detect the 4977 bp deletion in blood of healthy individuals over 20 years of age. While the 4977 bp deletion in blood is subject to a certain age dependence, it appears to be influenced by additional factors. A Primer-Shift-Assay amplifying four different deletion-specific fragments showed that the smaller fragments were amplified with a higher amplification efficiency than the larger fragments. The deletion-specific 389 bp fragment was demonstrated in 73% of individuals over 80 years of age, but in only 46% of individuals between 21 and 30 years old whereas the largest 802 bp deletion-specific fragment was detectable in 38% of subjects over 80 years of age, and in only 15% of individuals under 30 years of age. Deletion-specific fragments were not detected in a single individual under 20 years old, nor in fetal blood. In this work, we demonstrate for the first time the detection of 4977 bp specific fragments in blood of healthy individuals without the necessity of using a nested PCR. The deletion is detectable in postmortal and intravital blood, so that the occurrence of the 4977 bp deletion seems to be a physiological and not only a postmortal process.

Walker, V. E. and T. R. Skopek (1993). "A mouse model for the study of in vivo mutational spectra: Sequence specificity of ethylene oxide at the hprt locus." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **288**(1): 151.

<http://www.sciencedirect.com/science/article/B6T2C-47PG610-2N/2/2339058089135955862f3c991b955fa8>

We have developed an approach for determining mutational spectra in exon 3 of the hypoxanthineguanine phosphoribosyl transferase (hprt) gene in splenic T-lymphocytes of B6C3F1 mice. Hprt- mutants from treated animals were isolated by culturing splenic T-cells in microtiter dishes containing medium supplemented with IL-2, concanavalin A, and 6-thioguanine. DNA was extracted from 6-thioguanine-resistant co colonies and amplified by the polymerase chain reaction (PCR) using primers flanking the exon 3 region of hprt. Identification of samples containing mutant exon 3 sequences and purification of mutant DNA from contaminating wild-type hprt DNA was accomplished using denaturing gradient gel electrophoresis. Purified mutant sequences were then sequenced. This approach is being used to study the sequence specificity of ethylene oxide (ETO). 12-day-old mice were given single i.p. injections of 100 mg ETO/kg every other day or 30, 60, 90 or 120 mg ETO/kg daily for 5 days to achieve different cumulative doses of this compound. In mice exposed every other day, cumulative doses of 200, 600 and 900 mg ETO/kg produced average mutant frequencies of 15 ± 12.8 , 45 ± 13.2 , and $73 (70, 75) \times 10^{-6}$, respectively, 8 weeks after the first treatment. In mice exposed daily, cumulative doses of 150, 300, 450 and 600 mg ETO/kg produced average mutation frequencies of 4.2 ± 10.4 , 8.2 ± 10.4 , 11.1 ± 1.0 and $15.5 \pm 10.7 \times 10^{-6}$, respectively, 20 weeks after the first treatment. The mutant fraction in control mice was less than 3×10^{-6} . 123 hprt- mutants from mice exposed to 600 or 900 mg ETO/kg were isolated and analyzed for mutations in exon 3. 18 were located in exon 3 (14.6%). DNA sequencing revealed that 11/18 mutations were base-pair substitutions at 8 different sites in exon 3. Four AT transversions, three AT transitions, two GC transversions, and two GC transitions were observed. Three of the substitutions (2 AT $\rightarrow$ CG, 1 AT $\rightarrow$ GC) occurred at one base (203) in a single animal. The remaining 7 mutations, isolated from 4 different animals, were the same + 1 frameshift mutation in a run of 6 consecutive guanine bases (207-212) in exon 3. These results suggest the involvement of both modified guanine and adenine bases in ETO mutagenesis. The mouse T-cell cloning/sequencing assay for hprt described here represents a useful system for studying the molecular mechanism of chemically-induced mutation occurring in vivo at an endogenous gene.

Wang, G., L. M. Hallberg, et al. (1999). "Short Interspersed DNA Element-mediated detection of UVB-

induced DNA damage and repair in the mouse genome, in vitro, and in vivo in skin." Mutation Research/DNA Repair **433**(3): 147.

<http://www.sciencedirect.com/science/article/B6T2B-3WD5C3J-1/2/703efb76af1bb8b03f37c1b178dfca33>

We report a sensitive, SINE (Short Interspersed DNA Element)-mediated, PCR-based, DNA damage detection assay. Here, the SINE assay is used for detection of UVB-induced DNA damage and repair in cultured mouse cells and in vivo, in mouse skin. The unique feature of the SINE assay is its ability to support simultaneous amplification of multiple, random segments of genomic DNA. This can be accomplished due to the remarkable abundance, dispersion and conservation of SINEs in mammalian genomes. The most abundant SINEs in the mouse genome are the B1 elements, at a copy number of 50,000-80,000. Due to their strong sequence conservation, primers complementary to the B1 consensus sequence anneal to the majority of their targets in the genome. Consequently, long segments of genomic DNA located between pairs of B1 elements are efficiently amplified by PCR. Thus, in conjunction with the fact that many types of DNA adducts form blocks for thermostable polymerase, the B1 element anchored PCR makes a sensitive and versatile tool for assessing the overall integrity of the transcribed regions in mouse genome. We measured UVB-dose (0.1-3 kJ m⁻²) dependent formation of photoproducts in DNA from cultured cells, and after 20 h observed a substantial removal of damage at doses lower or equal to 0.6 kJ m⁻². The sensitivity of detection of UVB-photoproducts formation and repair was compared to that of the conventional, single locus-targeting QPCR. Using the SINE assay we also have shown the distribution of UVB and UVC induced DNA adducts at a single nucleotide resolution within the B1 elements in mouse DNA. Lastly, we demonstrated that the sensitivity of the SINE assay is adequate for measurement of UVB-dose (1-6 kJ m⁻²) dependent formation and subsequent removal of photoproducts in vivo, in mouse skin.

Wang, J. and J. Liu (2004). "Directly fishing out subtle mutations in genomic DNA with histidine-tagged *Thermus thermophilus* MutS." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **547**(1-2): 41.

<http://www.sciencedirect.com/science/article/B6T2C-4BP9P00-1/2/3d559b5d46bdf025d793730179f78f24>

Tth MutS, a mismatch repair protein from *Thermus thermophilus*, was reported to effectively recognize all eight possible types of base pair mismatches and insertions or deletions up to three base pairs at a wide temperature range up to 60 [deg]C. Here a procedure for directly fishing out subtle unknown mutations in bacterial genome with Tth MutS was described. Wild type genomic DNA and mutant one were mixed, digested with restriction enzymes, denatured and re-annealed. Hetero-duplex DNA carrying mispaired bases were bound to Tth MutS and recovered through Ni-NTA His-Bind(R) Resin. The recovered DNA was cloned into plasmids, producing a mini-library with inserts of the mutated regions. Further DNA sequencing and genetic complementation demonstrated that the method was extremely efficient in fishing out the mutations from total genomic DNA. Using this method, the mutations existed in a *Pseudomonas aeruginosa* mutant strain were screened, indicating that A/G transitions at nt 181 and nt 314 in chloramphenicol acetyltransferase (catB7) gene conferred this strain with a high chloramphenicol dosage resistant. Compared with those reported previously, this protocol can screen the mixed mutations more easily.

Wardell, T. M., E. Ferguson, et al. (2003). "Changes in the human mitochondrial genome after treatment of malignant disease." Mutation Research/Fundamental and Molecular Mechanisms of

Mutagenesis **525**(1-2): 19.

<http://www.sciencedirect.com/science/article/B6T2C-47MJ4X7-1/2/7553ae296cff6ed22a402d00e781fb93>

Mitochondrial DNA (mtDNA) is the only extrachromosomal DNA in human cells. The mitochondrial genome encodes essential information for the synthesis of the mitochondrial respiratory chain. Inherited defects of this genome are an important cause of human disease. In addition, the mitochondrial genome seems to be particularly prone to DNA damage and acquired mutations may have a role in ageing, cancer and neurodegeneration. We wished to determine if radiotherapy and chemotherapy used in the treatment of cancer could induce changes in the mitochondrial genome. Such changes would be an important genetic marker of DNA damage and may explain some of the adverse effects of treatment. We studied samples from patients who had received radiotherapy and chemotherapy for point mutations within the mtDNA control region, and for large-scale deletions. In blood samples from patients, we found a significantly increased number of point mutations compared to the control subjects. In muscle biopsies from 7 of 8 patients whom had received whole body irradiation as well as chemotherapy, the level of a specific mtDNA deletion was significantly greater than in control subjects. Our studies have shown that in patients who have been treated for cancer there is an increased level of mtDNA damage.

Webster, M. T., R. S. Wells, et al. (2002). "Analysis of variation in the human [beta]-globin gene cluster using a novel DHPLC technique." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **501**(1-2): 99.

<http://www.sciencedirect.com/science/article/B6T2C-45215VR-1/2/7bbc9d27c6067f5befc51293b213b901>

We have implemented a technique combining allele-specific PCR (AS-PCR) and denaturing high-performance liquid chromatography (DHPLC) to identify new polymorphic variants within an intergenic region in the [beta]-globin cluster. This technique is applicable to the detection of new variants in genomic regions where variation is apportioned into distinct classes of haplotype. Duplexes for DHPLC analysis were created by denaturation and re-annealing of a mixture of two AS-PCR products of known and unknown sequence from the same haplotypic class, permitting detection of new haplotypes in each class. A 454 bp fragment 3.5 kb 5' to the human [delta]-globin gene, which may have a gene regulatory function, was analysed in 840 chromosomes from a global sampling of human populations using this method. Two divergent haplotypes were found to predominate in all populations studied, possibly as a result of balancing selection.

Wenz, H. M., S. Ramachandra, et al. (1998). "Identification of known p53 point mutations by capillary electrophoresis using unique mobility profiles in a blinded study." Mutation Research/Mutation Research Genomics **382**(3-4): 121.

<http://www.sciencedirect.com/science/article/B6T2F-3T888C3-8/2/8ddb3f7806ab06aa14f2cbeb73c4de62>

This study is part of an ongoing project at the National Institute of Standards and Technology (NIST) that generates a panel of DNA clones containing the most common mutations found in the human p53 tumor suppressor gene. This panel will be made available as a reference source for evaluation and testing for p53 mutations. Single strand conformation polymorphism (SSCP) analysis has found widespread acceptance as a tool for simply and rapidly screening for

mutations, albeit with a detection rate that can be below 100%. We have begun to analyze mutations found in exon 7 of the p53 gene by SSCP using laser induced fluorescence capillary electrophoresis (LIF-CE). PCR fragments, containing single point mutations, were amplified from genomic DNA isolated from cell lines using primers labeled with two different fluorophores. This dual labeling approach allowed better traceability of mobility shifts as a function of the experimental conditions. While analyzing the clones H596, Colo320, Namalwa and wild type (reference samples) at different temperatures, ranging from 25 to 45[deg]C, it was observed that each mutation responded in a unique way to changes in temperature both in magnitude and direction of shifts relative to the wild type sample. In a blinded study, ten p53 exon 7 samples were matched automatically, using ABI PRISM Genotyper(R) software, against the four reference samples. From these 10 samples, six were correctly identified as containing one of the reference mutations, two corresponded to wild type, and two were correctly identified as non-reference mutations. This approach should prove helpful in the rapid screening of target sequences that are known to bear a limited number of mutations.

Wilson, V. L., K. R. Wade, et al. (2001). "Temporal delineation of sequential HPRT mutations arising in vivo in a T-cell clone with a mutator phenotype." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **473**(2): 181.

<http://www.sciencedirect.com/science/article/B6T2C-428FM7J-J/2/86373ec45d17beab4a92f35965f510c7>

Recurrent mutations in vivo in T-lymphocytes identify clonally restricted genomic instabilities in some individuals. Cell-based assays allow initial recognition of clones with mutator phenotypes, but genotypic selection is required to determine frequencies and temporal sequences of potentially independent mutational events isolated only as complex changes in the same allele. The present work illustrates how two single-base insertions in the HPRT gene recovered only as a double event in a cell-based assay were shown to arise as separate in vivo mutations, being individually present at frequencies of 10^{-4} and 10^{-5} , respectively, in peripheral blood. Full characterizations of mutator clones will allow elucidation of the earliest events in the emergence of genomic instability in human somatic cells.

Wilson, V. L., Q. Wei, et al. (1999). "Needle-in-a-haystack detection and identification of base substitution mutations in human tissues." Mutation Research/Mutation Research Genomics **406**(2-4): 79.

<http://www.sciencedirect.com/science/article/B6T2F-3X70SXM-5/2/26840eedec4ff7ec2497ece3ac779e55>

Background and induced germline mutagenesis and other genotoxicity studies have been hampered by the lack of a sufficiently sensitive technique for detecting mutations in a small cluster of cells or a single cell in a tissue sample composed of millions of cells. The most frequent type of genetic alteration is intragenic. The vast majority of oncogenic mutations in human and mammalian cancer involves only single base substitutions. We have developed universally applicable techniques that not only provide the necessary sensitivity and specificity for site specific mutagenesis studies, but also identify the point mutation. The exponential amplification procedures of polymerase chain reaction (PCR) and ligase chain reaction (LCR) have been combined with restriction endonuclease (RE) digestion to enable the selective enrichment and detection of single base substitution mutations in human oncogenic loci at a sensitivity of one mutant in more than 10^7 wild type alleles. These PCR/RE/LCR procedures have been successfully designed and used for codons 12 and 248 of the Ha-ras and p53 genes, respectively, both of which contain a natural MspI restriction endonuclease recognition sequence. These procedures have also been adapted for the detection and identification of mutations in

oncogenic loci that do not contain a natural restriction endonuclease recognition sequence. Using PCR techniques, a HphI site was incorporated into the codons 12/13 region of the human N-ras gene, which was then used for the selective enrichment of mutants at this oncogenic locus. These PCR/RE/LCR procedures for base substitution mutations in codon 12 of the N-ras gene were found to have the sensitivity of detection of at least one mutant allele in the presence of the DNA equivalent of 106 wild type cells. Only one peripheral blood leukocyte DNA specimen out of nine normal individuals displayed an observable Ha-ras mutation that was present at frequency between 10⁻⁵ and 10⁻⁶. These PCR/RE/LCR techniques for detecting and identifying base substitution mutations are universally applicable to almost any locus or base site within the human or animal genome. With the added advantage of the adjustability of both the amount of DNA (number of genomes) to be tested and the sensitivity (10⁻² to 10⁻⁷) of the assay selection or enrichment procedures, these PCR/RE/LCR techniques will be useful in addressing a broad range of important questions in mutagenesis and carcinogenesis.

Xu, Z., Y. Yu, et al. (1993). "Multiplex DNA amplification and solid-phase direct sequencing for mutation analysis at the hprt locus in Chinese hamster cells." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **288**(2): 237.

<http://www.sciencedirect.com/science/article/B6T2C-47PCNN3-8V/2/f15d99bffd31d39aa76a76c07989e236>

We report here the development of multiplex in vitro DNA amplification and solid-phase direct exon sequencing for the analysis of mutations at the hypoxanthine-guanine phosphoribosyltransferase (hprt) locus in Chinese hamster cells. 18 representative HPRT-deficient mutants, derived either spontaneously, or after exposure to UV light or ionizing radiation, were analyzed. All 9 hprt exons were simultaneously amplified via the polymerase chain reaction (PCR) for rapid deletion detection. 5 mutants involve single or multiple-exon deletions. Altered multiplex PCR patterns were detected in mutants Bsp-040, Bsp-065 and BGR-606. Subsequent direct sequence analysis reveals that Bsp-040 and Bsp-065 carry a 52-bp and a 13-bp intragenic DNA deletion in exon 3, respectively. BGR-606 contains a 223-bp insertion accompanied by a 10-bp deletion of intron sequence within exon 4 fragment. Other subtle DNA alterations identified by direct exon sequence analysis include single-base substitutions, small deletions and insertions, and RNA splicing mutations.

Yang, H., M. Mazur-Melnyk, et al. (1999). "A comparison of mutational specificity of mutations induced by S9-activated B[a]P and benzo[a]pyrene-7,8-diol-9,10-epoxide at the endogenous apt gene in CHO cells." Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis **423**(1-2): 23.

<http://www.sciencedirect.com/science/article/B6T2C-3VNR7B0-3/2/cb271a1c523d55b16bfef7cbb6d4f811>

We have determined the mutational specificity of S9-activated benzo[a]pyrene (B[a]P) at the endogenous apt locus in a hemizygous Chinese hamster ovary cell line. The apt gene of recovered mutants was amplified using the polymerase chain reaction (PCR) and directly sequenced. This spectrum was then compared to mutations recovered following treatment with the B[a]P metabolite, benzo[a]pyrene diol-epoxide (BPDE). No significant difference between the two spectra in the types of mutations produced, or their distribution was observed. This observation supports the hypothesis that BPDE is the reactive metabolite of B[a]P, responsible for the significant biological effects caused by this ubiquitous polycyclic aromatic hydrocarbon. The major mutation recovered was the G:C=>T:A transversion, and mutations were primarily localized within runs of guanines. We also confirmed our previous finding that mutation by B[a]P

is non-random, targeting events in runs of guanines flanked by adenine residues. This same target hotspot region is found in codon 61 of the human c-Ha-ras1 proto-oncogene. This may help explain the selective activation of this codon by BPDE.

Yu, J. J., C. Mu, et al. (1997). "A nucleotide polymorphism in ERCC1 in human ovarian cancer cell lines and tumor tissues." Mutation Research/Mutation Research Genomics **382**(1-2): 13.

<http://www.sciencedirect.com/science/article/B6T2F-3SY9RHW-3/2/cbadf6bf2b8ba92a9140c7999525dd27>

We studied the DNA sequence of the entire coding region of ERCC1 gene, in five cell lines established from human ovarian cancer (A2780, A2780/CP70, MCAS, OVCAR-3, SK-OV-3), 29 human ovarian cancer tumor tissue specimens, one human T-lymphocyte cell line (H9), and non-malignant human ovary tissue (NHO). Samples were assayed by PCR-SSCP and DNA sequence analyses. A silent mutation at codon 118 (site for restriction endonuclease MaeII) in exon 4 of the gene was detected in MCAS, OVCAR-3 and SK-OV-3 cells, and NHO. This mutation was a C->T transition, that codes for the same amino acid: asparagine. This transition converts a common codon usage (AAC) to an infrequent codon usage (AAT), whereas frequency of use is reduced two-fold. This base change was associated with a detectable band shift on SSCP analysis. For the 29 ovarian cancer specimens, the same base change was observed in 15 tumor samples and was associated with the same band shift in exon 4. Cells and tumor tissue specimens that did not contain the C->T transition, did not show the band shift in exon 4. Our data suggest that this alteration at codon 118 within the ERCC1 gene, may exist in platinum-sensitive and platinum-resistant ovarian cancer tissues.

Zhang, L., R. B. Hayes, et al. (2004). "Lack of increased genetic damage in 1,3-butadiene-exposed Chinese workers studied in relation to EPHX1 and GST genotypes." Mutation Research/Genetic Toxicology and Environmental Mutagenesis **558**(1-2): 63.

<http://www.sciencedirect.com/science/article/B6T2D-4BG45X0-1/2/f0ed3b1cc57d8ec4a2aa3fb870da4b5e>

1,3-Butadiene (BD) is an important industrial chemical and pollutant. Its ability to induce genetic damage and cause hematological malignancies in humans is controversial. We have examined chromosome damage by fluorescence in situ hybridization (FISH) and mutations in the HPRT gene in the blood of Chinese workers exposed to BD. Peripheral blood samples were collected and cultured from 39 workers exposed to BD (median level 2 ppm, 6 h time-weighted average) and 38 matched controls in Yanshan, China. No difference in the level of aneuploidy or structural changes in chromosomes 1, 7, 8, and 12 was detected in metaphase cells from exposed subjects in comparison with matched controls, nor was there an increase in the frequency of HPRT mutations in the BD-exposed workers. Because genetic polymorphisms in glutathione S-transferase (GST) enzymes and microsomal epoxide hydrolase (EPHX1) may affect the genotoxic effects of BD and its metabolites, we also related chromosome alterations and gene mutations to GSTT1, GSTM1 and EPHX1 genotypes. Overall, there was no effect of variants in these genotypes on numerical or structural changes in chromosomes 1, 7, 8 and 12 or on HPRT mutant frequency in relation to BD exposure, but the GST genotypes did influence background levels of both hyperdiploidy and HPRT mutant frequency. In conclusion, our data show no increase in chromosomal aberrations or HPRT mutations among workers exposed to BD, even in potentially susceptible genetic subgroups. The study is, however, quite small and the levels of BD exposure are not extremely high, but our findings in China do support those from a similar study conducted in the Czech Republic. Together, these studies suggest that low levels of occupational BD exposure do not pose a significant risk of genetic damage.

Zhang, Y., D. G. Monckton, et al. (2002). "Detection of radiation and cyclophosphamide-induced mutations in individual mouse sperm at a human expanded trinucleotide repeat locus transgene." Mutation Research/Genetic Toxicology and Environmental Mutagenesis **516**(1-2): 121.

<http://www.sciencedirect.com/science/article/B6T2D-45H93HP-1/2/ac9e1b1989d6a6d159aa45fe0791717b>

A method to measure the germline mutations induced by cancer treatment in humans is needed. To establish such a method we used a transgenic mouse model consisting of a human DNA repeat locus that has a high spontaneous mutation frequency as a biomarker. Alterations in repeat number were measured in individual sperm from mice hemizygous for an expanded (CTG)₁₆₂ human myotonic dystrophy type 1 (DM1) microsatellite repeat using single genome-equivalent (g.e.) PCR and detection by a DNA fragment analyzer. Mutation frequencies were measured in DNA from sperm from controls and sperm derived from stem spermatogonia, differentiating spermatogonia, and spermatocytes exposed to radiation and from spermatocytes of mice treated with cyclophosphamide. There was no increase above control levels in mutations, scored as >1 repeat changes, in any of the treated groups. However, moderately large deletion mutants (between 9 and 20 repeat changes) were observed at frequencies of 2.2% when spermatocytes were treated with cyclophosphamide and, 1.8 and 2.5% when spermatocytes and stem cells, respectively, were treated with radiation, which were significantly higher than the frequency of 0.3% in controls. Thus, radiation and cyclophosphamide induced deletions in the expanded DM1 trinucleotide repeat. PCR artifacts were characterized in sperm DNA from controls and from mice treated with radiation; all artifacts involved losses of more than 20 DM1 repeats, and surprisingly the artifact frequency was higher in treated sperm than in control sperm. The radiation-induced increase in the frequency of PCR artifacts might reflect alterations in sperm DNA that destabilize the genome not only during PCR amplification but also during early embryonic development.

Mutation Research Genomics (9)

Bellanne-Chantelot, C., S. Beaufils, et al. (1997). "Search for DNA sequence variations using a MutS-based technology." Mutation Research/Mutation Research Genomics **382**(1-2): 35.

<http://www.sciencedirect.com/science/article/B6T2F-3SY9RHW-5/2/e07fdfe956294f9c0abaa386b2beb8ac>

The search for DNA sequence variations (DSV) is emphasized with genetic studies of a large number of multifactorial diseases. Saturation of regions of interest with diallelic polymorphisms will be an essential step to pinpoint, through association studies, predisposing genes. We have developed a solid-phase method based on the ability of mismatch binding protein MutS to recognize single nucleotide mismatches. This approach was applied to the study of 83 sequence-tagged sites (STSs) extracted from an eight centimorgans (cM) chromosome 21 region. One-third of tested STSs were found to be polymorphic leading to a frequency of one DSV every 822 base pairs (bp). Sequencing of analyzed STSs showed the high reliability of the MutS-based technology for mismatches up to 2 bp in DNA fragments ranging in size from 200 bp to 1 kilobase (kb). The entire assay which is performed in a solid-phase format without the need of electrophoresis or sequencing, will provide an efficient tool for new polymorphism detection.

Cattaneo, M., M. Zollo, et al. (2001). "Allelic polymorphisms in the transcriptional regulatory region of human SEL1L." Mutation Research/Mutation Research Genomics **458**(3-4): 71.

<http://www.sciencedirect.com/science/article/B6T2F-453BS2D-2/2/6a0ce9b51263125ba3168099e89b3fef>

In this work, we explored the existence of genetic variants within the SEL1L transcriptional regulatory region by direct sequencing of the basal promoter. SEL1L is the human ortholog of the *Caenorhabditis elegans* gene *sel-1*, a negative regulator of LIN-12/NOTCH receptor proteins. To understand the relation in SEL1L transcription pattern observed in different epithelial cells, we analysed its promoter activity. We found it to be considerably higher only in pancreatic cells. We then looked for the presence of genetic variability within this region by sequencing the minimal promoter of 63 individuals (126 alleles); two new and associated polymorphic variants were found only in few lung carcinoma bearing patients. The functional effects of this polymorphism was analysed by transient transfection assay which resulted in a significant increase in the transcriptional activity of the gene.

Livak, K. J., W. A. Little, et al. (1998). "Polymorphisms in the human DNA ligase I gene (LIG1) including a complex GT repeat." Mutation Research/Mutation Research Genomics **406**(1): 1.

<http://www.sciencedirect.com/science/article/B6T2F-3V4J6S2-1/2/553b33ca561d521b0e225d038468c26b>

Sequencing of a human DNA ligase I cDNA clone derived from HeLa cells revealed two unreported differences with the published sequence: a single base change and a three-base deletion. Both differences are in exon 6, and were analyzed by amplifying a segment containing exon 5, intron 6, and exon 6. The first finding was that intron 6 is approximately 2.6 kb in size, not the 1 kb reported in the literature. By sequence analysis of amplified segments, the single-base difference in exon 6 was shown to be polymorphic, with HeLa cells heterozygous for the A/C difference. Analysis of 60 unrelated individuals found a frequency of 0.5 for each allele. Primer extension reactions across the exon 5/exon 6 boundary were performed on cDNA obtained from HeLa cells and human thymus. The results show that the three-base deletion is due to a variation in splicing. For both HeLa and thymus, two-thirds of the transcripts are like the published cDNA sequence and one-third have the three-base deletion. Finally, sequencing of part of intron 6 revealed the presence of a complex GT repeat consisting of a 48-50 nucleotide polypurine tract followed by a variable number of GT residues. This entire unit of polypurine tract plus GTs is repeated three times. Detection of the repeated sequences required the development of specialized cloning and PCR conditions. Analysis of a pedigree showed that this complex repeat is polymorphic.

Orimo, H., M. Ikejima, et al. (1998). "A novel missense mutation and frameshift mutations in the type II receptor of transforming growth factor- β gene in sporadic colon cancer with microsatellite instability." Mutation Research/Mutation Research Genomics **382**(3-4): 115.

<http://www.sciencedirect.com/science/article/B6T2F-3T888C3-7/2/d4fd8660014d77bbbbc6fc75a328df16>

Microsatellite instability of DNA samples of 79 sporadic colon cancer patients were analyzed. These samples were also screened to search mutations in the repeat sequences in the gene for

the type II receptor of transforming growth factor- β (TGF- β RII) using polymerase chain reaction (PCR), electrophoresis with urea gel, and PCR-single strand conformation polymorphism (PCR-SSCP) method. The incidence of microsatellite instability, defined as severe replication error phenotype (RER) with microsatellite alterations in more than three loci, was 6%. Deletion and insertion of an A residue in the (A)₁₀ region, which cause frameshift mutation, were found in four samples and their incidence in the samples with microsatellite instability was 80%. A novel nucleotide substitution of T for G at 1918, which causes missense mutation of arginine to leucine at codon 528, was found in a sample with microsatellite instability. The mutation at 1918 was in highly conservative amino acid residue.

Ponten, F., C. Williams, et al. (1997). "Genomic analysis of single cells from human basal cell cancer using laser-assisted capture microscopy." Mutation Research/Mutation Research Genomics **382**(1-2): 45.

<http://www.sciencedirect.com/science/article/B6T2F-3SY9RHW-6/2/6bbe1d386bbe9ca9645064499f98b6e>

In this study, we show that direct mutational analysis of genomic DNA can be performed on single somatic cells extracted from a frozen, immunohistochemically stained tissue section using laser-assisted capture microscopy. Eighty-nine single tumor cells were separately dissected from one case of human basal cell cancer (BCC) and p53 mutations were analyzed by direct semi-automated sequencing of PCR fragments. Amplification was obtained for at least one of the two analyzed exons from approximately 50% of the single tumor cells. Identical p53 mutations were found in widely spread areas of the tumor, suggesting a clonal proliferation originating from one cell. Interestingly, comparison between results of immunohistochemistry and genetic analysis of the single cells revealed the same p53 mutations irrespective of the p53 immunoreactivity. We propose that this approach has a great potential to allow investigation of genotypic differences in single cells and more specifically to resolve important and fundamental questions determining cancer heterogeneity.

Powell, E., L. Shi, et al. (2001). "Frequency and distribution in three ethnic populations of single nucleotide polymorphisms in the MICA gene." Mutation Research/Mutation Research Genomics **432**(3-4): 47.

<http://www.sciencedirect.com/science/article/B6T2F-4292MHS-1/2/4928e4a17e7dd3ddea3f929299350212>

The Homo sapiens major histocompatibility complex (MHC) class 1 chain related gene A (MICA) was scanned for novel single nucleotide polymorphisms (SNPs) using a panel of DNA samples from African-, Japanese- and Mexican-Americans. Overlapping primer-pairs were used to amplify products in the size range of 300 to 400 bp that were sequenced and scanned for SNPs using Phred, Phrap, Polyphred and Consed sequence analysis programs. A total of 16 SNPs were detected, six of which represent new variant nucleotides in the Homo sapiens MICA gene. Three of the variants also represent amino acid changes in the MICA protein. Differences among the three ethnic panels in the frequency of the variant nucleotides observed were inconsistent, but significant for seven of the SNPs detected. Though a small sample size, this study represents the first multi-population based analysis of the frequency and distribution of SNPs in the MICA gene, a locus that may be essential in the antigenic recognition by $\gamma\delta$ T cells.

Wenz, H. M., S. Ramachandra, et al. (1998). "Identification of known p53 point mutations by capillary electrophoresis using unique mobility profiles in a blinded study." Mutation Research/Mutation Research Genomics **382**(3-4): 121.

<http://www.sciencedirect.com/science/article/B6T2F-3T888C3-8/2/8ddb3f7806ab06aa14f2cbeb73c4de62>

This study is part of an ongoing project at the National Institute of Standards and Technology (NIST) that generates a panel of DNA clones containing the most common mutations found in the human p53 tumor suppressor gene. This panel will be made available as a reference source for evaluation and testing for p53 mutations. Single strand conformation polymorphism (SSCP) analysis has found widespread acceptance as a tool for simply and rapidly screening for mutations, albeit with a detection rate that can be below 100%. We have begun to analyze mutations found in exon 7 of the p53 gene by SSCP using laser induced fluorescence capillary electrophoresis (LIF-CE). PCR fragments, containing single point mutations, were amplified from genomic DNA isolated from cell lines using primers labeled with two different fluorophores. This dual labeling approach allowed better traceability of mobility shifts as a function of the experimental conditions. While analyzing the clones H596, Colo320, Namalwa and wild type (reference samples) at different temperatures, ranging from 25 to 45[deg]C, it was observed that each mutation responded in a unique way to changes in temperature both in magnitude and direction of shifts relative to the wild type sample. In a blinded study, ten p53 exon 7 samples were matched automatically, using ABI PRISM Genotyper(R) software, against the four reference samples. From these 10 samples, six were correctly identified as containing one of the reference mutations, two corresponded to wild type, and two were correctly identified as non-reference mutations. This approach should prove helpful in the rapid screening of target sequences that are known to bear a limited number of mutations.

Wilson, V. L., Q. Wei, et al. (1999). "Needle-in-a-haystack detection and identification of base substitution mutations in human tissues." Mutation Research/Mutation Research Genomics **406**(2-4): 79.

<http://www.sciencedirect.com/science/article/B6T2F-3X70SXM-5/2/26840eedec4ff7ec2497ece3ac779e55>

Background and induced germline mutagenesis and other genotoxicity studies have been hampered by the lack of a sufficiently sensitive technique for detecting mutations in a small cluster of cells or a single cell in a tissue sample composed of millions of cells. The most frequent type of genetic alteration is intragenic. The vast majority of oncogenic mutations in human and mammalian cancer involves only single base substitutions. We have developed universally applicable techniques that not only provide the necessary sensitivity and specificity for site specific mutagenesis studies, but also identify the point mutation. The exponential amplification procedures of polymerase chain reaction (PCR) and ligase chain reaction (LCR) have been combined with restriction endonuclease (RE) digestion to enable the selective enrichment and detection of single base substitution mutations in human oncogenic loci at a sensitivity of one mutant in more than 10⁷ wild type alleles. These PCR/RE/LCR procedures have been successfully designed and used for codons 12 and 248 of the Ha-ras and p53 genes, respectively, both of which contain a natural MspI restriction endonuclease recognition sequence. These procedures have also been adapted for the detection and identification of mutations in oncogenic loci that do not contain a natural restriction endonuclease recognition sequence. Using PCR techniques, a HphI site was incorporated into the codons 12/13 region of the human N-ras gene, which was then used for the selective enrichment of mutants at this oncogenic locus. These PCR/RE/LCR procedures for base substitution mutations in codon 12 of the N-ras gene were found to have the sensitivity of detection of at least one mutant allele in the presence of the DNA equivalent of 10⁶ wild type cells. Only one peripheral blood leukocyte DNA specimen out of nine normal individuals displayed an observable Ha-ras mutation that was present at frequency

between 10⁻⁵ and 10⁻⁶. These PCR/RE/LCR techniques for detecting and identifying base substitution mutations are universally applicable to almost any locus or base site within the human or animal genome. With the added advantage of the adjustability of both the amount of DNA (number of genomes) to be tested and the sensitivity (10⁻² to 10⁻⁷) of the assay selection or enrichment procedures, these PCR/RE/LCR techniques will be useful in addressing a broad range of important questions in mutagenesis and carcinogenesis.

Yu, J. J., C. Mu, et al. (1997). "A nucleotide polymorphism in ERCC1 in human ovarian cancer cell lines and tumor tissues." Mutation Research/Mutation Research Genomics **382**(1-2): 13.

<http://www.sciencedirect.com/science/article/B6T2F-3SY9RHW-3/2/cbadf6bf2b8ba92a9140c7999525dd27>

We studied the DNA sequence of the entire coding region of ERCC1 gene, in five cell lines established from human ovarian cancer (A2780, A2780/CP70, MCAS, OVCAR-3, SK-OV-3), 29 human ovarian cancer tumor tissue specimens, one human T-lymphocyte cell line (H9), and non-malignant human ovary tissue (NHO). Samples were assayed by PCR-SSCP and DNA sequence analyses. A silent mutation at codon 118 (site for restriction endonuclease MaeII) in exon 4 of the gene was detected in MCAS, OVCAR-3 and SK-OV-3 cells, and NHO. This mutation was a C->T transition, that codes for the same amino acid: asparagine. This transition converts a common codon usage (AAC) to an infrequent codon usage (AAT), whereas frequency of use is reduced two-fold. This base change was associated with a detectable band shift on SSCP analysis. For the 29 ovarian cancer specimens, the same base change was observed in 15 tumor samples and was associated with the same band shift in exon 4. Cells and tumor tissue specimens that did not contain the C->T transition, did not show the band shift in exon 4. Our data suggest that this alteration at codon 118 within the ERCC1 gene, may exist in platinum-sensitive and platinum-resistant ovarian cancer tissues.

Mutation Research Letters (7)

Andersson, B. and B. Lambert (1990). "Mutations induced by benzo[a]pyrene diolepoxide at the hprt locus in human T-lymphocytes in vitro." Mutation Research Letters **245**(2): 75.

<http://www.sciencedirect.com/science/article/B73H4-47P83MR-1C/2/7093b5820807d2b8beb8404809edb7b4>

Human T-lymphocytes have been treated with benzo[a]pyrene diolepoxide (BPDE) in vitro and T-cell clones mutated in the hprt gene have been isolated. The mutant frequencies in BPDE-treated T-cell cultures were on average 24-fold higher than those of untreated cultures. Thus, BPDE is a potent inducer of gene mutation in this system. In order to examine which types of mutations are induced by BPDE in human cells, 41 spontaneous and 44 BPDE-induced mutant clones have been characterized using the Southern blot technique. In addition, rearrangements of the T-cell-receptor[beta] and [gamma] loci have been used to determine the proportion of isolated clones that are unique, and thus likely to represent independent mutational events. Out of 23 independent spontaneous mutants 4 had large hprt alterations that could be detected on Southern blots. Two of these alterations, deletions of exons 2-6, have been confirmed using PCR of hprt cDNA and direct sequencing of the PCR product. All 33 independent BPDE-induced

mutants had normal hprt restriction patterns which indicates that BPDE is mainly a point mutagen in this system.

Fusco, J. C., L. J. Zimmerman, et al. (1992). "Large deletions are tolerated at the hprt locus of in vivo derived human T-lymphocytes." Mutation Research Letters **283**(4): 255.

<http://www.sciencedirect.com/science/article/B73H4-47PP1FH-9W/2/f42c09c4c7ba5f17678cbb778f96b7c4>

A cloning assay was used to recover hprt- T-lymphocytes from adult human males. Analysis of crude cellular extracts by polymerase chain reactions (PCRs) demonstrated that 7% (16/218) of the hprt mutation were due to total deletion of the hprt gene. 14 of the 16 mutants were examined by PCR for the presence of flanking DNA to determine the extent of the deletions. The deletion mutation in 13 mutants was at least 350 kb with 5 of these deletions being at least 700 kb. The largest deletions were greater than 15 times the size of the hprt gene. Therefore, large deletions are tolerated at the hprt locus of the T-lymphocytes.

Hovig, E., B. Smith-Sorensen, et al. (1991). "Constant denaturant gel electrophoresis, a modification of denaturing gradient gel electrophoresis, in mutation detection." Mutation Research Letters **262**(1): 63.

<http://www.sciencedirect.com/science/article/B73H4-47PP0XS-3H/2/8a72fe18176c6ba63e5a56dd46894bce>

Denaturing gradient gel electrophoresis (DGGE) is increasingly being utilized in mutational detection, both in characterization of variations in genomic DNA and in the generation of mutational spectra after in vitro and in vivo mutagenesis. The basis for this electrophoretic separation technique is strand dissociation of DNA fragments in discrete, sequence-dependent melting domains followed by an abrupt decrease in mobility. We have modified the DGGE by using constant denaturant gels corresponding to the specific melting domains of certain DNA fragments. This leads to increased resolution of mutants as fragments differing in as little as 1 base pair migrate with a consistently different mobility through the whole gel allowing separations of several centimeters. By using a set of constant denaturant gels it is also possible to obtain a better approximation of the location of the different mutations as each denaturant concentration will correspond to specific melting domains. We have used this technique to separate 6 out of 7 exon-3 hypoxanthine phosphoribosyltransferase (HPRT) mutants while using conventional DGGE we were only able to separate 3.

Kubota, Y., A. Shimada, et al. (1992). "Detection of [gamma]-ray-induced DNA damages in malformed dominant lethal embryos of the Japanese medaka (*Oryzias latipes*) using AP-PCR fingerprinting." Mutation Research Letters **283**(4): 263.

<http://www.sciencedirect.com/science/article/B73H4-47PP1FH-9X/2/4aba9503df047fc693eb79fbc5d2a081>

Adult male fish of the medaka HNI strain exposed to 9.5 Gy or 19 Gy (0.95 Gy/min) of [gamma]-rays were mated with non-irradiated female fish of the Hd-rR strain. Genomic DNA was prepared from malformed individual embryos which were expected to be dominant lethal and used for AP-PCR fingerprinting. By the use of a part of the T3 promoter sequence (20 mer), which to our

knowledge, is not found in the medaka genome as an arbitrary primer, we found polymorphisms in genomic fingerprints which could distinguish the parental strains. On the other hand, we found that the fingerprints of F1 hybrids were the sum of those of their parents. Based on those findings, we analyzed the fingerprints of genomic DNA of each severely malformed embryo, because we expect that radiation-induced genomic damages resulting in severe malformation and eventually in dominant lethals should be detected as changes in paternal fingerprints of F1 hybrids. Indeed, we succeeded in detecting changes in genomic DNA as loss of some paternal bands in fingerprints of malformed embryos. One of 10 malformed embryos obtained from 9.5 Gy [gamma]-irradiated males had lost one band of the paternal origin and 4 of 12 malformed embryos obtained from 19 Gy [gamma]-irradiated males had lost 5 bands. These results indicated a possibility that quantitative as well as qualitative estimation of [gamma]-ray-induced DNA damages can be made by this method which does not require the functional selection based on a specific target gene.

Swiger, R. R., B. Myhr, et al. (1994). "The LacZ transgene in mutaTM Mouse maps to chromosome 3." Mutation Research Letters **325**(4): 145.

<http://www.sciencedirect.com/science/article/B73H4-47S0RVY-5/2/9707d6793c146e7eb62c63362a3ed1ef>

Transgenic mouse models are being used with increasing frequency for mutational and toxicological studies. One such system, MutaMouse, contains a stably integrated lambda-gt10LacZ shuttle vector in the mouse genome. We describe the use of dual color fluorescence in situ hybridization (FISH) with *Mus musculus* whole chromosome paints and lambda DNA to map the integration site of the lambda transgene to band C on mouse chromosome 3.

Weber, C. A., J. M. Kirchner, et al. (1994). "Molecular analysis of CXPB mutations in the repair-deficient hamster mutants UV5 and UVL-13." Mutation Research Letters **324**(4): 147.

<http://www.sciencedirect.com/science/article/B73H4-47RSF9F-20/2/15a16b1b28a6ce4c901b75404b8e187e>

The cDNA sequence of the Chinese hamster xeroderma pigmentosum group D (CXPB) nucleotide excision repair gene was analyzed from three Chinese hamster ovary (CHO) cell lines: repair proficient strain AA8 and repair deficient, UV complementation group 2 strains UV5 and UVL-13. CXPB encodes a presumed ATP-dependent DNA helicase and is single copy in CHO lines due to the hemizyosity of chromosome 9. Comparison of the deduced wild-type AA8 CXPB protein sequence with that of the Chinese hamster V79 lung-derived cell line revealed two amino acid polymorphisms. Position 285 is glutamine in AA8 and arginine in V79, and position 298 is alanine in AA8 and threonine in V79. Comparison with the human XPD, *Saccharomyces cerevisiae* RAD3, and *Schizosaccharomyces pombe* rad15 homologs shows variability at these positions. Analysis of the CXPB sequence in the repair deficient CHO lines UV5 and UVL-13 revealed, in each case, a single base substitution resulting in an amino acid substitution. Position 116 is tyrosine in UV5 and cysteine in AA8, and the corresponding positions of XPD, RAD3, and rad15 are cysteine. Position 615 is glutamic acid in UVL-13 and glycine in AA8, and the corresponding positions of XPD, RAD3, and rad15 are glycine. In both UV5 and UVL-13, positions 285 and 298 are glutamine and alanine, respectively, as seen in AA8. These results suggest that cysteine 116 and glycine 615 are critical to the repair function of CXPB.

Yu, Y., Z. Xu, et al. (1994). "Adriamycin induces large deletions as a major type of mutation in CHO cells." Mutation Research Letters **325**(2-3): 91.

<http://www.sciencedirect.com/science/article/B73H4-47T1MDX-7/2/12eb7ef325f2aca45f4def082123631a>

Adriamycin (ADR), a commonly used cancer chemotherapy antibiotic, exhibits a variety of genotoxicities. In this study, we have examined the mutagenicity of ADR at the hypoxanthine-guanine phosphoribosyltransferase gene (hprt) in Chinese hamster ovary (CHO) cells and the xanthine-guanine phosphoribosyltransferase locus (gpt) in a pSV2gpt-transformed CHO cell line, AS52. Although ADR induced a dose-dependent increase of mutant frequency at both loci, it was more mutagenic to the gpt gene than to the hprt locus. Multiplex PCR analysis revealed that 35% of the 103 independent ADR-induced HPRT-deficient mutants carried large deletions. Among these deletion mutants, 33% were total gene deletions, 22% affected multiple exons, and 42% involved a single exon, of which most (9/15) were exon 1. The majority (63%) of ADR-induced AS52 mutants had a total deletion of the gpt gene. These observations indicate that ADR induces large deletions as a major type of gene mutation in mammalian cells, suggesting the involvement of reactive oxygen species as one mutagenic pathway in the mutagenesis of ADR.

N. Engl. J. Med. (4)

Hien, T. T., N. T. Liem, et al. (2004). "Avian Influenza A (H5N1) in 10 Patients in Vietnam." N. Engl. J. Med. **350**(12): 1179-1188.

<http://content.nejm.org/cgi/content/abstract/350/12/1179>

Background Recent outbreaks of avian influenza A (H5N1) in poultry throughout Asia have had major economic and health repercussions. Human infections with this virus were identified in Vietnam in January 2004. **Methods** We report the clinical features and preliminary epidemiologic findings among 10 patients with confirmed cases of avian influenza A (H5N1) who presented to hospitals in Ho Chi Minh City and Hanoi, Vietnam, in December 2003 and January 2004. **Results** In all 10 cases, the diagnosis of influenza A (H5N1) was confirmed by means of viral culture or reverse transcriptase-polymerase chain reaction with primers specific for H5 and N1. None of the 10 patients (mean age, 13.7 years) had preexisting medical conditions. Nine of them had a clear history of direct contact with poultry (median time before onset of illness, three days). All patients presented with fever (temperature, 38.5 to 40.0{degrees}C), respiratory symptoms, and clinically significant lymphopenia (median lymphocyte count, 700 per cubic millimeter). The median platelet count was 75,500 per cubic millimeter. Seven patients had diarrhea. In all patients, there were marked abnormalities on chest radiography. There was no definitive evidence of human-to-human transmission. Eight patients died, one patient has recovered, and one is recovering. **Conclusions** Influenza A (H5N1) infection, characterized by fever, respiratory symptoms, and lymphopenia, carries a high risk of death. Although in all 10 cases the infection appears to have been acquired directly from infected poultry, the potential exists for genetic reassortment with human influenzaviruses and the evolution of human-to-human transmission. Containment of influenza A (H5N1) in poultry throughout Asia is therefore urgently required.

Moreno, J. C., H. Bikker, et al. (2002). "Inactivating Mutations in the Gene for Thyroid Oxidase 2 (THOX2)

and Congenital Hypothyroidism." *N. Engl. J. Med.* **347**(2): 95-102.

<http://content.nejm.org/cgi/content/abstract/347/2/95>

Background Several genetic defects are associated with permanent congenital hypothyroidism. Immunologic, environmental, and iatrogenic (but not genetic) factors are known to induce transient congenital hypothyroidism, which spontaneously resolves within the first months of life. We hypothesized that molecular defects in the thyroid oxidase system, which is composed of at least two proteins, might be involved in the pathogenesis of permanent or transient congenital hypothyroidism in babies with defects in iodide organification, for which the oxidase system is required. **Methods** Nine patients were recruited who had idiopathic congenital hypothyroidism (one with permanent and eight with transient hypothyroidism) and an iodide-organification defect and who had been identified by the screening program for congenital hypothyroidism. The DNA of the patients and their relatives was analyzed for mutations in the genes for thyroid oxidase 1 (THOX1) and 2 (THOX2). **Results** The one patient with permanent and severe thyroid hormone deficiency and a complete iodide-organification defect had a homozygous nonsense mutation in the THOX2 gene that eliminates all functional domains of the protein. Three of the eight patients with mild transient congenital hypothyroidism and a partial iodide-organification defect had heterozygous mutations in the THOX2 gene that prematurely truncate the protein, thus abolishing its functional domains. **Conclusions** Biallelic inactivating mutations in the THOX2 gene result in complete disruption of thyroid-hormone synthesis and are associated with severe and permanent congenital hypothyroidism. Monoallelic mutations are associated with milder, transient hypothyroidism caused by insufficient thyroidal production of hydrogen peroxide, which prevents the synthesis of sufficient quantities of thyroid hormones to meet the large requirement for thyroid hormones at the beginning of life.

Ong, P. Y., T. Ohtake, et al. (2002). "Endogenous Antimicrobial Peptides and Skin Infections in Atopic Dermatitis." *N. Engl. J. Med.* **347**(15): 1151-1160.

<http://content.nejm.org/cgi/content/abstract/347/15/1151>

Background The innate immune system of human skin contains antimicrobial peptides known as cathelicidins (LL-37) and β -defensins. In normal skin these peptides are negligible, but they accumulate in skin affected by inflammatory diseases such as psoriasis. We compared the levels of expression of LL-37 and human β -defensin 2 (HBD-2) in inflamed skin from patients with atopic dermatitis and from those with psoriasis. **Methods** The expression of LL-37 and HBD-2 protein in skin-biopsy specimens from patients with psoriasis, patients with atopic dermatitis, and normal subjects was determined by immunohistochemical analysis. The amount of antimicrobial peptides in extracts of skin samples was also analyzed by immunodot blot analysis (for LL-37) and Western blot analysis (for HBD-2). Quantitative, real-time reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assays were used to confirm the relative expression of HBD-2 and LL-37 messenger RNA (mRNA) in the skin-biopsy specimens. These peptides were also tested for antimicrobial activity against *Staphylococcus aureus* with the use of a colony-forming assay. **Results** Immunohistochemical analysis confirmed the presence of abundant LL-37 and HBD-2 in the superficial epidermis of all patients with psoriasis. In comparison, immunostaining for these peptides was significantly decreased in acute and chronic lesions from patients with atopic dermatitis ($P=0.006$ and $P=0.03$, respectively). These results were confirmed by immunodot blot and Western blot analyses. Real-time RT-PCR showed significantly lower expression of HBD-2 mRNA and LL-37 mRNA in atopic lesions than in psoriatic lesions ($P=0.009$ and $P=0.02$, respectively). The combination of LL-37 and HBD-2 showed synergistic antimicrobial activity by effectively killing *S. aureus*. **Conclusions** A deficiency in the expression of antimicrobial peptides may account for the susceptibility of patients with atopic dermatitis to skin infection with *S. aureus*.

Shore, E. M., J. Ahn, et al. (2002). "Paternally Inherited Inactivating Mutations of the GNAS1 Gene in Progressive Osseous Heteroplasia." N. Engl. J. Med. **346**(2): 99-106.

<http://content.nejm.org/cgi/content/abstract/346/2/99>

Background Progressive osseous heteroplasia (POH), an autosomal dominant disorder, is characterized by extensive dermal ossification during childhood, followed by disabling and widespread heterotopic ossification of skeletal muscle and deep connective tissue. Occasional reports of mild heterotopic ossification in Albright's hereditary osteodystrophy (AHO) and a recent report of two patients with AHO who had atypically extensive heterotopic ossification suggested a common genetic basis for the two disorders. AHO is caused by heterozygous inactivating mutations in the GNAS1 gene that result in decreased expression or function of the alpha subunit of the stimulatory G protein (Gs{alpha}) of adenylyl cyclase. **Methods** We tested the hypothesis that GNAS1 mutations cause POH, using the polymerase chain reaction to amplify GNAS1 exons and exon-intron boundaries in 18 patients with sporadic or familial POH. **Results** Heterozygous inactivating GNAS1 mutations were identified in 13 of the 18 probands with POH. The defective allele in POH is inherited exclusively from fathers, a result consistent with a model of imprinting for GNAS1. Direct evidence that the same mutation can cause either POH or AHO was observed within a single family, in which the phenotype correlated with the parental origin of the mutant allele. **Conclusions** Paternally inherited inactivating GNAS1 mutations cause POH. This finding extends the range of phenotypes derived from haploinsufficiency of GNAS1, provides evidence that imprinting is a regulatory mechanism for GNAS1 expression, and suggests that Gs{alpha} is a critical negative regulator of osteogenic commitment in nonosseous connective tissues.

Nephrol. Dial. Transplant. (8)

Fries, J. W. U., T. Roth, et al. (2003). "A novel evaluation method for paraffinized human renal biopsies using quantitative analysis of microdissected glomeruli and VCAM-1 as marker of inflammatory mesangial cell activation." Nephrol. Dial. Transplant. **18**(4): 710-716.

<http://ndt.oupjournals.org/cgi/content/abstract/18/4/710>

Background. In the glomerular mesangium, immunologic and/or infectious activation of the inflammatory, NF- κ B-mediated signal pathway can induce a progression of already existing mesangial lesions in non-immunologic and immunologic glomerular disease. This progression is preceded by upregulated mesangial gene expression of which the vascular cell adhesion molecule-1, VCAM-1 (vascular cell adhesion molecule-1), is a well-established marker. Its evaluation on minimal tissue such as routinely paraffinized needle core biopsies is not established and needs the development of a novel evaluation method more meaningful than common immunohistology. **Methods.** By laser-microdissection, 10 glomeruli/case were isolated from 5 μ m thick tissue slices in a total of 15 cases of mesangial proliferation with different renal diseases (IgA nephropathy, lupus nephritis and mesangial proliferative lesions of unknown aetiology) vs transplant biopsies as negative and TNF α -treated cultured human mesangial cells as positive controls. After reverse transcription of isolated RNA, cDNA aliquots were quantified for VCAM-1 expression by real-time PCR using the threshold cycle (Ct) method, normalized for the housekeeping gene β -actin, and compared with qualitative RT-PCR results. **Results.** Unsuspected VCAM-1 transcript steady-state levels could be detected by real-

time PCR in agreement with qualitative PCR, while morphologic and immunohistologic analyses were unrevealing. As yields of RNA extraction in femtogram quantities cannot be measured spectrophotometrically, a Ct-ratio was formed between β -actin and VCAM-1 per case showing high VCAM-1 expression in lupus nephritis (1.39), and moderate expression in IgA nephropathy (1.08-1.23) vs TNF α -treated mesangial cells (0.97-1.23) and negative control cases (0.66-0.68). Conclusions. This is the first reported gene expression analysis method for routinely paraffinized human renal biopsies, demonstrating the power of combined laser-microdissection and PCR quantification as novel methods for the evaluation of minimal tissue beyond purely descriptive morphologic analysis.

Lam, S., R. N. van der Geest, et al. (2004). "Secretion of collagen type IV by human renal fibroblasts is increased by high glucose via a TGF- β -independent pathway." Nephrol. Dial. Transplant. **19**(7): 1694-1701.

<http://ndt.oupjournals.org/cgi/content/abstract/19/7/1694>

Background. Tubulointerstitial fibrosis is an important component of diabetic nephropathy, which is characterized by increased expression of interstitial extracellular matrix components and aberrant expression of the basement membrane component collagen type IV. The present study examined the effect of high ambient glucose and transforming growth factor- β 1 (TGF- β 1) on collagen secretion by human renal fibroblasts and proximal tubular epithelial cells (PTECs). Methods. Human renal fibroblasts (TK173) and PTECs (HK2) were used to examine the effects of high glucose (25 mM D-glucose) and TGF- β 1 (1 ng/ml) on collagen type I, III and IV secretion compared with control medium (5.5 mM glucose). Matrix components were measured by enzyme-linked immunosorbent assay (ELISA) and reverse transcription-polymerase chain reaction (RT-PCR). Results. Renal fibroblasts are the main producers of the interstitial components collagen type I and type III, while collagen type IV was secreted predominantly by PTECs. However, renal fibroblasts were also able to secrete collagen type IV. Secretion of collagen type IV by fibroblasts was increased upon stimulation with TGF- β 1, reaching levels comparable with those secreted by TGF- β 1-induced PTECs. Moreover, high glucose stimulated increased collagen type IV secretion. Importantly, this could not be attenuated by neutralizing pan-specific anti-TGF- β antibodies. Conclusions. These data show that renal fibroblasts secrete collagen type IV, which can be increased by high glucose independent of endogenous TGF- β . This suggests that as well as the increased expression of interstitial components, renal fibroblasts can contribute to the increased expression of the basement membrane component collagen type IV in tubulointerstitial fibrosis observed during diabetic nephropathy.

Langham, R. G., D. J. Kelly, et al. (2003). "Over-expression of platelet-derived growth factor in human diabetic nephropathy." Nephrol. Dial. Transplant. **18**(7): 1392-1396.

<http://ndt.oupjournals.org/cgi/content/abstract/18/7/1392>

Background. The pathogenetic mechanisms responsible for progressive renal impairment of diabetic nephropathy are still poorly understood, despite its growing incidence. Increasing evidence suggests that growth factors may contribute to the initiation and progressive fibrosis of diabetic nephropathy. In this study, the gene expression and protein distribution of platelet-derived growth factor-A and -B (PDGF-A and PDGF-B) in human diabetic nephropathy were examined. Methods. PDGF-A and PDGF-B mRNA levels in surplus renal biopsy tissue from seven patients with overt diabetic nephropathy and six nephrectomy samples were examined using quantitative reverse transcription-polymerase chain reaction (RT-PCR). In addition, each sample was also examined immunohistochemically to quantify and localize peptide expression of

each PDGF isoform. Results. Gene expression of PDGF-A and PDGF-B mRNA were increased 22- and 6-fold, respectively, in biopsies from patients with diabetic nephropathy compared with control tissue. Immunostaining also demonstrated increased peptide expression of both PDGF-A and PDGF-B in diabetic nephropathy, with each isoform showing a specific pattern of tissue distribution. Conclusions. The findings of increased gene and protein expression of PDGF in renal biopsies from patients with diabetic nephropathy imply a potential role for this prosclerotic growth factor in the development of the progressive fibrosis that characterizes human diabetic kidney disease.

Malaponte, G., V. Bevelacqua, et al. (2002). "IL-1{beta}, TNF-{alpha} and IL-6 release from monocytes in haemodialysis patients in relation to dialytic age." Nephrol. Dial. Transplant. **17**(11): 1964-1970.

<http://ndt.oupjournals.org/cgi/content/abstract/17/11/1964>

Background. It has been suggested that changes in immune response to infectious agents in patients on haemodialysis might be due to impaired monocyte function; uraemic and haemodialysed patients overproduce proinflammatory cytokines, such as interleukin-1 beta (IL-1{beta}), tumor necrosis factor-alpha (TNF-{alpha}) and interleukin-6 (IL-6). Methods. We quantitated the cytokines released into the plasma and into the supernatants of 24-h cultured purified monocytes, under basal conditions and after stimulation by lipopolysaccharide from *Escherichia coli*, in 15 healthy subjects (CON), 20 uraemic patients who had not yet started dialysis (CRF) and 60 haemodialysed patients (HD), who were divided into three groups of 20 patients corresponding to short-, medium- and long-term dialysis. Results. Monocytes from HD patients spontaneously secreted significantly higher levels of cytokines than those from controls and uraemic patients who had not yet started dialysis. After stimulation with lipopolysaccharide (LPS), cytokine levels in culture supernatants of cells from HD patients were significantly lower than those from controls and uraemic patients. Moreover, levels of cytokines in monocyte supernatants and plasma from short-, medium- and long-term haemodialysed patients decreased progressively with dialytic age. Monocytes from haemodialysed patients tended to be constitutively active, but their ability to secrete proinflammatory cytokines was inversely correlated with dialytic age. Conclusions. These results indicate that prolonged treatment with dialysis can be considered a form of chronic stress that causes the progressive activation of monocytes, which ultimately leads to monocyte exhaustion and dysfunction.

Odamaki, M., A. Kato, et al. (2004). "Counter-regulatory effects of procalcitonin and indoxyl sulphate on net albumin secretion by cultured rat hepatocytes." Nephrol. Dial. Transplant. **19**(4): 797-804.

<http://ndt.oupjournals.org/cgi/content/abstract/19/4/797>

Background. Although hypoalbuminaemia is a significant predictor of mortality in haemodialysis (HD) patients, the pathophysiological mechanisms involved remain to be determined. Albumin is a negative acute-phase reactant and many proinflammatory substances are elevated in HD patients. We investigated factors that may affect liver albumin synthesis. Methods. Hepatocytes were isolated from rat livers and were cultured with interleukin (IL)-4, IL-6, IL-12, tumor necrosis factor (TNF)-{alpha}, procalcitonin (PCT), a sensitive marker of infection, and indoxyl sulphate (IS), a uraemic toxin. Albumin levels in the supernatant were measured by enzyme-linked immunosorbent assay. Albumin mRNA expression was determined by reverse transcriptase polymerase chain reaction. Results. IL-6 and TNF-{alpha} significantly decreased albumin levels in a dose-dependent manner ($P < 0.01$ and $P < 0.05$, respectively). In contrast, IL-4 and IL-12 did not modulate albumin production. PCT and IS significantly and dose-dependently increased albumin levels (both $P < 0.01$). PCT increased albumin mRNA expression in the hepatocytes ($P = 0.05$) and dose-dependently abrogated IL-6-induced suppression of albumin synthesis ($P < 0.01$).

IS also blocked the IL-6-induced decrease in net albumin secretion ($P<0.01$). Conclusion. Our findings indicate that PCT and IS protect against suppression of hepatic albumin synthesis caused by proinflammatory cytokines, suggesting their potential role in preventing hypoalbuminaemia in HD patients.

Trochen, N., S. Ganapathipillai, et al. (2004). "Low prevalence of nonconservative mutations of serum and glucocorticoid-regulated kinase (SGK1) gene in hypertensive and renal patients." Nephrol. Dial. Transplant. **19**(10): 2499-2504.

<http://ndt.oupjournals.org/cgi/content/abstract/19/10/2499>

Background. The serum- and glucocorticoid-regulated kinase (SGK1) gene is an important mediator of aldosterone action, regulating the expression of the renal epithelial Na⁺ channel. In renal failure, blood pressure (BP) is markedly salt-dependent and increases with decreasing renal function. Mutations of the SGK1 gene affecting phosphorylation could be responsible for salt-mediated increases in BP and hypertension-related progression to end-stage renal disease (ESRD). Methods. The SGK1 gene was analysed for mutations in the exons 4, 5, 8 and 10-12, because of potential phosphorylation sites, in 591 subjects, including 311 ESRD patients (either dialysis or transplanted). In addition, an intron 6 single-nucleotide polymorphism (SNP) described previously was also investigated in this study. Genotyping was performed either by using a strategy based on single strand conformation polymorphism analysis of polymerase chain reaction (PCR) products and subsequent direct sequencing of identified gel shift variants or by using high throughput 5' nuclease allelic discrimination assay. Results. Two SNPs in coding regions of SGK1 potentially influencing the phosphorylation of Sgk1 were identified. Both SNPs were synonymous. The prevalence of the first variant, a previously reported SNP at codon 240 in exon 8, did not differ between ESRD patients (16.3%) and controls (15.7%). There was no association between the SNP in exon 8 and either BP within the control population or progression of renal disease in the ESRD population. The second SNP at codon 398 in exon 12 was identified in one patient only. Intron 6 and exon 8 SNPs were in strong linkage disequilibrium, but did not show any association with either BP or renal diseases. Conclusions. Based on statistical analysis homozygosity for nonconservative mutations in the coding region of the SGK1 gene is estimated at $< 1/300\ 000$ when a white Caucasian population is considered, arguing against an important role of mutations of this coding region in hypertension and hypertension-associated progression of renal disease.

Umekawa, T., K. Byer, et al. (2005). "Diphenyleneiodium (DPI) reduces oxalate ion- and calcium oxalate monohydrate and brushite crystal-induced upregulation of MCP-1 in NRK 52E cells." Nephrol. Dial. Transplant. **20**(5): 870-878.

<http://ndt.oupjournals.org/cgi/content/abstract/20/5/870>

Background. Our earlier studies have demonstrated upregulation of monocyte chemoattractant protein-1 (MCP-1) in NRK52E rat renal epithelial cells by exposure to oxalate (Ox) ions and crystals of calcium oxalate monohydrate (COM) or the brushite (Br) form of calcium phosphate. The upregulation was mediated by reactive oxygen species (ROS). This study was performed to investigate whether NADPH oxidase is involved in ROS production. Methods. Confluent cultures of NRK52E cells were exposed to Ox ions or COM and Br crystals. They were exposed for 1, 3, 6, 12, 24 and 48 h for isolation of MCP-1 mRNA and 24 h for enzyme-linked immunosorbent assay (ELISA) to determine the secretion of protein into the culture medium. We also investigated the effect of free radical scavenger, catalase, and the NADPH oxidase inhibitor diphenyleneiodium (DPI) chloride, on the Ox- and crystal-induced expression of MCP-1 mRNA and protein. The transcription of MCP-1 mRNA in the cells was determined using real-time

polymerase chain reaction. Hydrogen peroxide and 8-isoprostane were measured to investigate the involvement of ROS. Results. Exposure of NRK52E cells to Ox ions as well as the crystals resulted in increased expression of MCP-1 mRNA and production of the chemoattractant. Treatment with catalase reduced the Ox- and crystal-induced expression of both MCP-1 mRNA and protein. DPI reduced the crystal-induced gene expression and protein production but not Ox-induced gene expression and protein production. Conclusions. Exposure to Ox ions, and COM and Br crystals stimulates a ROS-mediated increase in MCP-1 mRNA expression and protein production. Reduction in ROS production, lipid peroxidation, low-density lipoprotein release, and inducible MCP-1 gene and protein in the presence of DPI indicates an involvement of NADPH oxidase in the production of ROS.

Umekawa, T., N. Chegini, et al. (2003). "Increased expression of monocyte chemoattractant protein-1 (MCP-1) by renal epithelial cells in culture on exposure to calcium oxalate, phosphate and uric acid crystals." Nephrol. Dial. Transplant. **18**(4): 664-669.

<http://ndt.oupjournals.org/cgi/content/abstract/18/4/664>

Background. During the development of non-infectious kidney stones, crystals form and deposit in the kidneys and become surrounded by monocytes/macrophages (M/M). We have proposed that in response to crystal exposure renal epithelial cells produce chemokines, which attract the M/M to the sites of crystal deposition. We investigated the expression of monocyte chemoattractant protein-1 (MCP-1) mRNA and protein by NRK52E rat renal tubular epithelial cells exposed to calcium oxalate (CaOx), brushite (Br, a calcium phosphate) and uric acid (UA) crystals. Methods. Confluent cultures of NRK52E cells were exposed to CaOx, Br or UA at a concentration of 250 {micro}g/ml (66.7 {micro}g/cm²). They were exposed for 1, 3, 6, 12, 24 and 48 h for isolation of mRNA and 24 h for ELISA to determine the secretion of protein into the culture medium. Since cells are known to produce free radicals on exposure to CaOx crystals we also investigated the effect of free radical scavenger catalase on the crystal induced expression of MCP-1 mRNA and protein. Results. Exposure of NRK52E cells to the crystals resulted in increased expression of MCP-1 mRNA and production of the chemoattractant. CaOx crystals were most provocative while UA the least. Treatment with catalase had a negative effect on the increased expression of both MCP-1 mRNA and protein, which indicates the involvement of free radicals in up-regulation of MCP-1 production. Conclusion. Exposure to both CaOx and calcium phosphate crystals stimulates increased production of MCP-1. Free radicals appear to be involved in this up-regulation. Results indicate that MCP-1, which is often associated with localized inflammation, may be one of the chemokine mediators associated with the deposition of various urinary crystals in the kidneys during kidney stone formation. Because of the small number of experiments performed here, results must be confirmed by more extensive studies with larger sample size.

Neurobiology of Aging(5)

Gaudreault, S. B., D. Dea, et al. (2004). "Increased caveolin-1 expression in Alzheimer's disease brain." Neurobiology of Aging **25**(6): 753.

<http://www.sciencedirect.com/science/article/B6T09-49YDBDW-4/2/b88369a1c0ea3d80bc0de19fe6d76404>

Increasing evidence suggests that cholesterol plays a central role in the pathophysiology of Alzheimer's disease (AD). Caveolin is a cholesterol-binding membrane protein involved in cellular cholesterol transport. We investigated the changes in the protein amount of hippocampal caveolin of autopsy-confirmed AD and aged-matched control subjects. Our results demonstrate that caveolin protein levels in the hippocampus and caveolin mRNA in the frontal cortex are up-regulated in AD by approximately two-fold, compared to control brains. These results suggest a relationship between caveolin-1 expression levels and a dysregulation of cholesterol homeostasis at the plasma membrane of brain cells. In support of this hypothesis, a significant increase in caveolin protein levels has also been observed in hippocampal tissue from ApoE-deficient (knockout) and aged wild-type mice; two situations associated with modifications of transbilayer distribution of cholesterol in brain synaptic plasma membranes. These results indicate that caveolin over-expression is linked to alterations of cholesterol distribution in the plasma membrane of brain cells and are consistent with the notion of a deterioration of cholesterol homeostasis in AD.

Kolker, D. E., M. H. Vitaterna, et al. (2004). "Effects of age on circadian rhythms are similar in wild-type and heterozygous Clock mutant mice." *Neurobiology of Aging* **25**(4): 517.

<http://www.sciencedirect.com/science/article/B6T09-4B0WSP3-2/2/71f57ec7e63032ad239f6adb284461cd>

The amplitudes of many circadian rhythms, at the behavioral, physiological, cellular, and biochemical levels, decrease with advanced age. Previous studies suggest that the amplitude of the central circadian pacemaker is decreased in old animals. Recently, it has been reported that expression of several circadian clock genes, including Clock, is lower in the master circadian pacemaker of old rodents. To test the hypothesis that decreased activity of a circadian clock gene renders animals more susceptible to the effects of aging, we analyzed the circadian rhythm of locomotor activity in young and old wild-type and heterozygous Clock mutant mice. We found that the effects of age and the Clock mutation were additive. These results indicate that age-related changes in circadian rhythmicity occur equally in wild-type and heterozygous Clock mutants, suggesting that the Clock mutation does not render mice more susceptible to the effects of age on the circadian pacemaker.

Sleegers, K., T. den Heijer, et al. "ACE gene is associated with Alzheimer's disease and atrophy of hippocampus and amygdala." *Neurobiology of Aging In Press, Corrected Proof*
<http://www.sciencedirect.com/science/article/B6T09-4DW3F56-1/2/1abc64810a3b6be1fae5551eba2e31fe>

Despite biological support for a role of angiotensin converting enzyme (ACE) in Alzheimer's disease (AD), studies assessing the ACE I/D polymorphism in AD are conflicting. We re-evaluated this association in the Rotterdam Study, a population-based cohort study. The mechanism of association was further explored by adjusting for vascular factors, and by analysing atrophy, white matter lesions and infarcts on MRI in non-demented individuals. Genotypes were available for 6488 participants. During average follow-up of 6 years 250 subjects developed AD. MRI data were available for 494 non-demented participants. Homozygosity for the I-allele conferred a slightly increased risk of AD compared to carrying a D-allele (RR 1.12 (95% CI 0.99-1.25)). This increase was only significant in women, and independent of vascular factors (RR 1.39 (95% CI 1.14-1.69)). Non-demented women with the II genotype had smaller hippocampal and amygdalar volumes. Vascular pathology was not significantly associated with ACE. This suggests a modest but significant increase in risk of AD and early AD pathology in women homozygous for the ACE I-allele independent of vascular factors.

Sugaya, K., R. Greene, et al. (1998). "Septo-hippocampal cholinergic and neurotrophin markers in age-induced cognitive decline." Neurobiology of Aging **19**(4): 351.

<http://www.sciencedirect.com/science/article/B6T09-3V3DPMM-9/2/c1e3d540e9a51378625cc81300c3e340>

Messenger RNA (mRNA) molecules encoding proteins related to the presynaptic cholinergic and neurotrophin systems were quantitated in the hippocampus and basal forebrain of Long-Evans rats with spatial learning ability assessed in the Morris water maze. The reverse transcriptase-polymerase chain reaction showed that the mRNAs for the low-affinity neurotrophin receptor (p75-NTR) and the growth-associated protein GAP-43 were decreased in level in the basal forebrain of aged-impaired rats. In the hippocampus of these aged-impaired rats, the mRNA for VGF, another neurotrophin-inducible gene, also was decreased. In situ hybridization histochemistry revealed that mRNAs for nerve growth factor (NGF) and brain-derived neurotrophic factor increased in level in the aged rat hippocampus; when age effects were removed, NGF mRNA level remained significantly correlated with maze performance. Enzyme-linked immunosorbent assay indicated that NGF protein was expressed at normal levels in the aged rat hippocampus. These mRNA and protein alterations may signify that a defect in neurotrophin signaling exists in the brains of aged Long-Evans rats, underlying reduced plasticity responses in the basal forebrain cholinergic system.

Zohar, O., C. G. Pick, et al. "Age-dependent differential expression of BACE splice variants in brain regions of tg2576 mice." Neurobiology of Aging **In Press, Corrected Proof**

<http://www.sciencedirect.com/science/article/B6T09-4F6CRKB-2/2/e09f1f7d384f64db0356043810b8f198>

Plaques found in the brains of patients suffering from Alzheimer's disease (AD) mainly consist of [beta]-amyloid (A[beta]), which is produced by sequential cleaving of amyloid precursor protein (APP) by two proteolytic enzymes, [beta]- and [gamma]-secretases. Any change in the fine balance between these enzymes and their substrate may contribute to the etio-pathogenesis of AD. Indeed, the protein level and enzymatic activity of [beta]-secretase (BACE), but not its mRNA level, were found elevated in brain areas of AD patients who suffer a high load of A[beta] plaque formation. Similarly, increased BACE activity but no mRNA change was observed in a transgenic mouse model of AD, tg2576, in which over expression of the Swedish mutated human APP leads to A[beta] plaque formation and learning deficits. Based on the recent demonstration of four BACE splice variants with different enzymatic activity, the discrepancy between BACE activity and mRNA expression may be explained by the altered BACE alternative splicing. To test this hypothesis, we studied the expression of all BACE splice variants in different brain areas of tg2576 mice at age of 4 months and 1 year old. We found developmental and regional differences between wild-type and tg2576 mice. Our results indicate that over expression of APP in tg2576 mice leads to the altered alternative splicing of BACE and the increase of its enzymatically more active splice variant (I-501).

Benussi, L., R. Ghidoni, et al. (2003). "Alzheimer disease-associated cystatin C variant undergoes impaired secretion." Neurobiology of Disease **13**(1): 15.

<http://www.sciencedirect.com/science/article/B6WNK-48GF25K-2/2/25196c4352eb138c979df7f465fd8197>

CST3 is the coding gene for cystatin C (CysC). CST3 B/B homozygosity is associated with an increased risk of developing Alzheimer disease. We performed CysC analysis on human primary skin fibroblasts obtained from donors carrying A/A, A/B, and B/B CST3. Pulse-chase experiments demonstrated that the release of the B variant of CysC has a different temporal pattern compared to that of the A one. Fibroblasts B/B homozygous displayed a reduced secretion of CysC due to a less efficient cleavage of the signal peptide, as suggested by high-resolution Western blot analysis and by in vitro assay. In the brain, the reduced level of CysC may represent the molecular factor responsible for the increased risk of Alzheimer disease.

Duan, R.-S., Z. Chen, et al. (2004). "CCR5 deficiency does not prevent P0 peptide 180-199 immunized mice from experimental autoimmune neuritis." Neurobiology of Disease **16**(3): 630.

<http://www.sciencedirect.com/science/article/B6WNK-4CHRGJ2-2/2/87d5071b45bfa7b31685d63652be3064>

Experimental autoimmune neuritis (EAN) is an inflammatory autoimmune demyelinating disease of peripheral nervous system (PNS) and represents an animal model of Guillain-Barre syndrome (GBS) in man. The inflammatory cell infiltrating into the PNS is a prerequisite for developing EAN. To explore the role of CC chemokine receptor 5 (CCR5) in the inflammatory process of EAN, we induced EAN in CCR5-deficient (CCR5^{-/-}) mice with P0 protein peptide 180-199. We found that CCR5^{-/-} mice showed a similar EAN clinical course and severity as well as profile of infiltrating macrophages and T cells in cauda equina (CE) of EAN and the same levels of spleen mononuclear cell (MNC) response to antigen and mitogen when compared with CCR5^{+/+} control mice. However, increased IP-10 and MIP-1[beta] production in sciatic nerves were seen in CCR5^{-/-} mice. These results suggest that CCR5 deficiency does not prevent P0 peptide 180-199-immunized mice from EAN. Increased MIP-1[beta] and IP-10 in sciatic nerves may compensate the CCR5 deficiency and contribute to inflammatory cells infiltrating to the PNS.

Hsu, S.-H., M.-J. Lee, et al. (2004). "Cutaneous and sympathetic denervation in neonatal rats with a mutation in the delta subunit of the cytosolic chaperonin-containing t-complex peptide-1 gene." Neurobiology of Disease **16**(2): 335.

<http://www.sciencedirect.com/science/article/B6WNK-4C5HRSF-1/2/6960e57402b303b7191df20cdaaf2120>

The mutilated-foot rat (mf rat) is an autosomal recessive mutant with characteristic digit deformities in adult animals, and this phenotype mimics many aspects of human sensory neuropathy. The genetics of mf rats was recently elucidated. To understand whether the genotype is responsible for cutaneous denervation before clinically overt mutilation in adult mf rats, we investigated skin innervation in postnatal day 7 (P7) mf rats and compared the patterns with P7 wild-type rats. The mf rat carries a G->A mutation in the gene encoding the delta subunit of the cytosolic chaperonin-containing t-complex peptide-1 (Cct4). In the footpad skin of P7 mf rats, there was a >90% loss of epidermal nerves (0.7-7.9% of P7 wild-type rats) as indicated by neuronal markers including protein gene product 9.5 (PGP 9.5), growth-associated protein 43 (GAP43), calcitonin gene-related peptide (CGRP), and substance P (SP). The epidermis of hairy

skin in hind feet was completely denervated in mf rats as well. Compared with an approximately 80% reduction in the size of dermal nerve fascicles and a parallel loss of nerve fibers, the nearly complete absence of epidermal innervation suggests further sensory nerve degeneration at the level of nerve terminals in the epidermis. In contrast, the loss of epidermal nerves in the abdominal skin of mf rats was less extensive than that in the footpad skin of mf rats; CGRP (+) and SP (+) fibers were moderately reduced (28.3-56.4% of levels of wild-type rats) with normal amounts of PGP 9.5 (+) and GAP43 (+) nerves. Sympathetic innervation as assessed by tyrosine hydroxylase immunoreactivity was absent from the footpad and abdominal skin of mf rats. In conclusion, there is regional skin denervation with diffuse sympathetic denervation in P7 mf rats. These results suggest that the mutation in *Cct4* underlies cutaneous nerve degeneration in mf rats.

Savioz, A., B. M. Riederer, et al. (2003). "Tau and neurofilaments in a family with frontotemporal dementia unlinked to chromosome 17q21-22." Neurobiology of Disease **12**(1): 46.

<http://www.sciencedirect.com/science/article/B6WKN-47TFDMV-4/2/3237bbae0b24417687c5dc2c79edb892>

A Swiss frontotemporal dementia (FTD) kindred with extrapyramidal-like features and without motor neuron disease shows a brain pathology with ubiquitin-positive but tau-negative inclusions. Tau and neurofilament modifications are now studied here in three recently deceased family members. No major and specific decrease of tau was observed as described by others in, e.g., sporadic cases of FTD with absence of tau-positive inclusions. However, a slight decrease of tau, neurofilament, and synaptic proteins, resulting from frontal atrophy was detected. In parallel, polymorphic markers on chromosome 17q21-22, the centromeric region of chromosome 3 and chromosome 9, were tested. Haplotype analysis showed several recombination events for chromosomes 3 and 17, but patients shared a haplotype on chromosome 9q21-22. However as one of the patients exhibited Alzheimer and vascular dementia pathology with uncertain concomitant FTD, this locus is questionable. Altogether, these data indicate principally that the Swiss kindred is unlinked to locus 17q21-22, and that tau is not at the origin of FTD in this family.

Yeh, T.-H., H.-M. Hwang, et al. (2005). "Glutamate transporter function of rat hippocampal astrocytes is impaired following the global ischemia." Neurobiology of Disease **18**(3): 476.

<http://www.sciencedirect.com/science/article/B6WKN-4FCRFFY-1/2/0d0d990281e5bed1e711a5d46b0c9665>

Astroglial glutamate transporters, GLT-1 and GLAST, play an essential role in removing released glutamate from the extracellular space and are essential for maintaining a low concentration of extracellular glutamate in the brain. It was hypothesized that impaired function of glial glutamate transporters induced by transient global ischemia may lead to an elevated level of extracellular glutamate and subsequent excitotoxic neuronal death. To test this hypothesis, in the present study, we performed whole-cell patch-clamp recording of hippocampal CA1 astrocytes in control or postischemic slices, and measured glutamate transporter activity by recording glutamate-evoked transporter currents. Six to 24 h after global ischemia, maximal amplitude of glutamate transporter currents recorded from postischemic CA1 astrocytes was significantly reduced. Western blotting analysis indicated that transient global ischemia decreased the protein level of GLT-1 in the hippocampal CA1 area without affecting GLAST protein level. Further real-time quantitative RT-PCR assays showed that global ischemia resulted in a decrease in GLT-1 mRNA level of hippocampal CA1 region. Global ischemia-induced reduction in GLT-1 expression and glutamate transporter function of CA1 astrocytes precedes the initiation of delayed neuronal death in CA1 pyramidal layer. The present study provides the evidence that transient global

ischemia downregulates glutamate transporter function of hippocampal CA1 astrocytes by decreasing mRNA and protein levels of GLT-1.

Neurochemistry International (6)

Burgher, K. L., J. A. Heroux, et al. (1997). "CYCLIC AMP POTENTIATION OF CYTOKINE-INDUCED NITRIC OXIDE SYNTHASE ACTIVITY IN A MURINE ASTROCYTE CELL LINE." Neurochemistry International **30**(4-5): 483.

<http://www.sciencedirect.com/science/article/B6T0B-3PPV0BK-J/2/78acc765042e7a5993718812c87dc7f4>

Astrocytes in culture have been previously shown to express inducible nitric oxide synthase (iNOS) following treatment with cytokines such as interleukin-1[beta] (IL-1[beta]) and interferon-[gamma] (IFN-[gamma]). We report here on the effects of the cyclic nucleotide analogues 8-bromo-cyclic AMP and 8-bromo-cyclic GMP on cytokine-stimulated iNOS gene expression in a cultured murine astrocyte cell line. In these cells, neither 8-bromo-cyclic AMP nor 8-bromo-cyclic GMP alone was able to stimulate iNOS activity. Similarly, neither IL-1[beta] nor IFN-[gamma] was capable of independently stimulating iNOS expression. Co-stimulation with both cytokines, however, resulted in measurable increases in iNOS activity, and correlated to increases in iNOS mRNA levels. The addition of 8-bromo-cyclic AMP, but not 8-bromo-cyclic GMP, was found to further enhance the expression of iNOS activity induced by IL-1[beta] and IFN-[gamma] co-stimulation. This potentiation effect of 8-bromo-cyclic AMP correlated to a further elevation in iNOS mRNA levels over that produced by cytokine co-stimulation alone. However, 8-bromo-cyclic AMP co-treatment with either cytokine alone did not stimulate iNOS activity, indicating that the signal transduction pathway(s) involved in the potentiation effect of 8-bromo-cyclic AMP is functional only in the presence of both cytokines. These results indicate that cyclic AMP-mediated processes can participate in modulating the expression of astrocyte iNOS when the appropriate combinations of stimulatory cytokines are present. (c) 1997 Elsevier Science Ltd. All rights reserved

Citron, B. A., E. J. Gregory, et al. (2000). "Regulation of the dual function tissue transglutaminase/G[alpha]h during murine neuromuscular development: gene and enzyme isoform expression." Neurochemistry International **37**(4): 337.

<http://www.sciencedirect.com/science/article/B6T0B-409VHRV-2/2/662ae57b98425516559fdebe84fecc7e>

Coagulation Factor XIII (F. VIII), a member of the transglutaminase (TGase) superfamily, is activated by thrombin, cross-links fibrin and stabilizes clots. Another member of this family, tissue TGase (tTG), having similar enzymatic activity, is implicated in neural development and synapse stabilization. Our previous studies indicated that synapse formation and maintenance at the neuromuscular junction (NMJ) involved components of the coagulation cascade in development. Others then showed that either F. XIII or tTG were localized at NMJs in a developmentally-regulated fashion. In the current studies, we addressed the temporal course of skeletal muscle tTG gene expression and found maximal expression at birth and continuing into the immediate postnatal period. Subcellular fractionation revealed a relatively constant particulate isoform of

TGase activity which predominated in early embryonic muscle development. In contrast, cytosolic TGase specific activity became the major isoform in the postnatal period. The timing of muscle TGase activity correlated well with expression of tTG mRNA and we now present novel data of Tgm 2 gene expression for tTG in skeletal muscle. Confirming and extending the previous studies, TGase becomes localized at NMJs in the early, further ramifying in the late, neonatal period. These data suggest that the early pulse of particulate activity could coincide with the period of myoblast cell death in embryonic muscle. On the other hand, the peak cytosolic TGase activity occurs in the neonatal period, correlating temporally with muscle prothrombin expression during activity-dependent synapse elimination and possibly the source of the enzyme localized to the NMJ extracellular matrix resulting in synaptic stabilization.

Eva, C., M. Fusco, et al. (1994). "Intracerebroventricular administration of nerve growth factor affects muscarinic cholinergic receptors in the cerebral cortex of neonatal rats." Neurochemistry International **24**(1): 57.

<http://www.sciencedirect.com/science/article/B6T0B-4859KB0-RS/2/dc5d5f73d384115f92a8e760a10b46dc>

The repeated intracerebroventricular administration of nerve growth factor (5 [mu]g/2.5 [mu]l) to neonatal rats induced the activation of choline acetyltransferase in forebrain cholinergic neurons that was paralleled by a concomitant change in the density of muscarinic cholinergic receptors in the cerebral cortex. The administration of nerve growth factor altered muscarinic binding sites in a biphasic fashion during postnatal development. A significant stimulation of the developmental increase in the density of muscarinic binding sites occurred in nerve growth factor-treated animals at days 2 and 3 after birth. Conversely, nerve growth factor induced a significant decrease in the receptor number at postnatal days 8 and 14. Muscarinic receptor number returned to control values after treatment, suggesting that nerve growth factor-induced changes to muscarinic cholinergic receptors are reversible. Nerve growth factor administration did not affect muscarinic cholinergic receptor density in striatal membranes and did not alter the relative content of cortical messenger RNAs encoding m1 and m3 muscarinic cholinergic receptor subtypes at postnatal day 14, as determined by reverse transcriptase-polymerase chain reaction. The up- and down-regulation of muscarinic cholinergic receptors induced by nerve growth factor during postnatal development may be temporally related events associated with concomitant changes in the activity of choline acetyltransferase.

Jurado, S., J. Sanchez-Prieto, et al. (2004). "Elements of the nitric oxide/cGMP pathway expressed in cerebellar granule cells: biochemical and functional characterisation." Neurochemistry International **45**(6): 833.

<http://www.sciencedirect.com/science/article/B6T0B-4CFV5RD-3/2/ee0144de706c56c70e085b7bc5400bdd>

It is known that the nitric oxide (NO)/cGMP pathway affects neuronal development and the expression of the different proteins is developmentally dependent in several brain areas. However, so far there are no data on the expression of the proteins involved in this signalling system during the development of the cerebellar granule cell, one of the most widely used models of neuronal development. This study was accordingly designed to analyse the developmental regulation of neuronal nitric oxide synthase (nNOS), soluble guanylyl cyclase subunits ([alpha]1, [alpha]2 and [beta]1) and cGMP-dependent protein kinases (cGK I and cGK II) in cerebellar granule cells through real time-polymerase chain reaction (RT-PCR) and Western blotting. We were able to detect guanylyl cyclase subunits and cGK I and cGK II in cerebellar granule cells at every stage of development examined (cells freshly isolated from 7-day-old rat pups, and cells

cultured for 7 days or 14 days). Expression levels, nevertheless, varied significantly at each stage. nNOS, $\alpha 2$ and $\beta 1$ and cGK II levels increased during granule cell development, while $\alpha 1$ and cGK I showed an opposite behaviour pattern; the levels of these latter proteins diminished as the cells matured. The functionality of this pathway was assessed by stimulating cells kept in culture for 7 days with DEA/NO or with N-methyl-L-aspartate (NMDA). Cells responded by increasing intracellular cGMP and activating cGMP-dependent protein kinase activity, which effectively phosphorylated two well-known substrates of this activity, the vasodilator stimulated phosphoprotein (VASP) and the cAMP response element binding protein (CREB). In summary, through both functional and biochemical tests, this is the first demonstration of a complete NO/cGMP signalling transduction pathway in cerebellar granule cells. Our results also indicate the developmental regulation of the proteins in this system.

Pahud, G., S. Bontron, et al. (2001). "Modulation of choline acetyltransferase synthesis by okadaic acid, a phosphatase inhibitor, and KN-62, a CaM kinase inhibitor, in NS-20Y neuroblastoma." Neurochemistry International **38**(1): 75.

<http://www.sciencedirect.com/science/article/B6T0B-40NMS2G-9/2/fff0ec7bf8ffbb75384aefd49fe320f>

Choline-O-acetyltransferase (ChAT) is the enzyme which catalyses the biosynthesis of the neurotransmitter acetylcholine in cholinergic neurons. Here we show that in mouse cholinergic NS-20Y neuroblastoma cells cultured in the presence of either okadaic acid (serine/threonine phosphatases 1 and 2A inhibitor) or KN-62 (CaM kinase inhibitor) ChAT activity and mRNA either increased or decreased as a function of the drug concentration, respectively. After 24 h exposure, okadaic acid exerted a dramatic effect on cell morphology; cells became round and had no more neurites. On the contrary, KN-62 induced a slight morphological differentiation of the cells. The present results suggest that phosphatases 1 and 2A and CaM kinase could mediate regulation of ChAT gene expression.

Wang, S., F. M. Longo, et al. (1993). "Induction of glucose regulated protein (grp78) and inducible heat shock protein (hsp70) mRNAs in rat brain after kainic acid seizures and focal ischemia." Neurochemistry International **23**(6): 575.

<http://www.sciencedirect.com/science/article/B6T0B-4859JGT-G8/2/9df24473b75ad98cecb2af28573e9a0>

Specific probes were obtained using PCR cloning from rat brain for the 78 kDa glucose regulated (grp78), inducible 72 kDa (hsp70) as well as constitutive 73 kDa (hsc73) heat shock mRNAs. Grp78 and hsc73 were expressed in normal rat brain whereas hsp70 was not. Subcutaneous injection kainic acid (10 mg/kg) produced seizures and induced all three mRNAs. The induction of grp78 and hsp70 mRNAs occurred within 2 h, peaked between 6-8 h, persisted for 48 h, and returned to control levels by 72 h. Expression of the grp78 and hsp70 mRNAs after focal ischemia progressively increased with occlusion durations from 15-120 min in the cerebral cortex. Though grp78 and hsp70 mRNAs were induced modestly in the striatum by 15 min of ischemia, longer durations of ischemia were characterized by little change in the grp78 mRNA levels and relatively lower levels of hsp70 expression. This result indicates that progressive increases in the duration of ischemia in brain, prior to infarction, may produce proportional increases in transcription of the heat shock genes. However, once the duration of ischemia is long enough to produce infarction, this severely limits the availability of ATP which blocks transcription of the heat shock genes. In conclusion, concurrent induction of the heat shock genes suggests that kainic acid seizures and focal ischemia induce several different stress responses in brain cells caused by denaturation of proteins, changes of protein synthesis, and changes of protein glycosylation.

NeuroImage (1)

Graff-Guerrero, A., C. De la Fuente-Sandoval, et al. "Frontal and limbic metabolic differences in subjects selected according to genetic variation of the SLC6A4 gene polymorphism." **NeuroImage In Press, Corrected Proof** <http://www.sciencedirect.com/science/article/B6WNP-4FH5K71-1/2/ebd252755c28c0a2f90f60f39dd64b55>

Allelic variants in the promoter region of the serotonin transporter (5-HTT) gene have been implicated in several psychiatric disorders and personality traits. In particular, two common alleles in a variable repeat sequence of the promoter region (SLC6A4) have been differentially associated with a display of abnormal levels of anxiety and affective illness in individuals carrying the "s" allele. The aim of this study was to compare the basal cerebral metabolic activity of non-psychiatric subjects in fronto-limbic structures to determine whether differences exist in basal metabolic activity within this functional polymorphism. PET scans with fluorine-18 fluorodeoxyglucose as radiotracer were performed in 71 non-psychiatric subjects previously screened for psychopathology and subsequently genotyped for SLC6A4; PET images were compared with SPM2 according to s/s (n = 27), s/l (n = 25), and l/l (n = 19) groups considering a significance threshold in a priori selected areas of $P < 0.001$ and an extent threshold [greater-than-or-equal]5 voxels. The analysis showed an effect of interest among the three genotype groups in right anterior cingulate gyrus (ACC), left middle frontal gyrus, and left posterior cingulate gyrus (PCC). Comparison between l/l vs. s/s showed increased metabolism for l/l in left middle frontal gyrus and an increase for s/s in right ACC and left PCC. Comparison between s/s vs. s/l showed an increase for s/s in left PCC and right ACC. Increased basal metabolism in fronto-limbic structures for the s/s group may be conceived as an "overactive metabolic state" of these structures, possibly related to an increased susceptibility for developing an anxiety-depression spectrum disorder.

Neurology (10)

Criscuolo, C., S. Banfi, et al. (2004). "A novel mutation in SACS gene in a family from southern Italy." Neurology **62**(1): 100-102.

<http://www.neurology.org/cgi/content/abstract/62/1/100>

A form of autosomal recessive spastic ataxia (ARSACS) has been described in the Charlevoix and Saguenay regions of Quebec. So far a frameshift and a nonsense mutation have been identified in the SACS gene. The authors report a new mutation (1859insC), leading to a frameshift with a premature termination of the gene product sacsin, in two sisters from consanguineous parents. The phenotype is similar to previously described patients with ARSACS.

Du Pasquier, R. A., S. Corey, et al. (2003). "Productive infection of cerebellar granule cell neurons by JC virus in an HIV+ individual." Neurology **61**(6): 775-782.

<http://www.neurology.org/cgi/content/abstract/61/6/775>

Background: In the setting of severe immunosuppression, the polyomavirus JC (JCV) can cause a lytic infection of oligodendrocytes. This demyelinating disease of the CNS white matter (WM) is called progressive multifocal leukoencephalopathy (PML). JCV has a very narrow host-cell range and productive infection of neurons has never been demonstrated. Patient, methods, and results: An HIV-1-infected patient presented with signs of pyramidal tract and cerebellar dysfunction. Brain MRI revealed T2 hyperintensities in the WM of both frontal lobes and cerebellar atrophy. His disease progressed despite therapy and he died 6 months later. In addition to classic PML findings in the frontal lobe WM, autopsy revealed scattered foci of tissue destruction in the internal granule cell layer (IGCL) of the cerebellum. In these foci, enlarged granule cell neurons identified by the neuronal markers MAP-2 and NeuN reacted with antibodies specific for the polyomavirus VP1 capsid protein. Electron microscopy showed 40 nm viral particles, consistent with polyomaviruses, in these granule cell neurons. In addition, JCV DNA was detected by PCR after laser capture microdissection of cells from the areas of focal cell loss. Finally, in situ hybridization studies demonstrated that many granule cell neurons were infected with JCV but did not contain viral proteins. Sequence analysis of the JCV regulatory region from cerebellar virions showed a tandem repeat pattern also found in PML lesions of the frontal lobe WM. Conclusion: JCV can productively infect granule cell neurons of the IGCL of the cerebellum. This suggests a role for JCV infection of neurons in cerebellar atrophy occurring in HIV-infected individuals.

Iivonen, S., E. Corder, et al. (2004). "Polymorphisms in the CYP19 gene confer increased risk for Alzheimer disease." Neurology **62**(7): 1170-1176.

<http://www.neurology.org/cgi/content/abstract/62/7/1170>

Background: Brain aromatase may be neuroprotective by increasing the local estrogen levels in injured neurons. Aromatase is encoded by the CYP19 gene located at 15q21.1, a chromosomal region in linkage disequilibrium (LD) with Alzheimer disease (AD) in this sample. Objective: To investigate whether nine single-nucleotide polymorphisms (SNP) spanning the CYP19 gene were associated with AD. Methods: Three hundred ninety-four patients were compared with 469 nondemented control subjects using single-locus and haplotype approaches. Haplotypes were identified using the expectation/maximization algorithm and latent class analysis, which included additional information on age, sex, and APOE polymorphism. Results: Allelic and genotypic frequencies for three adjacent SNP differed between AD and control groups. Both haplotype approaches identified an approximately 60% increase ($p = 0.02$) in the risk of AD for one haplotype and similar levels of excess risk irrespective of APOE polymorphism and gender. Conclusion: Genetic variation in the brain aromatase gene may modify the risk for AD.

Kim, J. Y., J. M. Hwang, et al. (2005). "Mitochondrial DNA content is decreased in autosomal dominant optic atrophy." Neurology **64**(6): 966-972.

<http://www.neurology.org/cgi/content/abstract/64/6/966>

Background: Autosomal dominant optic atrophy (ADOA) is the commonest form of inherited optic neuropathy. Mutations in the OPA1 gene encoding a dynamin-related mitochondrial protein underlie ADOA and may perturb the biogenesis and maintenance of mitochondria. Objective: To investigate the mutation spectrum of the OPA1 gene and assess alterations in mitochondrial

content caused by OPA1 mutations. Methods: Sixteen Korean patients with clinically suspected ADOA were studied. The mutation spectrum of the OPA1 gene was analyzed by PCR single-strand conformation polymorphism and sequencing, and mitochondrial DNA (mtDNA) content was quantified by real-time PCR. Results: Eight different mutations were found, including five novel mutations. Quantitative real-time PCR analysis showed excellent linearity and precision for the determination of mtDNA copy numbers. The number of mtDNA copies per cell in patients with OPA1 gene mutations (ages 7 to 40) was significantly lower than those in all normal control subjects ($p = 0.037$), particularly lower than in normal control subjects ages 10 to 39 ($p = 0.022$). Conclusion: The mutation spectrum of the OPA1 gene disclosed marked genetic heterogeneity and the mitochondrial DNA content was found to be lower in autosomal dominant optic neuropathy, which provides direct evidence for a pathogenetic role of mutations of the OPA1 gene.

Lus, G., E. Nelis, et al. (2003). "Charcot-Marie-Tooth disease with giant axons: A clinicopathological and genetic entity." Neurology **61**(7): 988-990.

<http://www.neurology.org/cgi/content/abstract/61/7/988>

The authors report an Italian family with autosomal-dominant Charcot-Marie-Tooth disease (CMT) in which there were giant axons in the sural nerve biopsy. Linkage to the known CMT2 loci (CMT2A, CMT2B, CMT2D, CMT2F) and mutations in the known CMT2 genes (Cx32, MPZ, NEFL), GAN, NEFM, and CMT1A duplication/HNPP deletion were excluded. This family with CMT and giant axons has a pathologic and genetic entity distinct from classic CMT.

Murray, J. M. and S. Morgello (2004). "Polyomaviruses and primary central nervous system lymphomas." Neurology **63**(7): 1299-1301.

<http://www.neurology.org/cgi/content/abstract/63/7/1299>

The authors investigated the potential association of human primary CNS non-Hodgkin lymphoma (PCNSL) with polyomavirus in HIV-1 infected and uninfected individuals. Immunohistochemical analysis of CNS biopsies from 19 HIV-negative and 17 HIV-positive patients and PCR analysis of 12 HIV-negative and 14 HIV-positive patients revealed that the lymphomas were uniformly negative for polyomaviruses. The authors conclude that polyomaviruses are unlikely to be related to the pathogenesis of most PCNSL.

Nelis, E., S. Erdem, et al. (2002). "Mutations in GDAP1: Autosomal recessive CMT with demyelination and axonopathy." Neurology **59**(12): 1865-1872.

<http://www.neurology.org/cgi/content/abstract/59/12/1865>

Background: Mutations in the ganglioside-induced differentiation-associated protein 1 gene (GDAP1) were recently shown to be responsible for autosomal recessive (AR) demyelinating Charcot-Marie-Tooth disease (CMT) type 4A (CMT4A) as well as AR axonal CMT with vocal cord paralysis. Methods: The coding region of GDAP1 was screened for the presence of mutations in seven families with AR CMT in which the patients were homozygous for markers of the CMT4A locus at chromosome 8q21.1. Results: A nonsense mutation was detected in exon 5 (c.581C>G, S194X), a 1-bp deletion in exon 6 (c.786delG, G262fsX284), and a missense mutation in exon 6 (c.844C>T, R282C). Conclusions: Mutations in GDAP1 are a frequent cause of AR CMT. They

result in an early-onset, severe clinical phenotype. The range of nerve conduction velocities (NCV) is variable. Some patients have normal or near normal NCV, suggesting an axonal neuropathy, whereas others have severely slowed NCV compatible with demyelination. The peripheral nerve biopsy findings are equally variable and show features of demyelination and axonal degeneration.

Orland, J. R., J. Engstrom, et al. (2003). "Prevalence and clinical features of HTLV neurologic disease in the HTLV Outcomes Study." Neurology **61**(11): 1588-1594.

<http://www.neurology.org/cgi/content/abstract/61/11/1588>

Background: Almost 20 years after its discovery, the prevalence and clinical course of human T-lymphotropic virus type I (HTLV-I)-associated myelopathy (HAM, also known as tropical spastic paraparesis [TSP]) remain poorly defined. Whereas the causative association of HTLV-I and HAM/TSP is generally recognized, controversy still surrounds the relationship between HTLV-II and HAM/TSP. Methods: The HTLV Outcomes Study (HOST--formerly Retrovirus Epidemiology Donor Study [REDS]) is a prospective cohort study including 160 patients with HTLV-I, 405 patients with HTLV-II, and 799 uninfected controls who have been followed every 2 years since 1990-1992. Clinical outcomes are measured by health interviews and examinations, and blood samples are obtained. Results: Six cases of HTLV-I-associated myelopathy (3.7%, 95% CI 1.4 to 8.0) and four cases of HTLV-II myelopathy (1.0%, 95% CI 0.3 to 2.5) have been diagnosed since the formation of the cohort. There have been no cases of HAM/TSP diagnosed among HTLV-negative subjects (0.0%, 95% CI 0.0 to 0.5). Clinical features of the cases include lower extremity hyperreflexia, variably associated with weakness, spasticity, and bladder dysfunction. Conclusions: Systematic screening of HTLV-infected blood donors reveals a high prevalence of HAM/TSP. The clinical course of HAM/TSP appears highly variable. HTLV-II-associated myelopathy generally presents with milder and more slowly progressive signs and symptoms.

Remes, A. M., S. Finnila, et al. (2004). "Hereditary dementia with intracerebral hemorrhages and cerebral amyloid angiopathy." Neurology **63**(2): 234-240.

<http://www.neurology.org/cgi/content/abstract/63/2/234>

Background: Deposition of the {beta}-amyloid peptide (A{beta}) in neuritic plaques is a hallmark of Alzheimer disease (AD). Mutations in genes encoding amyloid precursor protein (APP) and presenilin 1 and 2 (PSEN1, PSEN2) are associated with increased accumulation of A{beta} in neuritic plaques or in the walls of cerebral vessels. Intracerebral hemorrhage occasionally affects patients with AD. Methods: A Finnish family with dementia in four generations and with frequent co-occurrence of dementia and intracerebral hemorrhage was identified. Clinical features of 14 family members with a cognitive decline were evaluated. All exons in genes encoding APP, PSEN1, PSEN2, cystatin C, transthyretin, gelsolin, and ITM2B were sequenced, and an association study of APP was conducted by identification of single-nucleotide polymorphisms. Results: Neuropathologic examination revealed Alzheimer-type changes with A{beta} in neuritic plaques and vessel walls, but the cognitive profile of the patients differed from that in AD, as the visuoconstructive functions and verbal fluency were well preserved even in the moderate stage of the disease. In addition to cognitive decline, five patients had had lobar intracerebral hemorrhages and one was diagnosed with hemosiderin deposits in MRI, suggesting previous cerebral microbleeds. No causative mutations were identified in candidate genes associated with amyloid diseases, but linkage to APP region could not be entirely excluded. Conclusions: The family presents an autosomal dominant form of {beta}-amyloidogenic disease that resembles the Italian, Flemish, and Iowa types of AD. No amyloidogenic mutations were identified, but the role of the APP region could not be entirely excluded.

Steele, A. J., A. Al-Chalabi, et al. (2005). "Detection of serum reverse transcriptase activity in patients with ALS and unaffected blood relatives." Neurology **64**(3): 454-458.

<http://www.neurology.org/cgi/content/abstract/64/3/454>

Background: Retroviral involvement in the etiology of sporadic ALS has been suspected for several years since the recognition that both murine and human retroviruses can cause motor neuron disease-like syndromes. In a pilot study, an increased prevalence of a retroviral marker (reverse transcriptase [RT] activity) was demonstrated in the serum of British patients with ALS. The current investigation was designed to confirm and extend these findings in a geographically distinct patient cohort under blinded testing conditions. Methods: A highly sensitive product-enhanced RT assay was employed to test coded sera obtained from 30 American patients with sporadic ALS and from 14 of their blood relatives, 16 of their spouses, and 28 nonrelated, nonspousal control subjects. Results: Serum RT activity was detected in a higher proportion of ALS patients (47%) than in non-blood-related controls (18%; $p = 0.008$). The prevalence of RT activity in the serum of spousal controls (13%) was similar to that in other non-blood-related controls. Unexpectedly, the prevalence of serum RT activity in blood relatives of ALS patients (43%) approached that in the ALS patients themselves. Conclusions: These results confirm that patients with ALS have a significantly higher prevalence of serum reverse transcriptase (RT) activity than that seen in unrelated control subjects. The finding of a similarly increased prevalence in blood relatives of ALS patients raises the possibility that the observed RT activity might be due to an inherited endogenous retrovirus.

Neuromuscular Disorders (17)

Bereznai, B., A. Winkler, et al. (1997). "A novel SOD1 mutation in an Austrian family with amyotrophic lateral sclerosis." Neuromuscular Disorders **7**(2): 113.

<http://www.sciencedirect.com/science/article/B6T9T-3RGSWVD-7/2/606ddd9721daa672e14185924f166b2e>

We report on an Austrian pedigree with autosomal dominant amyotrophic lateral sclerosis (ALS), diagnosed in six patients from two generations. The only surviving clinically affected family member was examined in our ALS clinic. Historical information on other affected individuals was obtained from knowledgeable family members. The mean $\pm$ S.D. age of onset of the disease was 54 $\pm$ 6.9 years, with a range of 43-66 years. The duration of the index patient's disease until death was 8 months. Using single strand conformational polymorphism (SSCP) analysis, we studied the index patient's exons 1, 2 and 4 of the Cu/Zn superoxide dismutase gene (SOD1) on chromosome 21. A variant banding pattern was observed for exon 1. Sequencing studies showed a previously underscribed T to A missense mutation at position 8 in exon 1 of the SOD1 gene. This mutation results in the elimination of an Eco571 restriction site. Whereas the index patient was heterozygous for this restriction site, 50 unrelated healthy controls and an unaffected brother were not. The mutation lies in a region involved in dimer contact in the three-dimensional structure of the SOD1 protein. This region comprises other known sites for ALS-causing mutations.

Beroud, C., A. Carrie, et al. (2004). "Dystrophinopathy caused by mid-intronic substitutions activating cryptic exons in the DMD gene." Neuromuscular Disorders **14**(1): 10.

<http://www.sciencedirect.com/science/article/B6T9T-4B1SG79-4/2/edf3469750c0b25aee2953e2da97f4f0>

In the course of a mutation search performed by muscle dystrophin transcript analysis in 72 Duchenne and Becker Muscular Dystrophies (DMD/BMD) patients without gross gene defect, we encountered four unrelated cases with additional out-of-frame sequences precisely intercalated between two intact exons of the mature muscle dystrophin mRNA. An *in silico* search of the whole dystrophin genomic sequence revealed that these inserts correspond to cryptic exons flanked by one strong and one weak consensus splice site and located in the mid-part of large introns (introns 60, 9, 1M, and 62, respectively). In each case we identified an intronic point mutation activating the cryptic donor or acceptor splice site. The patients exhibited a BMD/intermediate phenotype consistent with the presence of reduced amounts of normally spliced transcript and normal dystrophin. The frequency of this new type of mutation is not negligible (6% of our series of 65 patients with 'small' mutations). It would be missed if the exploration of the DMD gene is exclusively performed on exons and flanking sequences of genomic DNA.

Ceccarini, M., P. Macioce, et al. (2002). "Expression of dystrophin-associated proteins during neuronal differentiation of P19 embryonal carcinoma cells." Neuromuscular Disorders **12**(1): 36.

<http://www.sciencedirect.com/science/article/B6T9T-44HXJS4-7/2/602253887aae167493fe28e0cfc8c82>

The dystrophin gene that is defective in Duchenne muscular dystrophy shows a complex transcriptional control based on several promoters driving independent cell-type-specific expression of different isoforms. Dystrophin isoforms together with dystroglycan, a transmembrane protein which in turn binds to extracellular matrix, are the core of a complex of proteins, the dystrophin-associated protein (DAP) complex, which also comprises cytoplasmic elements like dystrobrevin. Whereas the molecular organization of DAP complex in muscle is well documented, the composition of a similar complex in the nervous system remains largely unknown. We followed by competitive PCR the expression of DAP complex components during retinoic acid (RA)-induced neuronal differentiation of P19 cells. Transcripts for the full-length dystrophin, Dp427, and the short isoform, Dp71, as well as for [alpha]-dystrobrevin 2 increased in parallel with days in culture after RA stimulation, while dystroglycan, [alpha]-dystrobrevin 1 and 3, and [beta]-dystrobrevin were constitutively expressed. The upregulation of some of the components of the dystrophin complex during neuronal maturation suggests functional flexibility of the complex in the nervous system, where specific associations between different isoforms of DAP complex components could possibly organize distinct DAP complex-like complexes.

Garofalo, O., D. A. Figlewicz, et al. (1993). "Androgen receptor gene polymorphisms in amyotrophic lateral sclerosis." Neuromuscular Disorders **3**(3): 195.

<http://www.sciencedirect.com/science/article/B6T9T-4834G83-3/2/de13ce58f5974460441ed75b25e47e9e>

Amyotrophic lateral sclerosis (ALS) is more common in men than in women (male to female ratio of approximately 2: 1), suggesting a role for a sex-linked factor in the disease. The recent identification of a mutation of the androgen receptor gene in Kennedy's disease or X-linked

bulbospinal neuronopathy, a rare form of progressive lower motor neurone degeneration, also associated with clinical signs of androgen insensitivity, raises the possibility that androgen function may be disturbed in other motor neurone disorders, including ALS. The Kennedy's disease mutation consists of an increased size of a highly polymorphic CAG repeat sequence in the first exon of the androgen receptor gene, coding for a polyglutamine tract. We have analysed this CAG repeat sequence in a large number of patients with typical sporadic ALS and in normal controls, in order to test the hypothesis that this polymorphism of the androgen receptor gene may influence susceptibility for ALS. We report that the distribution of alleles relating to the size of the CAG repeat sequence of the androgen receptor gene is similar in ALS and controls, indicating that polymorphisms of the CAG repeat sequence of the androgen receptor gene play a limited role, if any, in susceptibility to ALS.

Le Guern, E., N. Ravise, et al. (1994). "Linkage analyses between dominant X-linked Charcot-Marie-Tooth disease, and 15 Xq11-Xq21 microsatellites in a new large family: Three new markers are closely linked to the gene." Neuromuscular Disorders **4**(5-6): 463.

<http://www.sciencedirect.com/science/article/B6T9T-4834GD8-2V/2/af1ed13117c80e64f29e5cb7e53a925d>

X-linked dominant inheritance was suspected in a large family with Charcot-Marie-Tooth disease since no male to male transmission was observed, and since the sensory and motor neuropathy was more severe in males than in females. To test linkage to the dominant X-linked Charcot-Marie-Tooth disease (DCMTX) locus in Xq13, genotypes of 19 affected and 19 unaffected individuals from this family were determined for 4 microsatellite markers. Close linkage to mfd66 (DXS453) was found by bipoint analysis ($Z_{\max} = 4.8$ at $[\theta] = 0.00$). Multipoint analysis mapped the gene between the androgen receptor and DXYS1. In addition, linkage analysis performed with 11 microsatellite markers, derived from a high density map spanning 16 cM on Xq11-Xq21 revealed 3 new tightly linked loci: afm287zgl (DXS1216), afm261zh5 and afm207zg5 (DXS995). Multipoint analysis localized the DCMTX gene to a 7.5 cM interval between afm123xd4 (DXS988) and afm116xg1 (DXS986). Combined analysis with these new microsatellites provides a powerful tool for carrier detection because of their high informativity and the small genetic distance (< 10 cM) between the markers flanking the gene.

Mercuri, E., C. Cini, et al. (2003). "Muscle magnetic resonance imaging in patients with congenital muscular dystrophy and Ullrich phenotype." Neuromuscular Disorders **13**(7-8): 554.

<http://www.sciencedirect.com/science/article/B6T9T-497Y5H5-5/2/d1325b3f1301f67b17b1a8fb84bd0664>

The aim of this study was to evaluate muscle magnetic resonance imaging findings in patients with congenital muscular dystrophy and Ullrich phenotype. Fifteen children with congenital muscular dystrophy and Ullrich phenotype were included in the study. All patients had collagen VI studies in muscle and, when family structure was informative, linkage studies to the collagen 6 loci. Three of the 15 patients had reduced collagen in muscle. One of the three was from an informative family and linked to one of the collagen 6 loci. Another patient was linked to one of the collagen 6 loci but had normal expression of collagen in muscle. The remaining 11 all had normal collagen expression in muscle. Only two of these 11 were from informative families and linkage to collagen 6 loci was excluded in them. All patients had muscle magnetic resonance imaging of their leg muscles using transverse T1 sequences. With the exception of the two patients in whom linkage to the collagen 6 loci was excluded, the other 13 patients showed the same pattern of selective involvement on magnetic resonance imaging of thigh muscles. This consisted of relative sparing of sartorius, gracilis, adductor longus and rectus. This pattern was also found in the case

linked COL6A1/A2 locus but with normal collagen. This finding, and the striking clinical and magnetic resonance imaging concordance between patients with normal and reduced collagen VI in muscle suggest that collagen VI could still be the culprit in several cases with normal collagen expression, or alternatively a primary defect in a protein that closely interacts with collagen VI. Mutation analysis of the collagen 6 genes in cases with normal collagen VI expression is needed to resolve this issue.

Nelis, E., S. Erdem, et al. (2002). "A novel homozygous missense mutation in the myotubularin-related protein 2 gene associated with recessive Charcot-Marie-Tooth disease with irregularly folded myelin sheaths." Neuromuscular Disorders **12**(9): 869.

<http://www.sciencedirect.com/science/article/B6T9T-45NY2F2-1/2/54e12c08076b4f3a090a7c1746503559>

Mutations in the myotubularin-related protein 2 gene on chromosome 11q22 are known to cause autosomal recessive Charcot-Marie-Tooth disease with irregularly folded myelin sheaths. We screened the coding region of the myotubularin-related protein 2 gene in a Turkish consanguineous Charcot-Marie-Tooth disease family compatible with linkage to chromosome 11q22. A homozygous cytosine to thymine missense mutation at nucleotide position 847, resulting in an amino acid substitution of arginine to tryptophan at codon 283, was detected in exon 9 of the MTMR2 gene. This is the second homozygous missense mutation associated with recessive Charcot-Marie-Tooth disease with focally folded myelin sheaths.

Nishino, I., N. Minami, et al. (1998). "MTM1 gene mutations in Japanese patients with the severe infantile form of myotubular myopathy." Neuromuscular Disorders **8**(7): 453.

<http://www.sciencedirect.com/science/article/B6T9T-3TYW442-3/2/11e0e6a582c4bc568cacdb1921d43145>

The severe infantile form of myotubular myopathy is a fatal muscle disease that predominantly affects male infants and is characterized by severe weakness and hypotonia from birth. X-linked myotubular myopathy was found to be associated with mutations in the MTM1 gene in Xq28 encoding the putative tyrosine phosphatase, myotubularin. We screened the MTM1 gene for mutations in seven Japanese patients (six males and one female) who had the diagnosis of severe infantile form of myotubular myopathy. We found five mutations, including three novel mutations based on sequence analysis of RT-PCR fragments covering the entire open reading frame. Two patients (one male and one female), who had similar clinicopathologic features, did not have any mutation in the MTM1 gene open reading frame, suggesting that they may have had an autosomal recessive disease.

Quijano-Roy, S., L. Galan, et al. (2002). "Severe progressive form of congenital muscular dystrophy with calf pseudohypertrophy, macroglossia and respiratory insufficiency." Neuromuscular Disorders **12**(5): 466.

<http://www.sciencedirect.com/science/article/B6T9T-44R2NF6-1/2/8170018baf1cd6190890f6d877326b26>

A novel form of congenital muscular dystrophy in four unrelated patients is proposed. Congenital hypotonia, markedly increased CK, calf pseudohypertrophy and proximal weakness were

common early findings. Two cases were severely affected since infancy and never walked. The phenotypical homogeneity was not very evident until advanced stages of the disease. All the patients showed catastrophic progression of the weakness, severe restrictive respiratory insufficiency, macroglossia, peculiar extreme amyotrophy of hands and feet, and a round and 'puffy' face. All patients became tetraplegic and required mechanical ventilation. Two cases had signs of mild cardiac involvement. The only non-tracheotomised patient died of respiratory complications. No mental retardation or specific brain abnormalities were observed. All patients showed secondary deficit of laminin [alpha]2 and up-regulation of laminin [alpha]5 in muscle. Expression of [alpha]-dystroglycan was severely reduced in two available muscle samples. The known loci for congenital muscular dystrophies were excluded in the only consanguineous case by linkage analysis. Clinical, immunohistochemical and genetic findings strongly suggest a distinct entity.

Roest, P. A. M., A. C. Van Der Tuijn, et al. (1996). "Application of in vitro myo-differentiation of non-muscle cells to enhance gene expression and facilitate analysis of muscle proteins." Neuromuscular Disorders **6**(3): 195.

<http://www.sciencedirect.com/science/article/B6T9T-3VX9862-9/2/ca79e57f7136a1269ab20ab8e1945521>

Introduction of the myogenic-determination gene MyoD forces non-muscle cell cultures into myogenesis, thereby inducing expression of muscle-specific proteins and facilitating their analysis. In several MyoD-transfected fibroblasts, immunohistochemical detection showed expression of desmin after three days, of titin after five days and of dystrophin after seven days. Cell fusion (myotube formation) could be observed after five days. After nine days a fraction of the cells showed a striated titin pattern, indicating an advanced state of muscle differentiation. Dystrophin (the protein absent in Duchenne Muscular Dystrophy patients) can be detected in MyoD-transfected and differentiated fibroblasts from healthy individuals, and is absent in those of patients. MyoD-transfection increases transcription of the dystrophin gene, facilitating RNA-based mutation detection. Using RNA from MyoD-transfected, differentiated fibroblasts of a deceased patient with an unknown, non-deletion mutation, we were able to identify a CGA->TGA nonsense mutation in the rod domain at basepair 6492 and to establish a rapid mutation specific test for future diagnosis of the mutation in his relatives.

Sambuughin, N., T. E. Nelson, et al. (2001). "Identification and functional characterization of a novel ryanodine receptor mutation causing malignant hyperthermia in North American and South American families." Neuromuscular Disorders **11**(6-7): 530.

<http://www.sciencedirect.com/science/article/B6T9T-43T7K72-3/2/cff4fa066fba2002b5e02c50cdfa711f>

Malignant hyperthermia is a pharmacogenetic disorder associated with mutations in Ca²⁺ regulatory proteins. It manifests as a hypermetabolic crisis triggered by commonly used anesthetics. Malignant hyperthermia susceptibility is a dominantly inherited predisposition to malignant hyperthermia that can be diagnosed by using caffeine/halothane contracture tests. In a multigenerational North American family with a severe form of malignant hyperthermia that has caused four deaths, a novel RYR1 A2350T missense mutation was identified in all individuals testing positive for malignant hyperthermia susceptibility. The same A2350T mutation was identified in an Argentinean family with two known fatal MH reactions. Functional analysis in HEK-293 cells revealed an altered Ca²⁺ dependence and increased caffeine sensitivity of the expressed mutant protein thus confirming the pathogenic potential of the RYR1 A2350T mutation.

Santoro, L., R. Carrozzo, et al. (2000). "A novel SURF1 mutation results in Leigh syndrome with peripheral neuropathy caused by cytochrome c oxidase deficiency." Neuromuscular Disorders **10**(6): 450.

<http://www.sciencedirect.com/science/article/B6T9T-40PXM5-B/2/fcc0d451a44243f4fe076990c9315c88>

We report on a 5-year-old boy with clinical and neuroradiological evidence of Leigh syndrome and peripheral neuropathy. Skeletal muscle biopsy showed decreased cytochrome c oxidase stain. Ultrastructurally, the nerve biopsy showed a defect of myelination. Biochemical analyses of muscle homogenate showed cytochrome c oxidase deficiency (15% residual activity). SURF1 gene analysis identified a novel homozygous nonsense mutation which predicts a truncated surf1 protein.

Sterrenburg, E., R. Turk, et al. (2004). "Large-scale gene expression analysis of human skeletal myoblast differentiation." Neuromuscular Disorders **14**(8-9): 507.

<http://www.sciencedirect.com/science/article/B6T9T-4CHRD4/2/683edecd290e8d628830d15801cc82d7>

To study pathways involved in human skeletal myogenesis, we profiled gene expression in human primary myoblast cells derived from three individuals using both oligonucleotide and cDNA microarrays. Following stringent statistical testing (false-positive rate 0.4%), we identified 146 genes differentially expressed over time. Interestingly, 86 of these genes have not been reported to be involved in myogenesis in mouse cell lines. This demonstrates the additional value of human primary cell cultures in the study of muscle differentiation. Many of the identified genes play a role in muscle regeneration, indicating the close relationship of this process with muscle development. In addition, we found overlap with expression profiling studies in muscle from Duchenne muscular dystrophy patients, confirming ongoing muscle regeneration in Duchenne muscular dystrophy. Further study of these genes can bring new insights into the process of muscle differentiation, and they are candidate genes for neuromuscular disorders with an as yet unidentified cause.

Talim, B., A. Ferreiro, et al. (2000). "Merosin-deficient congenital muscular dystrophy with mental retardation and cerebellar cysts unlinked to the LAMA2, FCMD and MEB loci." Neuromuscular Disorders **10**(8): 548.

<http://www.sciencedirect.com/science/article/B6T9T-41GWMX-2/2/837592970186a7f7ff526e9310c84798>

We report a case of congenital muscular dystrophy with secondary merosin deficiency, structural involvement of the central nervous system and mental retardation in an 8-year-old girl from a consanguineous family. She had early-onset hypotonia, generalized muscle wasting, with weakness especially of the neck muscles, joint contractures, mental retardation and high creatine kinase. Muscle biopsy showed dystrophic changes with partial deficiency of the laminin [alpha]2 chain. Cranial magnetic resonance imaging revealed multiple small cysts in the cerebellum, without cerebral cortical dysplasia or white matter changes. The laminin [alpha]2 chain (6q2), Fukuyama type congenital muscular dystrophy (9q31-q33) and muscle-eye-brain disease (1p32-p34) loci were all excluded by linkage analysis. We suggest that this case represents a new entity

in the nosology of congenital muscular dystrophy.

Villanova, M., E. Mercuri, et al. (2000). "Congenital muscular dystrophy associated with calf hypertrophy, microcephaly and severe mental retardation in three Italian families: evidence for a novel CMD syndrome." Neuromuscular Disorders **10**(8): 541.

<http://www.sciencedirect.com/science/article/B6T9T-41GWMXM-1/2/1d23d78ab1de80cf0a2853eacc4aa28f>

We describe four Italian patients (aged 3, 4, 12, and 13 years) affected by a novel autosomal form of recessive congenital muscular dystrophy. These patients were from three non-consanguineous families and presented an almost identical phenotype. This was characterized by hypotonia at birth, joint contractures associated with severe psychomotor retardation, absent speech, inability to walk and almost no interest in their surroundings. In addition, all patients had a striking enlargement of the calf and quadriceps muscles. Ophthalmologic examination revealed no structural ocular abnormalities in any of the children; one patient had severe myopia. In all cases a magnetic resonance imaging of the brain showed an abnormal posterior cranial fossa with enlargement of the cisterna magna and variable hypoplasia of the vermis of the cerebellum. Abnormality of the white matter was also present in all patients, in the form of patchy signal most evident in the periventricular areas. Serum CK was grossly elevated in all. The muscle biopsy from all cases showed dystrophic changes compatible with congenital muscular dystrophy. Immunofluorescence studies showed mild to moderate partial deficiency of laminin [alpha]2 chain. Linkage analysis in the only informative family excluded the known loci for congenital muscular dystrophy, including laminin [alpha]2 chain on chromosome 6q2, the Fukuyama congenital muscular dystrophy locus on 9q3 and the muscle-eye-brain disease on chromosome 1p3. We propose that this represent a novel severe variant of congenital muscular dystrophy, with associated central nervous system involvement.

Wilton, S. D., F. Lloyd, et al. (1999). "Specific removal of the nonsense mutation from the mdx dystrophin mRNA using antisense oligonucleotides." Neuromuscular Disorders **9**(5): 330.

<http://www.sciencedirect.com/science/article/B6T9T-3WWWV9D0-9/2/2f185ec20f26a95200eb1f90327ce23e>

The mdx mouse, which carries a nonsense mutation in exon 23 of the dystrophin gene, has been used as an animal model of Duchenne muscular dystrophy to evaluate cell or gene replacement therapies. Despite the mdx mutation, which should preclude the synthesis of a functional dystrophin protein, rare, naturally occurring dystrophin-positive fibres have been observed in mdx muscle tissue. These dystrophin-positive fibres are thought to have arisen from an exon-skipping mechanism, either somatic mutations or alternative splicing. Increasing the frequency of these fibres may offer another therapeutic approach to reduce the severity of Duchenne muscular dystrophy. Antisense oligonucleotides have been shown to block aberrant splicing in the human [beta]-globin gene. We wished to use a similar approach to re-direct normal processing of the dystrophin pre-mRNA and induce specific exon skipping. Antisense 2'-O-methyl-oligonucleotides, directed to the 3' and 5' splice sites of introns 22 and 23, respectively in the mdx pre-mRNA, were used to transfect myoblast cultures. The 5' antisense oligonucleotide appeared to efficiently displace factors normally involved in the removal of intron 23 so that exon 23 was also removed during the splicing of the dystrophin pre-mRNA. Approximately 50% of the dystrophin gene mRNAs were missing this exon 6 h after transfection of primary mdx myotubes, with all transcripts showing skipping of exon 23 after 24 h. Deletion of exon 23 does not disrupt the reading frame and should allow the synthesis of a shorter but presumably functional Becker-like dystrophin. Molecular intervention at dystrophin pre-mRNA splicing has the potential to

reduce the severity of a Duchenne mutation to the milder Becker phenotype.

Winand, N. J., M. Edwards, et al. (1994). "Deletion of the dystrophin muscle promoter in feline muscular dystrophy." Neuromuscular Disorders **4**(5-6): 433.

<http://www.sciencedirect.com/science/article/B6T9T-4834GD8-2R/2/b0f583d277d75e93228ad56fb4ec75af>

We have characterized the mutation in a feline model of DMD that selectively eliminates expression of the muscle and Purkinje neuronal dystrophin isoforms. The cortical neuronal isoform was expressed at a detectable level in skeletal muscle in the absence of the muscle promoter and levels of PCR products representing cortical neuronal-type transcripts in dystrophic muscle were comparable to those of normal feline skeletal muscle. Although localized at the sarcolemma, cortical neuronal dystrophin apparently failed to protect skeletal muscle. Neuronal transcripts could not be amplified from feline heart, indicating that these promoters are not active in this tissue in the cat.

Neuron(31)

Baldwin, T. J., M.-L. Tsaor, et al. (1991). "Characterization of a mammalian cDNA for an inactivating voltage-sensitive K⁺ channel." Neuron **7**(3): 471.

<http://www.sciencedirect.com/science/article/B6WSS-4C6KNF1-13/2/62a61df890a033305fc03220ef0f8ca2>

A cDNA clone encoding a K⁺ channel polypeptide with 72% amino acid sequence identity to *Drosophila* Shal was isolated from rat hippocampus. Functional expression of the cDNA in *Xenopus* oocytes generated 4-amino-pyridine-sensitive K⁺ channels displaying rapid inactivation kinetics. The fastest component of inactivation was slowed by the deletion of 3 basic residues in the amino-terminal region. Northern blots revealed that the mRNA encoding this K⁺ channel polypeptide was expressed at a similar level in the brain and in the heart. In situ hybridization revealed that the mRNA encoding this K⁺ channel appeared concentrated in the hippocampus, dentate gyrus, and habenular nucleus in the brain. Thus, this K⁺ channel polypeptide is likely to form some of the A-type K⁺ channels expressed in the mammalian nervous system and heart.

Bito, H., M. Nakamura, et al. (1992). "Platelet-activating factor (PAF) receptor in rat brain: PAF mobilizes intracellular Ca²⁺ in hippocampal neurons." Neuron **9**(2): 285.

<http://www.sciencedirect.com/science/article/B6WSS-4C6CSNN-BR/2/0fa2896a304e83aff07014c1b9ed7bb7>

Platelet-activating factor (PAF), an alkylether phospholipid, is produced in the brain when it is subjected to various stimuli. Using a *Xenopus* oocyte expression system, we obtained evidence for functional PAF receptor mRNA expression in rat brain. The presence of the PAF receptor was confirmed and shown to be quite ubiquitous in the CNS by RNA blot and radioligand binding

studies. To investigate the neuronal functions of PAF, intracellular Ca^{2+} increase elicited by nanomolar PAF application was analyzed in cultured rat hippocampal cells. Fractions of NMDA-responsive cells and non-NMDA-responsive cells were shown to respond to PAF, suggesting a potential role for PAF in the Ca^{2+} signaling pathway in the hippocampus.

Buckanovich, R. J., J. B. Posner, et al. (1993). "Nova, the paraneoplastic Ri antigen, is homologous to an RNA-binding protein and is specifically expressed in the developing motor system." *Neuron* **11**(4): 657.

<http://www.sciencedirect.com/science/article/B6WSS-4C6KNC8-B/2/ec54365c79dc68386065ba537a45d487>

Paraneoplastic opsoclonus-ataxia, a disorder of motor control, develops in breast or lung cancer patients who harbor an antibody (Ri) that recognizes their tumors and a nuclear neuronal protein of 55 kd. We have characterized a gene, Nova, encoding an antigen recognized by the Ri antibody. Nova encodes a novel, highly conserved protein, homologous to the RNA-binding protein hnRNP K, the yeast splicing protein MER1, and a motif in several retroviral proteases. Northern blot analysis detects Nova transcripts only in brain, and several alternatively spliced forms are present in brain and tumor cells. Nova expression is restricted to the ventral brain stem and spinal cord in E18 mice. Since Nova encodes a target antigen in the motor disorder paraneoplastic opsoclonus-ataxia that is expressed in the developing subcortical motor system, it is a likely participant in both the pathogenesis of paraneoplastic opsoclonus-ataxia and the developmental biology of the motor system. The homology between Nova and hnRNP K suggests that Nova regulates RNA splicing or metabolism in a specific subset of developing neurons.

Cirelli, C., C. M. Gutierrez, et al. (2004). "Extensive and Divergent Effects of Sleep and Wakefulness on Brain Gene Expression." *Neuron* **41**(1): 35.

<http://www.sciencedirect.com/science/article/B6WSS-4BF62JX-7/2/65104ebf9590932783eb67bc9ca3f75d>

Sleep is present in all species where it has been studied, but its functions remain unknown. To investigate what benefits sleep may bring at the cellular level, we profiled gene expression in awake and sleeping rats by using high-density microarrays. We find that ~10% of the transcripts in the cerebral cortex change their expression between day and night and demonstrate that half of them are modulated by sleep and wakefulness independent of time of day. We also show that molecular correlates of sleep are found in the cerebellum, a structure not known for generating sleep rhythms. Finally, we show that different functional categories of genes are selectively associated with sleep and wakefulness. The ~100 known genes whose expression increases during sleep provide molecular support for the proposed involvement of sleep in protein synthesis and neural plasticity and point to a novel role for sleep in membrane trafficking and maintenance.

Coolen, M. W., K. M. J. Van Loo, et al. (2005). "Gene Dosage Effect on [gamma]-Secretase Component Aph-1b in a Rat Model for Neurodevelopmental Disorders." *Neuron* **45**(4): 497.

<http://www.sciencedirect.com/science/article/B6WSS-4FH5K0X-6/2/8b949b3873d792a58c45d666967ae7a0>

Summary A combination of genetic factors and early life events is thought to determine the vulnerability of an individual to develop a complex neurodevelopmental disorder like schizophrenia. Pharmacogenetically selected, apomorphine-susceptible Wistar rats (APO-SUS) display a number of behavioral and pathophysiological features reminiscent of such disorders. Here, we report microarray analyses revealing in APO-SUS rats, relative to their counterpart APO-UNSUS rats, a reduced expression of Aph-1b, a component of the [gamma]-secretase enzyme complex that is involved in multiple (neuro)developmental signaling pathways. The reduced expression is due to a duplicon-based genomic rearrangement event resulting in an Aph-1b dosage imbalance. The expression levels of the other [gamma]-secretase components were not affected. However, [gamma]-secretase cleavage activity was significantly changed, and the APO-SUS/-UNSUS Aph-1b genotypes segregated with a number of behavioral phenotypes. Thus, a subtle imbalance in the expression of a single, developmentally important protein may be sufficient to cause a complex phenotype.

Crino, P. B. and J. Eberwine (1996). "Molecular Characterization of the Dendritic Growth Cone: Regulated mRNA Transport and Local Protein Synthesis." Neuron **17**(6): 1173.

<http://www.sciencedirect.com/science/article/B6WSS-41838Y2-G/2/7afca99796c01375fe90995efce47e1c>

The molecular mechanisms that regulate growth cone guidance of dendrite outgrowth remain to be elucidated. We hypothesized that mRNA localization in dendritic growth cones and their local protein synthesis may be important for growth cone functioning. The appearance of 23 of 31 growth cone mRNAs was developmentally regulated. Also, alteration of growth cone morphology affected the relative levels of three mRNAs. Finally, using single dendrite transfection, it was shown that local protein synthesis occurs in dendrites and growth cones. A heterogeneous population of mRNAs exists in dendritic growth cones of cultured hippocampal neurons whose relative abundances are developmentally regulated and can vary with changes in growth cone physiology. The demonstration of protein synthesis in growth cones suggests that translation of the localized mRNAs may contribute to regulation of growth cone motility and dendrite outgrowth.

Daubas, P., A. Devillers-Thiery, et al. (1990). "Differential expression of the neuronal acetylcholine receptor [alpha]2 subunit gene during chick brain development." Neuron **5**(1): 49.

<http://www.sciencedirect.com/science/article/B6WSS-4CF6DTR-P/2/fac8fb9855432883c955feeb3136133f>

In situ hybridization histochemistry reveals localized expression of the nicotinic acetylcholine receptor (nAChR) [alpha]2 subunit mRNA restricted to the lateral spiriform nucleus cleus (SpL) of the chick diencephalon. The [alpha]2 nAChR transcripts are not detected in immature SpL neurons at 4.5-5 days of embryonic development. They begin to accumulate in the SpL at embryonic day 11 and increase until the newborn stage. Specific [alpha]2 cDNA amplification by the polymerase chain reaction shows that during this period, the absolute content of [alpha]2 mRNA increases about 20-fold. The expression of the [alpha]2 nAChR gene is thus developmentally regulated and appears concomitant with the entry of cholinergic fibers into the SpL, as demonstrated by choline acetyltransferase immunohistochemistry.

Dihanich, M., M. Kaser, et al. (1991). "Prothrombin mRNA is expressed by cells of the nervous system." Neuron **6**(4): 575.

<http://www.sciencedirect.com/science/article/B6WSS-4C6CS41-W/2/fde20b272ca3b0ffc0f6600c1d2acb5a>

Thrombin, a serine protease of the blood coagulation system, has additional effects on cells in vitro. It is mitogenic for fibroblasts and astrocytes and contributes to the regulation of neurite outgrowth and astrocyte stellation. Until now the expression of thrombin or its precursor prothrombin in tissues other than liver has not been demonstrated conclusively because of difficulty in avoiding serum contamination. Using sensitive mRNA detection methods, we show here that prothrombin is expressed not only in the liver, but also in the brain throughout development. Polymerase chain reaction, Northern, and in situ hybridization studies demonstrate the presence of prothrombin transcripts in the olfactory bulb, the cortex, the cerebellum, and other regions of the rat and human nervous system, as well as in neural cell lines. These results support an involvement of (pro)thrombin in the regulation of cellular events in the nervous system.

Earl Patton, D., J. W. West, et al. (1993). "A peptide segment critical for sodium channel inactivation functions as an inactivation gate in a potassium channel." *Neuron* **11**(5): 967.

<http://www.sciencedirect.com/science/article/B6WSS-4C6CSF1-6P/2/dc136daf38f9434e7189f87433e59c62>

The short cytoplasmic peptide segment connecting domains III and IV of voltage-gated sodium channels (III-IV linker) is essential for fast inactivation. To test the functional similarity between the III-IV linker and the potassium channel inactivation particle, we attached the III-IV linker to the amino terminus of a noninactivating potassium channel. This chimeric channel inactivated rapidly and displayed biophysical properties similar to Shaker A-type potassium channels. Recovery from inactivation in the chimeric channels was accelerated by high external potassium, consistent with the idea that potassium ions passing through the channel displaced the III-IV linker inactivation particle. A mutation that completely abolishes fast inactivation in rat brain sodium channels also completely abolished inactivation in the chimera. These results demonstrate that the sodium channel III-IV linker can function as a fast inactivation gate and suggest a functional relationship between the fast inactivation processes of sodium and potassium channels.

Erlander, M. G., N. J. K. Tillakaratne, et al. (1991). "Two genes encode distinct glutamate decarboxylases." *Neuron* **7**(1): 91.

<http://www.sciencedirect.com/science/article/B6WSS-4C6CSHX-88/2/ac1648c2123aa3db13a52d3b349312a6>

[gamma]-Aminobutyric acid (GABA) is the most widely distributed known inhibitory neurotransmitter in the vertebrate brain. GABA also serves regulatory and trophic roles in several other organs, including the pancreas. The brain contains two forms of the GABA synthetic enzyme glutamate decarboxylase (GAD), which differ in molecular size, amino acid sequence, antigenicity, cellular and subcellular location, and interaction with the GAD co-factor pyridoxal phosphate. These forms, GAD65 and GAD67, derive from two genes. The distinctive properties of the two GADS provide a substrate for understanding not only the multiple roles of GABA in the nervous system, but also the autoimmune response to GAD in insulin-dependent diabetes mellitus.

Gabay, L., S. Lowell, et al. (2003). "Deregulation of Dorsoventral Patterning by FGF Confers Trilineage

Differentiation Capacity on CNS Stem Cells In Vitro." Neuron **40**(3): 485.

<http://www.sciencedirect.com/science/article/B6WSS-4B3MJP2-9/2/42fc51933cc0d10172407bbf5ff13df7>

The CNS is thought to develop from self-renewing stem cells that generate neurons, astrocytes, and oligodendrocytes. Other data, however, have suggested that astrocytes and oligodendrocytes are generated from separate progenitor populations. To reconcile these observations, we have prospectively isolated progenitors that do or do not express Olig2, an oligodendrocyte bHLH determination factor. Both Olig2⁻ and Olig2⁺ progenitors can behave as tripotential CNS stem cells (CNS-SCs) in vitro. Growth in FGF-2 causes induction of Olig2 in the former population, permitting oligodendrocyte differentiation; extinction of Olig2 in the latter cells permits astrocyte differentiation. The induction of Olig2 by FGF-2 is mediated, in part, via endogenous Sonic Hedgehog. These data indicate that clonogenic competence to generate neurons, astrocytes, and oligodendrocytes reflects a deregulation of dorsoventral patterning during expansion in vitro, raising the question of whether such trifotent cells actually exist in vivo.

Garbern, J. Y., F. Cambi, et al. (1997). "Proteolipid Protein Is Necessary in Peripheral as Well as Central Myelin." Neuron **19**(1): 205.

<http://www.sciencedirect.com/science/article/B6WSS-418392H-P/2/e32eeb35760fff3d1eaf97c2bf64cef1>

Alternative products of the proteolipid protein gene (PLP), proteolipid protein (PLP) and DM20, are major components of compact myelin in the central nervous system, but quantitatively minor constituents of Schwann cells. A family with a null allele of PLP has a less severe CNS phenotype than those with other types of PLP mutations. Moreover, individuals with PLP null mutations have a demyelinating peripheral neuropathy, not seen with other PLP mutations of humans or animals. Direct analysis of normal peripheral nerve demonstrates that PLP is localized to compact myelin. This and the clinical and pathologic observations of the PLP null phenotype indicate that PLP/DM20 is necessary for proper myelin function both in the central and peripheral nervous systems.

Hollmann, M., J. Boulter, et al. (1993). "Zinc potentiates agonist-induced currents at certain splice variants of the NMDA receptor." Neuron **10**(5): 943.

<http://www.sciencedirect.com/science/article/B6WSS-4C6KNVS-7B/2/2b408fdc494fd5a742861d1ff2a9b4fa>

We have determined the gene structure for the NMDA receptor subunit gene NMDAR1. We found eight splice variants that arise from different combinations of a single 5' terminal exon insertion and three different 3' terminal exon deletions, relative to NMDAR1. We analyzed the modulation by Zn²⁺ of currents through homomeric receptors assembled from these splice variants and found that, in addition to its well-known inhibitory effect at high concentrations, Zn²⁺ potentiates agonist-induced currents at submicromolar concentrations (EC₅₀ = 0.50 [μM]). This potentiation is observed only with a subset of NMDAR1 splice variants that show additional differences in pharmacological properties. Zn²⁺ potentiation is rapidly reversible, noncompetitive with either glutamate or glycine, and voltage independent. Zn²⁺ potentiation is mimicked by Cd²⁺, Cu²⁺, and Ni²⁺, but not by Mn²⁺, Co²⁺, Fe³⁺, Sn²⁺, or Hg²⁺. Our results suggest a possible role for Zn²⁺ as a positive modulator of NMDA receptors in certain regions of the brain.

Hollmann, M., C. Maron, et al. (1994). "N-glycosylation site tagging suggests a three transmembrane domain topology for the glutamate receptor GluRI." *Neuron* **13**(6): 1331.

<http://www.sciencedirect.com/science/article/B6WSS-4C71KX7-2T/2/95a1d8430b5b85f206a59ba76abc2dab>

We investigated the transmembrane topology of the glutamate receptor GluR1 by introducing N-glycosylation sites as reporter sites for an extracellular location of the respective site. Our data show that the N-terminus is extracellular, whereas the C-terminus is intracellular. Most importantly, we found only three transmembrane domains (designed TMD A, TMD B, and TMD C), which correspond to the previously proposed TMDs I, III, and IV, respectively. Contrary to earlier models, the putative channel-lining hydrophobic domain TMD II does not span the membrane, but either lies in close proximity to the intracellular face of the plasma membrane or loops into the membrane without traversing it. Furthermore, the region between TMDs III and IV, in previous models believed to be intracellular, is an entirely extracellular domain.

Hu, Y., A. Barzilai, et al. (1993). "5-HT and cAMP induce the formation of coated pits and vesicles and increase the expression of clathrin light chain in sensory neurons of aplysia." *Neuron* **10**(5): 921.

<http://www.sciencedirect.com/science/article/B6WSS-4C6KNVS-78/2/572a39f842ff8c7e70f315855db37c79>

In the course of studying proteins involved in long-term facilitation in Aplysia, we found that 5-HT and cAMP, a second messenger activated by 5-HT, lead to the removal of a set of N-CAM-related cell adhesion molecules (apCAMs) from the surface membrane of sensory neurons by means of receptor-mediated endocytosis. Here we describe that, as part of this coordinated program for endocytosis, 5-HT and cAMP also induce in the sensory neurons an increase in the density of coated pits and coated vesicles and an increase in the expression of the light chain of Aplysia clathrin (apClathrin). The clathrin-related endocytosis seems designed to internalize and redistribute apCAMs and other surface membrane proteins in the sensory neurons, and thus it appears to constitute one of the initial steps in the growth of new synaptic connections that accompanies long-term facilitation.

Jasmin, B. J., R. K. Lee, et al. (1993). "Compartmentalization of acetylcholinesterase mRNA and enzyme at the vertebrate neuromuscular junction." *Neuron* **11**(3): 467.

<http://www.sciencedirect.com/science/article/B6WSS-4C6CS5Y-1W/2/81dfb42eb4d74c63b76e0a5b3ad57811>

Acetylcholinesterase (AChE) is concentrated at the vertebrate neuromuscular synapse. To determine whether increased transcript levels could underlie this selective accumulation, we employed a quantitative reverse transcription polymerase chain reaction-based assay to determine mRNA copy number in samples as small as single neuromuscular junctions (NMJs) and a microassay to measure AChE enzyme activity at single synapses. Our results show that AChE mRNA is an intermediate transcript at NMJs, whereas in noninnervated regions of muscle fibers, AChE transcripts are either undetectable or rare. In contrast, α -actin transcript levels in the same samples are similar in junctional and extrajunctional regions. However, compared with AChE enzyme activity and α -actin mRNA levels, the levels of AChE transcripts at NMJs are highly variable. These results indicate that AChE mRNA and protein expression are

compartmentalized at the vertebrate NM) and provide a direct approach toward dissecting the molecular events leading from synaptic activation to plastic changes in gene expression at single vertebrate synapses.

Knaus, P., B. Marqueze-Pouey, et al. (1990). "Synaptoporin, a novel putative channel protein of synaptic vesicles." Neuron **5**(4): 453.

<http://www.sciencedirect.com/science/article/B6WSS-4C6CS34-8/2/8e50ff0be839294bfaafd927e3436d48>

By homology screening of a rat brain library, we have isolated cDNAs that encode a novel member of the synaptophysin/connexin channel protein superfamily. The deduced protein, named synaptoporin, displays 58% amino acid identity to synaptophysin, with highly conserved transmembrane segments, but a divergent cytoplasmic tail. Northern blot analysis and PCR amplification of RNA from different rat tissues indicate expression of synaptoporin transcripts in the CNS. Antibodies against a synthetic peptide or a fusion construct encompassing the cytoplasmic tail region of synaptoporin detect a polypeptide of 37 kd that copurifies with small synaptic vesicles. Our data suggest the existence of a family of vesicular channel proteins whose members may be differently distributed among synaptic vesicle subpopulations.

Krichevsky, A. M. and K. S. Kosik (2001). "Neuronal RNA Granules: A Link between RNA Localization and Stimulation-Dependent Translation." Neuron **32**(4): 683.

<http://www.sciencedirect.com/science/article/B6WSS-44J1095-G/2/9a8fcdc4d6d17b59c240fd038e0f9cbb>

RNA granules are a macromolecular structure observed in neurons, where they serve as motile units that translocate mRNAs. Isolated RNA granules are highly enriched in Staufen protein and ultrastructurally contain densely packed clusters of ribosomes. With depolarization, many mRNAs, including those involved in plasticity, rapidly shift from the RNA granule fraction to polysomes. Depolarization reorganizes granules and induces a less compact organization of their ribosomes. RNA granules are not translationally competent, as indicated by the failure to incorporate radioactive amino acids and the absence of eIF4E, 4G, and tRNAs. We concluded that RNA granules are a local storage compartment for mRNAs under translational arrest but are poised for release to actively translated pools. Local release of mRNAs and ribosomes from granules may serve as a macromolecular mechanism linking RNA localization to translation and synaptic plasticity.

Larsson, H. P. and F. Elinder (2000). "A Conserved Glutamate Is Important for Slow Inactivation in K⁺ Channels." Neuron **27**(3): 573.

<http://www.sciencedirect.com/science/article/B6WSS-41FKPNH-R/2/d0eddf92375a1b4cfd779676505cb056>

Voltage-gated ion channels undergo slow inactivation during prolonged depolarizations. We investigated the role of a conserved glutamate at the extracellular end of segment 5 (S5) in slow inactivation by mutating it to a cysteine (E418C in Shaker). We could lock the channel in two different conformations by disulfide-linking 418C to two different cysteines, introduced in the Pore-S6 (P-S6) loop. Our results suggest that E418 is normally stabilizing the open conformation

of the slow inactivation gate by forming hydrogen bonds with the P-S6 loop. Breaking these bonds allows the P-S6 loop to rotate, which closes the slow inactivation gate. Our results also suggest a mechanism of how the movement of the voltage sensor can induce slow inactivation by destabilizing these bonds.

Ma, J.-x., S. Znoiko, et al. (2001). "A Visual Pigment Expressed in Both Rod and Cone Photoreceptors." Neuron **32**(3): 451.

<http://www.sciencedirect.com/science/article/B6WSS-44FC84P-C/2/03ecf63f5881683ffa5bdac1660c76d1>

Rods and cones contain closely related but distinct G protein-coupled receptors, opsins, which have diverged to meet the differing requirements of night and day vision. Here, we provide evidence for an exception to that rule. Results from immunohistochemistry, spectrophotometry, and single-cell RT-PCR demonstrate that, in the tiger salamander, the green rods and blue-sensitive cones contain the same opsin. In contrast, the two cells express distinct G protein transducin [alpha] subunits: rod [alpha] transducin in green rods and cone [alpha] transducin in blue-sensitive cones. The different transducins do not appear to markedly affect photon sensitivity or response kinetics in the green rod and blue-sensitive cone. This suggests that neither the cell topology or the transducin is sufficient to differentiate the rod and the cone response.

Majercak, J., D. Sidote, et al. (1999). "How a Circadian Clock Adapts to Seasonal Decreases in Temperature and Day Length." Neuron **24**(1): 219.

<http://www.sciencedirect.com/science/article/B6WSS-418PW08-R/2/57ab5e568da1d0c83c5f89aa25443419>

We show that a thermosensitive splicing event in the 3' untranslated region of the mRNA from the period (*per*) gene plays an important role in how a circadian clock in *Drosophila* adapts to seasonally cold days (low temperatures and short day lengths). The enhanced splicing of this intron at low temperatures advances the steady state phases of the *per* mRNA and protein cycles, events that significantly contribute to the preferential daytime activity of flies on cold days. Because the accumulation of PER is also dependent on the photosensitive TIMELESS (TIM) protein, long photoperiods partially counteract the cold-induced advances in the oscillatory mechanism by delaying the daily increases in the levels of TIM. Our findings also indicate that there is a temperature-dependent switch in the molecular logic governing cycles in *per* mRNA levels.

Mulhardt, C., M. Fischer, et al. (1994). "The spastic mouse: Aberrant splicing of glycine receptor [beta] subunit mRNA caused by intronic insertion of LI element." Neuron **13**(4): 1003.

<http://www.sciencedirect.com/science/article/B6WSS-4C6KNYX-8W/2/a0f11587446afc96aa3e911fd2e78389>

Mice homozygous for the spastic mutation (*spa*) suffer from a complex motor disorder resulting from reduced CNS levels of the adult glycine receptor isoform GlyRA, which is composed of ligand-binding α and structural [beta] polypeptides. The [beta] subunit-encoding gene (*Glyrb*) was mapped near the *spa* locus on mouse chromosome 3. In *spa/spa* mice, aberrant splicing of the [beta] subunit pre-mRNA strikingly diminishes the CNS contents of fulllength transcripts, whereas

truncated [beta] subunit mRNAs accumulate. This is a result of exon skipping, which causes translational frameshifts and premature stop codons. Intron 5 of the *spa Glyrb* gene contains an L1 transposable element that apparently is causal for the aberrant splicing of [beta] subunit transcripts.

Niclas, J., F. Navone, et al. (1994). "Cloning and localization of a conventional kinesin motor expressed exclusively in neurons." *Neuron* **12**(5): 1059.

<http://www.sciencedirect.com/science/article/B6WSS-4C58XVP-B/2/1afee2f9a639ed7f394993fa94c42ee2>

Kinesin is a microtubule-based motor protein involved in organelle transport in neuronal and nonneuronal cells. Although a single kinesin motor has been thought to serve all cell types, we document here that neurons express a second conventional kinesin heavy chain (nKHC) that is 65% identical in amino acid sequence to the ubiquitously expressed kinesin heavy chain (uKHC). By preparing antibodies which distinguish between the two KHCs, we demonstrate that nKHC is a nucleotide-dependent microtubule-binding protein which partially cofractionates with membrane organelles. Immunolocalization experiments show that nKHC is distributed throughout the CNS but is highly enriched in subsets of neurons. In hippocampal neurons in culture, uKHC is distributed uniformly throughout the neuron, whereas nKHC is selectively concentrated in the cell body. These results demonstrate that mammalian neuronal tissue contains two conventional kinesin motors which may serve distinct functions in microtubule-based transport.

Patton, D. E., T. Silva, et al. (1997). "RNA Editing Generates a Diverse Array of Transcripts Encoding Squid Kv2 K⁺ Channels with Altered Functional Properties." *Neuron* **19**(3): 711.

<http://www.sciencedirect.com/science/article/B6WSS-418393K-T/2/0284fd45276f2ee563bda9f2b760578b>

We have cloned a Kv2 potassium channel from squid optic lobe termed sqKv2. Multiple overlapping sqKv2 cDNA clones differed from one another at specific positions by purine transitions. To test whether the purine transitions were generated by RNA editing, we compared a 360 nucleotide genomic sequence with corresponding cDNA sequences (encoding S4-S6) isolated from individual animals and lying on a single gene and exon. cDNA sequences differed from genomic sequence at 17 positions, resulting in 28 unique sequences. There was invariably an adenosine in the genomic sequence and a guanosine in the edited cDNA sequences. Two of the edits altered the rates of channel closure and slow inactivation. These results extend selective RNA editing to invertebrate taxa and represents a novel mechanism for the posttranscriptional modulation of voltage-gated ion channels.

Puchalski, R. B., J.-C. Louis, et al. (1994). "Selective RNA editing and subunit assembly of native glutamate receptors." *Neuron* **13**(1): 131.

<http://www.sciencedirect.com/science/article/B6WSS-4C71M2W-5P/2/24170ad6a7cf011df89897aa88afa383>

RNA editing and subunit assembly of ionotropic glutamate receptors (GluRs) were examined in an oligodendrocyte progenitor cell line, CG4, which expresses GluR2-GluR4, GluR6, GluR7, KA1, and KA2. AMPA-evoked currents rapidly desensitize, whereas kainate-evoked currents contain a

steady-state component with a nearly linear current-voltage relation and a fast desensitizing component that is inwardly rectifying. The Q/R site is edited >95% to the arginine codon in GluR2(Q607) mRNA, and <5% in GluR6(Q621) mRNA. Immunoprecipitation experiments demonstrate that GluR6 and/or GluR7 subunits assemble with KA2, but not with GluR2-GluR4. These results indicate that oligodendrocyte progenitor cells selectively edit and assemble glutamate receptors into at least two functionally and structurally distinct heteromeric channels.

Simonen, M., V. Pedersen, et al. (2003). "Systemic Deletion of the Myelin-Associated Outgrowth Inhibitor Nogo-A Improves Regenerative and Plastic Responses after Spinal Cord Injury." Neuron **38**(2): 201.

<http://www.sciencedirect.com/science/article/B6WSS-48FMKMF-7/2/336dfc0464575367367f6fba3a9df804>

To investigate the role of the myelin-associated protein Nogo-A on axon sprouting and regeneration in the adult central nervous system (CNS), we generated Nogo-A-deficient mice. Nogo-A knockout (KO) mice were viable, fertile, and not obviously afflicted by major developmental or neurological disturbances. The shorter splice form Nogo-B was strongly upregulated in the CNS. The inhibitory effect of spinal cord extract for growing neurites was decreased in the KO mice. Two weeks following adult dorsal hemisection of the thoracic spinal cord, Nogo-A KO mice displayed more corticospinal tract (CST) fibers growing toward and into the lesion compared to their wild-type littermates. CST fibers caudal to the lesion--regenerating and/or sprouting from spared intact fibers--were also found to be more frequent in Nogo-A-deficient animals.

Tietjen, I., J. M. Rihel, et al. (2003). "Single-Cell Transcriptional Analysis of Neuronal Progenitors." Neuron **38**(2): 161.

<http://www.sciencedirect.com/science/article/B6WSS-48FMKMF-7/2/944cb8d1bb9ae3bee9042334946d5490>

The extraordinary cellular heterogeneity of the mammalian nervous system has largely hindered the molecular analysis of neuronal identity and diversity. In order to uncover mechanisms involved in neuronal differentiation and diversification, we have monitored the expression profiles of individual neurons and progenitor cells collected from dissociated tissue or captured from intact slices. We demonstrate that this technique provides a sensitive and reproducible representation of the single-cell transcriptome. In the olfactory system, hundreds of transcriptional differences were identified between olfactory progenitors and mature sensory neurons, enabling us to define the large variety of signaling pathways expressed by individual progenitors at a precise developmental stage. Finally, we show that regional differences in gene expression can be predicted from transcriptional analysis of single neuronal precursors isolated by laser capture from defined areas of the developing brain.

Tsaur, M.-L., M. Sheng, et al. (1992). "Differential expression of K⁺ channel mRNAs in the rat brain and down-regulation in the hippocampus following seizures." Neuron **8**(6): 1055.

<http://www.sciencedirect.com/science/article/B6WSS-4D8F37C-22/2/1da9708d3f5c47616d02c11462bae75d>

K⁺ channels are major determinants of membrane excitability. Differences in neuronal excitability within the nervous system may arise from differential expression of K⁺ channel genes, regulated spatially in a cell type-specific manner, or temporally in response to neuronal activity. We have compared the distribution of mRNAs of three K⁺ channel genes, Kv1.1, Kv1.2, and Kv4.2 in rat brain, and examined activity-dependent changes following treatment with the convulsant drug pentylenetetrazole. Both regional and cell type-specific differences of K⁺ channel gene expression were found. In addition, seizure activity caused a reduction of Kv1.2 and Kv4.2 mRNAs in the dentate granule cells of the hippocampus, raising the possibility that K⁺ channel gene regulation may play a role in long-term neuronal plasticity.

Westaway, D., C. A. Mirenda, et al. (1991). "Paradoxical shortening of scrapie incubation times by expression of prion protein transgenes derived from long incubation period mice." Neuron 7(1): 59.

<http://www.sciencedirect.com/science/article/B6WSS-4C6CSHX-85/2/a1cab262f06910589dcda5890474602f>

Prolonged incubation times for experimental scrapie in I/LnJ mice are dictated by a dominant gene linked to the prion protein gene (Prn-p). Transgenic mice were analyzed to discriminate between an effect of the I/LnJPrn-pb allele and a distinct incubation time locus designated Prn-i. Paradoxically, 4 independent Prn-pb transgenic mouse lines had scrapie incubation times shorter than nontransgenic controls, instead of the anticipated prolonged incubation periods. Aberrant or over-expression of the Prn-pb transgenes may dictate abbreviated incubation times, masking genuine Prnp/Prn-i congruence; alternatively, a discrete Prn-i gene lies adjacent to Prn-p.

Zack, D. J., J. Bennett, et al. (1991). "Unusual topography of bovine rhodopsin promoter-lacZ fusion gene expression in transgenic mouse retinas." Neuron 6(2): 187.

<http://www.sciencedirect.com/science/article/B6WSS-4C6KP83-9P/2/26b759d3ad348ca13af245a4177e6da2>

To define the cis-acting DNA elements required for rhodopsin expression, we generated lines of transgenic mice carrying sequences upstream of the bovine rhodopsin gene fused to the E. coli [beta]-galactosidase gene (lacZ). Upstream sequences extending from -2174 to +70 bp, from -734 to +70 bp, and from -222 to +70 by direct photoreceptor-specific expression. All three -2174 lines demonstrate a superior-temporal to inferior-nasal gradient of expression across the retina, whereas lines carrying the shorter constructs demonstrate either spatially continuous expression across the retina, discrete clusters of expression, or both. As a complementary approach to defining regulatory elements, we compared DNA sequences 5' of the murine, bovine, and human rhodopsin genes. Significant homology between all three species was found just upstream of the transcription start site and at approximately 1.5 kb upstream.

Zackenfeld, K., R. W. Oppenheim, et al. (1995). "Evidence for an important role of IGF-I and IGF-II for the early development of chick sympathetic neurons." Neuron 14(4): 731.

<http://www.sciencedirect.com/science/article/B6WSS-4CWYS0K-7/2/44032b7f27baa26cf6a00e8cdd7b72d5>

The ability of immature neurons from chick lumbosacral sympathetic ganglia to proliferate in vitro

was used to identify factors that affect neurogenesis. Under serum-free culture conditions, insulin-like growth factor I (IGF-1), IGF-II, or insulin caused an increase in the proportion of cells that incorporated [3H]thymidine. In addition, IGFs also stimulated neurite outgrowth from these immature sympathetic neurons. IGF-I and IGF-11 mRNA was found to be expressed in E7 sympathetic ganglia during the period of neurogenesis. IGF-I was detectable in fibroblasts, whereas IGF-II mRNA was expressed by neurons, glia, and fibroblasts. Elimination of endogenous IGFs by neutralizing antibodies resulted in a reduction of neuron proliferation and neuron number, whereas elevation of IGF levels by treatment with IGF-I increased sympathetic neuron proliferation in vivo. These findings suggest an important role of IGFs for the development of sympathetic neurons and imply a general role of IGFs in the control of neurogenesis and neurite outgrowth.

Neuropeptides (1)

Lambert, R. W., K. Campton, et al. (2002). "Langerhans cell expression of neuropeptide Y and peptide YY." Neuropeptides **36**(4): 246.

<http://www.sciencedirect.com/science/article/B6WNR-46VBHJD-1/2/32d5833b5d3f354fa21d73b5b3e7393f>

Neuropharmacology (4)

Arslan, G., C. M. Filipeanu, et al. (2000). "P2Y receptors contribute to ATP-induced increases in intracellular calcium in differentiated but not undifferentiated PC12 cells." Neuropharmacology **39**(3): 482.

<http://www.sciencedirect.com/science/article/B6T0C-3YDFYV0-G/2/bf6ea69e39e06a8bff07960156702bf3>

ATP-induced Ca²⁺ transients were examined in individual PC12 cells of a well defined clone, before and after treatment with nerve growth factor (NGF) to induce a neurone-like phenotype. Using reverse transcriptase PCR these cells were found to express mRNA for several P2 receptors. In undifferentiated cells the ATP-induced Ca²⁺ response was entirely dependent on Ca²⁺ influx, could not be mimicked by UTP, [alpha],[beta]-methylene ATP or dibenzoyl ATP or be blocked by pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS). ATP had no significant effect on levels of cyclic AMP or inositol 1,4,5-trisphosphate (InsP3). These results suggest that in undifferentiated PC12 cells ATP mainly acts on a P2X receptor, possibly the P2X4 subtype. After treatment with NGF for 7 days the ATP response was increased and partially sensitive to PPADS. A component of the ATP-induced Ca²⁺ increase was due to mobilisation of intracellular Ca²⁺ stores and another to capacitative Ca²⁺ entry. UTP caused an increase in intracellular Ca²⁺, and InsP3 formation could be stimulated by ATP and UTP. ATP also caused a small increase in cyclic AMP, but this was abolished in the presence of indomethacin. Thus, after NGF treatment ATP acts partially via a P2Y receptor, possibly the P2Y2 subtype.

Jones, K. W. and S. J. Peroutka (1998). "Step-wise analysis of polymorphisms in the human dopamine D2 receptor gene." Neuropharmacology **37**(6): 803.

<http://www.sciencedirect.com/science/article/B6T0C-3T3JC4N-1B/2/d64e8d714a6c329c1348596c57a6e61e>

Evolutionary analysis of neurotransmitter receptor systems has previously focused on interspecies differentiation. Recently, emphasis has shifted to intragenic evolution within a single species and the functional relevance associated with intraspecies variations. For example, multiple polymorphisms have been identified within the human dopamine D2 receptor (DRD2) gene, many of which have been used in clinical association studies. In an attempt to evaluate the intragenic evolution of the DRD2 gene, genotypes from 116 humans were determined using five biallelic markers which reside within a 30 kb span of the DRD2 gene, that are non-polymorphic in other higher order primates. Only seven different haplotypes, out of a theoretical maximum of 32, were present in the study group of 232 chromosomes. Moreover, five of the seven haplotypes accounted for 99% (n=230/232) of the human haplotypes. A phylogenetic tree was generated from the haplotypic data using a maximum parsimony algorithm. The relationship of the haplotypes within the phylogenetic tree is consistent with a progressive step-wise nucleotide conversion within the human gene. These data indicate that specific haplotypic subtypes of the human DRD2 gene exist within the human population and allow for the possibility that functional differences may exist between the DRD2 subtypes. Therefore, future studies focused on a functional analysis of the entire human DRD2 haplotype, as opposed to individual polymorphisms, may provide important insights into the functional relevance of molecular variations within the human DRD2 gene.

Kim, H., B. A. Flanagan, et al. (2003). "The mouse Chrna4 A529T polymorphism alters the ratio of high to low affinity [alpha]4[beta]2 nAChRs." Neuropharmacology **45**(3): 345.

<http://www.sciencedirect.com/science/article/B6T0C-48SBTF0-5/2/73526943b68a17bc83a3bd2ced635b25>

Previously, a missense polymorphism was identified in the mouse nicotinic receptor [alpha]4 subunit gene, Chrna4. This polymorphism leads to an Ala/Thr variation at amino acid position 529 of the [alpha]4 subunit. Chrna4 A529T is associated with several measures of acute sensitivity to nicotine as well as with mouse strain differences in nicotine-stimulated 86Rb⁺ efflux from synaptosomes. Here, we report that the variant forms of the mouse [alpha]4 subunit confer functional differences when expressed with the [beta]2 subunit in a heterologous system. [alpha]4[beta]2 receptors containing the T529 variant of the [alpha]4 subunit exhibited a higher EC₅₀ value for the high affinity receptor population and an apparent reduced sensitivity to blockade by DH[beta]E relative to [alpha]4[beta]2 receptors containing the A529 variant of the [alpha]4 subunit. Moreover, the proportion of the total agonist-elicited current contributed by the high affinity [alpha]4[beta]2 receptor population was greater for [alpha]4[beta]2 receptors containing the [alpha]4(T529) variant (64%) than the [alpha]4[beta]2 receptors containing the [alpha]4(A529) variant (41%). These data suggest that the polymorphism in the mouse [alpha]4 subunit is located in a previously unidentified functional domain of the receptor subunit that influences receptor function, including regulation of the affinity population ratio of [alpha]4[beta]2 receptors.

Nakamichi, N., G. Chidlow, et al. (2003). "Effects of intraocular injection of a low concentration of zinc on

the rat retina." Neuropharmacology **45**(5): 637.

<http://www.sciencedirect.com/science/article/B6T0C-4967C3C-3/2/098c4bf023bcb3db69a6a3241ac8fc26>

The main aim of this study was to investigate whether intraocular injection of low concentrations of zinc (no greater than 10 [μM]) aid the survival of ganglion cells in the rat retina after excitotoxic (NMDA) and ischemia/reperfusion injuries. We also determined whether low amounts of zinc cause any detectable retinal toxicity. Intraocular injection of NMDA caused substantial reductions in the mRNA levels of the ganglion cell-specific markers Thy-1 and neurofilament light (NF-L). Co-injection of 0.1 or 1 nmol zinc neither diminished nor exacerbated the effect of NMDA on the levels of these mRNAs. Likewise, ischemia/reperfusion caused significant decreases in the levels of Thy-1 and NF-L mRNAs and in the b-wave amplitude of the electroretinogram. These effects were not counteracted by injection of zinc. Intraocular injection of NMDA caused marked toxicological effects in retinal glial cells, including upregulations of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), glial fibrillary acidic protein (GFAP), basic fibroblast growth factor (FGF-2) and ciliary neurotrophic factor (CNTF). Interestingly, injection of 1 nmol zinc caused no changes in the levels of COX-2 and iNOS, yet produced similar, although quantitatively less pronounced, changes in FGF-2, GFAP and CNTF. The upregulations of FGF-2 and CNTF suggest that increasing zinc intake may benefit injured retinal neurons. However, this was not found to be the case in the present studies, perhaps due to the acute nature of the injury paradigms utilised.

Neuropsychopharmacology (3)

Hua, L. V., M. Green, et al. (2001). "Molecular Cloning of a Novel Isoform of Diphosphoinositol Polyphosphate Phosphohydrolase: A Potential Target of Lithium Therapy." Neuropsychopharmacology **24**(6): 640.

<http://www.sciencedirect.com/science/article/B6T9V-42WG3XJ-6/2/f9cafc9dc75efe04c636e6960d21cb0b>

The mechanisms underlying the therapeutic effects of lithium are largely unknown but may involve progressive adaptive alterations at the level of gene expression. Using differential display PCR, we identify a novel cDNA fragment, the expression of which was increased in the rat frontal cortex after 5 weeks of lithium administration. A full-length cDNA (2954-nt) was cloned by arrayed cDNA library screening, and sequencing of the clone revealed an open reading frame of 537-bp encoding a 179-residue protein. Amino acid sequence comparisons revealed that our clone is a member of the Nudix hydrolase family, with the highest percentage of homology (95%) being with a subtype of human diphosphoinositol polyphosphate phosphohydrolase, hDIPP2. Northern blot analysis revealed that chronic lithium treatment significantly increased rDIPP2 mRNA levels in frontal cortex, but not in hippocampus, midbrain, and cerebellum. The effect of lithium on rDIPP2 mRNA expression was not shared by two other anticonvulsant mood stabilizers, carbamazepine and valproate. Time-course studies showed that 1-week of lithium had no effect on rDIPP2 mRNA abundance in the frontal cortex. Our results suggest that DIPP2 may represent a biologically relevant target of lithium therapy, further supporting the notion that abnormalities in inositol phosphate metabolism may be significant in the pathophysiology and pharmacotherapy of bipolar disorder.

Huang, Y.-y., R. Grailhe, et al. (1999). "Relationship of Psychopathology to the Human Serotonin1B Genotype and Receptor Binding Kinetics in Postmortem Brain Tissue." Neuropsychopharmacology **21**(2): 238.

<http://www.sciencedirect.com/science/article/B6T9V-3WWW9KP-7/2/8d4d81f7a380f359f456daa715e9147c>

Knockout of the 5-HT1B gene in mice results in increased aggression, as well as alcohol and cocaine consumption. Given the clinical association of aggression, suicide, alcoholism, and substance abuse, we studied relationship of psychopathology to the human 5-HT1B receptor gene (N = 178) and postmortem human 5-HT1B receptor binding (N = 96) in the brain. The sample comprised: 71 suicide victims, 107 nonsuicides, 45 with a history of major depression and 79 without, 64 with a history of a alcoholism or substance abuse and 60 without, as well as 36 with a history of pathological aggression and 42 without. Single-strand conformational polymorphism (SSCP) analysis and DNA sequencing techniques were used to screen the coding region of the human 5-HT1B receptor gene in genomic DNA isolated from postmortem human brain tissue. Two common polymorphisms were identified in the 5-HT1B receptor gene, involving a silent C to T substitution at nucleotide 129 and a silent G to C substitution at nucleotide 861 of the coding region. These polymorphisms were found with the same frequency in the suicide and the nonsuicide groups and in those with and without a history of major depression, alcoholism, or pathological aggression. The binding indices (Bmax and KD of the 5-HT1B receptor in prefrontal cortex also did not differ in suicides and controls, major depression, alcoholism, and cases with a history of pathological aggression. The C129 or G861 allele had 20% fewer 5-HT1B receptor compared to the 129T or 861C allele. We did not identify a relationship between suicide, major depression, alcoholism, or pathological aggression with 5-HT1B receptor binding indices or genotype.

Lu, W., L. M. Monteggia, et al. (2002). "Repeated Administration of Amphetamine or Cocaine Does Not Alter AMPA Receptor Subunit Expression in the Rat Midbrain." Neuropsychopharmacology **26**(1): 1.

<http://www.sciencedirect.com/science/article/B6T9V-44NM021-1/2/e1a417cf45fb2ffae21bd179bc8e475a>

We previously reported that ventral tegmental area (VTA) dopamine neurons are supersensitive to AMPA when recorded three days after discontinuing repeated amphetamine or cocaine administration. By increasing dopamine cell activity, this may contribute to the induction of behavioral sensitization. The goal of this study was to determine if increased sensitivity to AMPA reflects increased AMPA receptor expression in the midbrain. Immunolabeling for GluR1, GluR2, GluR2/3, and GluR4 was quantified by immunohistochemistry with 35S-labeled secondary antibodies in VTA, substantia nigra, and a transitional area. First, rats were treated for five days with saline or amphetamine (5 mg/kg) and killed three or 14 days after the last injection. No significant changes in immunolabeling were observed for any subunit at either withdrawal time. GluR1 immunolabeling was further examined in rats killed 16-18 hrs or 24 hrs after a single injection of amphetamine or repeated injections of saline, amphetamine (5 mg/kg x 5 days) or cocaine (20 mg/kg x 7 days). No significant differences were observed in any region. Finally, neither repeated amphetamine or cocaine administration significantly altered GluR1 mRNA levels as quantified by reverse transcriptase-polymerase chain reaction. Our results suggest that enhanced responsiveness of VTA dopamine neurons to AMPA after withdrawal from repeated stimulant administration involves mechanisms more complex than increased expression of AMPA receptor subunits.

Neuroscience (37)

Berghs, C. A. F. M., N. Korteweg, et al. (1999). "Co-expression in *Xenopus* neurons and neuroendocrine cells of messenger RNA homologues of exocytosis proteins DOC2 and munc18-1." Neuroscience **92**(2): 763.

<http://www.sciencedirect.com/science/article/B6T0F-3WKXYXY-14/2/528946f6fe4d80e052ad9e6a6fe37a3c>

The proteins munc18-1 and DOC2 are assumed to play a role in docking of synaptic vesicles in neurotransmitter exocytosis at the presynaptic junction. As the proteins are known to interact, they should co-exist within neurons. We have tested this hypothesis for exocytosis of both classical and peptidergic messengers, by investigating the distribution of the messenger RNAs of munc18-1 and DOC2 homologues in the brain and pituitary gland of the clawed toad *Xenopus laevis*, using in situ hybridization. For this purpose we cloned a partial complementary DNA encoding *Xenopus* unc18 (xunc18) and used a corresponding RNA probe, together with an RNA probe for *Xenopus* DOC2. At the messenger RNA level DOC2 and xunc18 were found to be expressed throughout the *Xenopus* brain. All brain nuclei expressing DOC2-messenger RNA showed xunc18-messenger RNA expression as well. Co-expression was shown at the individual cell level in consecutive sections of large-sized neurons. A strong expression was demonstrated in the suprachiasmatic and magnocellular nuclei and in peptidergic endocrine cells in the intermediate and anterior lobes of the pituitary gland, suggesting roles of DOC2 and xunc18 in messenger release from peptidergic secretory systems. Combined in situ hybridization and immunocytochemical analyses show that neuropeptide Y-containing cells in the suprachiasmatic nucleus also express DOC2 and xunc18 messenger RNAs. Since these cells have a high secretory activity, controlling the activity of the pituitary pars intermedia, the levels of expression of DOC2 and xunc18 may be indicators for neuronal secretory activity. The present data represent the first evidence for the co-existence of DOC2 and munc18-1 and suggest co-ordinate action of these proteins at the level of brain nuclei, individual neurons and endocrine cells.

Boudreau-Lariviere, C., H. Sveistrup, et al. (1996). "Ciliary neurotrophic factor: regulation of acetylcholinesterase in skeletal muscle and distribution of messenger RNA encoding its receptor in synaptic versus extrasynaptic compartments." Neuroscience **73**(2): 613.

<http://www.sciencedirect.com/science/article/B6T0F-3VXNHH5-W/2/167a8ab62f33da5e84fabac03f9fc3ca>

Several recent studies have shown that the ciliary neurotrophic factor exerts myotrophic effects in addition to its well-characterized neurotrophic actions on various neuronal populations. Since expression of acetylcholinesterase in skeletal muscle has been shown to be regulated by putative yet unknown nerve-derived trophic factors, we tested the hypothesis that the ciliary neurotrophic factor is a neurotrophic agent capable of influencing expression of acetylcholinesterase in adult rat skeletal muscle in vivo. To this end, we first determined the impact of daily ciliary neurotrophic factor administration on expression of acetylcholinesterase in both intact and denervated rat soleus muscles. The results of our experiments indicate that although chronic administration of ciliary neurotrophic factor partially counteracted the atrophic response of soleus muscles to surgical denervation, thus confirming its myotrophic effects, it failed to either increase

acetylcholinesterase expression in intact muscles or prevent the decrease normally occurring in seven-day denervated muscles. In fact, acetylcholinesterase messenger RNA and enzyme levels were further reduced by ciliary neurotrophic factor treatment in denervated muscles without significant modifications in the pattern of acetylcholinesterase molecular forms. Conversely, transcript levels of the ϵ subunit of the acetylcholine receptor in intact and denervated soleus muscles treated with the ciliary neurotrophic factor were similar to those observed in their respective counterparts from vehicle-treated animals. In addition, we also determined whether transcripts encoding the receptor for the ciliary neurotrophic factor selectively accumulate in junctional domains of rat skeletal muscle fibres. In contrast to the preferential localization of transcripts encoding acetylcholinesterase and the ϵ subunit of the acetylcholine receptor within the postsynaptic sarcoplasm, messenger RNAs for the ciliary neurotrophic factor receptor appeared homogeneously distributed between junctional and extra-junctional compartments of both diaphragm and extensor digitorum longus muscle fibres, with no compelling evidence for a selective accumulation within the postsynaptic sarcoplasm. These data show that the ciliary neurotrophic factor exerts an inhibitory influence on expression of acetylcholinesterase in muscle fibres. Furthermore, the lack of an effect on expression of the ϵ acetylcholine receptor transcripts indicates that treatment with ciliary neurotrophic factor does not lead to general adaptations in the expression of all synaptic proteins. Given the distribution of transcripts encoding the ciliary neurotrophic factor receptor along multinucleated muscle fibres, we propose a model whereby the ciliary neurotrophic factor, or a related unknown molecule that also utilizes the receptor for the ciliary neurotrophic factor, contributes to the maintenance of low levels of enzyme activity in extra-junctional regions of muscle fibres by acting as a repressor of acetylcholinesterase expression that functions directly or indirectly via a pretranslational regulatory mechanism. Accordingly, these results further highlight the complexity of the regulatory mechanisms presiding over acetylcholinesterase expression *in vivo*.

Bouret, S., D. Croix, et al. (2002). "Galanin modulates the activity of proopiomelanocortin neurons in the isolated mediobasal hypothalamus of the male rat." *Neuroscience* **112**(2): 475.

<http://www.sciencedirect.com/science/article/B6T0F-45Y6RG0-19/2/f3034fe5fb1b4ebba9c0fd59d62e7aa>

It has become apparent that galanin as well as proopiomelanocortin-derived peptides, such as [beta]-endorphin, play an important role in the hypothalamic circuitry that regulates neuroendocrine functions and appetite behavior. We have recently shown that GalR1 and GalR2 galanin receptor mRNAs are expressed in proopiomelanocortin neurons of the arcuate nucleus, suggesting a direct modulatory action of galanin on the proopiomelanocortin neuronal system. In the present study, we investigated the effect of galanin on [beta]-endorphin release and proopiomelanocortin mRNA expression from male rat mediobasal hypothalamic fragments incubated *ex vivo*. Galanin induced a decrease of spontaneous [beta]-endorphin release within the first 30-60 min of incubation and this effect was blocked by the galanin receptor antagonist galantide. Co-incubation of galanin with FK-506 (tacrolimus), a calcineurin inhibitor, suppressed the inhibitory effect of galanin on [beta]-endorphin release, suggesting that calcineurin is involved in the galanin-evoked decrease in [beta]-endorphin release. Measurement of [beta]-endorphin levels in the tissues at the end of the incubation period (120 min) revealed that galanin caused a two-fold increase of [beta]-endorphin peptide concentration in the mediobasal hypothalamic tissues. Concurrently, galanin induced an increase in the mean density of silver grains overlying proopiomelanocortin neurons after 60 min of incubation, an effect antagonized by galantide. Finally, reverse transcription-polymerase chain reaction analysis revealed that the mRNAs for the three galanin receptor subtypes (i.e. GalR1, GalR2, and GalR3) were expressed in the incubated mediobasal hypothalamic fragments. Taken as a whole, our results indicate that galanin plays a modulatory role on proopiomelanocortin neurons and this interrelation contributes to the elucidation of the neural circuitry that controls, among others, gonadotropin-releasing hormone function.

Brauer, A. U., N. E. Savaskan, et al. (2003). "Cholecystokinin expression after hippocampal deafferentiation: molecular evidence revealed by differential display-reverse transcription-polymerase chain reaction." *Neuroscience* **121**(1): 111.

<http://www.sciencedirect.com/science/article/B6T0F-497YTGN-1/2/cf7c4ad055c398113e637dd8b0a5843c>

The cortical information flow via the perforant path represents a major excitatory projection to the hippocampus. Lesioning this projection leads to massive degeneration and subsequently to reorganization in its termination zones as well as in primary non-affected subfields of the hippocampus. The molecular mechanisms and factors which are involved in the postlesional events are poorly defined. Using a differential display reverse transcription-polymerase chain reaction (DDRT-PCR) strategy, we located one band which occurred only in control hippocampus lanes and almost disappeared in the lanes of lesioned hippocampi. By sequencing, we identified the corresponding gene as cholecystokinin (CCK). Northern blot analysis confirmed a decreased transcription of CCK after lesion. In situ hybridization analysis was performed for localization and quantification of altered CCK transcription. We noted a significant downregulation of CCK transcription in the hippocampus (20%) and in the contralateral cortex (12%) 1-day after lesion (dal) and an increased signal in the ipsilateral cortex (10.5%). This pattern was altered, showing upregulation of CCK mRNA expression, reaching its highest level of 70% above control levels at 5 dal. In the hippocampus, the control level was reached again at 21 dal, whereas the cortex reached the control level at 10 dal. In comparison, the mRNA transcripts of the receptors CCKA and CCKB remained unchanged. Since CCK-containing neurons are involved in the modulation of pyramidal and granule cell excitability, our data indicate a time course correlation between CCK mRNA expression and postlesional axonal sprouting response in the hippocampus.

Burvenich, S., K. Unsicker, et al. (1998). "Calcitonin gene-related peptide promotes differentiation, but not survival, of rat mesencephalic dopaminergic neurons in vitro." *Neuroscience* **86**(4): 1165.

<http://www.sciencedirect.com/science/article/B6T0F-3TN4YT0-1H/2/7e2dba0c46cc10b3755444b4885619bb>

The aim of this study was to investigate putative effects of calcitonin gene-related peptide on developing dopaminergic neurons in the ventral mesencephalon. To determine a time-point for a physiological role of calcitonin gene-related peptide in the development of this system, we first investigated calcitonin gene-related peptide messenger RNA expression in the ventral mesencephalon of Wistar rats at embryonic days (E) 11-19. Calcitonin gene-related peptide messenger RNA was not detectable at E11, i.e. prior to the appearance of dopaminergic neurons in this area. From E14 to E19, calcitonin gene-related peptide messenger RNA was expressed in increasing amounts. We therefore investigated the effects of calcitonin gene-related peptide on serum-free cell cultures established from the E14 midbrain floor. Addition of calcitonin gene-related peptide (200 ng/ml) every other day significantly increased neuronal differentiation, including longer tyrosine hydroxylase-positive neurites, enhanced immunoreactivity for growth-associated protein-43 and increased dopaminergic uptake per neuron. These effects were maximal after seven to eight days. Calcitonin gene-related peptide acted synergistically with fibroblast growth factor-2 on these parameters. In contrast to fibroblast growth factor-2, however, calcitonin gene-related peptide did not promote survival of tyrosine hydroxylase-immunoreactive neurons. Lack of calcitonin gene-related peptide expression in the mesencephalon at E11 was paralleled by a lack of effect of calcitonin gene-related peptide on early presumptive dopaminergic neurons in terms of eliciting this phenotype. Our data suggest that calcitonin gene-related peptide may act physiologically as a differentiation-promoting factor for phenotypically defined dopaminergic neurons during a time period when dopaminergic neurons assemble in the

ventral mesencephalon and grow axons towards their targets.

Butler, M., C. S. Hayes, et al. (2005). "Spinal distribution and metabolism of 2'-O-(2-methoxyethyl)-modified oligonucleotides after intrathecal administration in rats." *Neuroscience* **131**(3): 705.

<http://www.sciencedirect.com/science/article/B6T0F-4FDMYB3-4/2/7824b58cebb7df0d9017755894a31a00>

Intrathecal (IT) delivery of antisense oligodeoxynucleotides (ASO) has been used to study the function of specific gene products in spinal nociception. However, a lack of systematic studies on the spinal distribution and kinetics of IT ASO is a major hurdle to the utilization of this technique. In the present study, we injected rats IT with 2'-O-(2-methoxyethyl) modified phosphorothioate ASO (2'-O-MOE ASO) and examined anatomical and cellular location of the ASO in the spinal cord and dorsal root ganglia (DRG) by immunocytochemistry. At 0.5 h after a single IT injection, immunostaining for ISIS 13920 (a 2'-O-MOE ASO targeting h-ras) localized superficially in the lumbar spinal cord, while at 24 h the immunostaining was distributed throughout the spinal cord and was predominantly intracellular. Double staining with cell type specific antibodies indicated that the ASO was taken up by both glia and neurons. ASO immunoreactivity was also observed in DRG after IT ISIS 13920. Capillary gel electrophoresis analysis showed that ISIS 22703, a 2'-O-MOE ASO targeting the [alpha] isozyme of protein kinase C (PKC), remained intact in spinal cord tissue and cerebrospinal fluid up to 24 h after the injection and no metabolites were detected. In contrast, after IT ISIS 11300, an unmodified phosphorothioate ASO with the same sequence as ISIS 22703, no full-length compound was detectable at 24 h, and metabolites were seen as early as 0.5 h. IT treatment with ISIS 22703 at doses that effectively down-regulated PKC[alpha] mRNA in spinal cord did not affect the mRNA expression in DRG. In summary, 2'-O-MOE ASO displayed high stability in spinal tissue after IT delivery, efficiently distributed to spinal cord, and internalized into both neuronal and non-neuronal cells. ASO are able to reach DRG after IT delivery; however, higher doses may be required to reduce target gene in DRG as compared with spinal cord.

Calver, A. R., A. D. Medhurst, et al. (2000). "The expression of GABAB1 and GABAB2 receptor subunits in the CNS differs from that in peripheral tissues." *Neuroscience* **100**(1): 155.

<http://www.sciencedirect.com/science/article/B6T0F-416BVJ9-G/2/01856ed5e168001d63c775b3dbeab972>

GABAB receptors are G-protein-coupled receptors that mediate the slow and prolonged synaptic actions of GABA in the CNS via the modulation of ion channels. Unusually, GABAB receptors form functional heterodimers composed of GABAB1 and GABAB2 subunits. The GABAB1 subunit is essential for ligand binding, whereas the GABAB2 subunit is essential for functional expression of the receptor dimer at the cell surface. We have used real-time reverse transcriptase-polymerase chain reaction to analyse expression levels of these subunits, and their associated splice variants, in the CNS and peripheral tissues of human and rat. GABAB1 subunit splice variants were expressed throughout the CNS and peripheral tissues, whereas surprisingly GABAB2 subunit splice variants were neural specific. Using novel antisera specific to individual GABAB receptor subunits, we have confirmed these findings at the protein level. Analysis by immunoblotting demonstrated the presence of the GABAB1 subunit, but not the GABAB2 subunit, in uterus and spleen. Furthermore, we have shown the first immunocytochemical analysis of the GABAB2 subunit in the brain and spinal cord using a GABAB2-specific antibody. We have, therefore, identified areas of non-overlap between GABAB1 and GABAB2 subunit expression in tissues known to contain functional GABAB receptors. Such areas are of interest as they may well contain novel GABAB receptor subunit isoforms, expression of which would enable the

GABAB1 subunit to reach the cell surface and form functional GABAB receptors.

Champagne, D., D. Pearson, et al. (2003). "The cholesterol-lowering drug Probucol increases apolipoprotein e production in the hippocampus of aged rats: implications for Alzheimer's disease." Neuroscience **121**(1): 99.

<http://www.sciencedirect.com/science/article/B6T0F-4991NC7-1/2/bfc5d4fcd9c069033c42a3bcf0c32f13>

Several recent epidemiological studies have proposed that cholesterol-lowering drug Statin may provide protection against Alzheimer's disease (AD). Probucol is a non-Statin cholesterol-lowering drug and a potent inducer of apolipoprotein E (apoE) production in peripheral circulation. A recent clinical study using Probucol in elderly AD subjects revealed a concomitant stabilisation of cognitive symptoms and significant increases in apoE levels in the cerebral spinal fluid in these patients. To gain insight into the mechanisms underlying these effects, we treated a cohort of aged male rats (26-month-old) with oral dose of Probucol for 30 days. Specifically, we examined the effects of Probucol on apoE production and its receptors (low density lipoprotein receptor [LDLr] and low density lipoprotein receptor-related protein [LRP]), astroglial marker of cell damage (glial fibrillary acidic protein [GFAP]), markers of neuronal synaptic plasticity and integrity (synaptosomal associated protein of 25 kDa [SNAP-25] and synaptophysin) as well as cholesterol biosynthesis (3-hydroxy-3-methylglutaryl coenzyme A reductase [HMGCoAr]) in the hippocampus. We report that Probucol induces the production of apoE and one of its main receptors, LRP, increases HMGCoAr (rate-limiting enzyme in cholesterol synthesis), substantially attenuates age-related increases in glial activation, and induces production of synaptic marker SNAP-25, a molecule commonly associated with synaptogenesis and dendritic remodeling. These findings suggest that Probucol could promote neural and synaptic plasticity to counteract the synaptic deterioration associated with brain aging through an apoE/LRP-mediated system. Consistent with the beneficial effects of other cholesterol-lowering drugs such as the Statin, Probucol could also offers additional benefits based on apoE neurobiology.

Craig, C. G., R. D'sa, et al. (1999). "Migrational analysis of the constitutively proliferating subependyma population in adult mouse forebrain." Neuroscience **93**(3): 1197.

<http://www.sciencedirect.com/science/article/B6T0F-3XMGMXY-1B/2/c0e7e3e6a29f4ac49d73d9fbfdcb137d>

Initial experiments to evaluate the in vivo fate(s) of constitutively proliferating subependymal cells determined that, following in vivo labeling of this population by infection with a retrovirus containing a [beta]-galactosidase reporter gene, there was a progressive and eventually complete loss of histochemically [beta]-galactosidase-positive cells within the lateral ventricle subependyma with increasing survival times of up to 28 days after retroviral infection. Subsequent experiments were designed to ascertain the potential contributions of: (i) the migration of subependymal cells away from the forebrain lateral ventricles; and (ii) the down-regulation of the retroviral reporter gene expression. Retroviral lineage tracing experiments demonstrate that a major in vivo fate for constitutively proliferating subependymal cells is their rostral migration away from the walls of the lateral ventricle to the olfactory bulb. Although down-regulation of retroviral reporter gene expression does not contribute to the loss of detection of [beta]-galactosidase-labeled cells from the lateral ventricle subependyma, it does result in an underestimation of the absolute number of retrovirally labeled cells in the olfactory bulb at longer survival times. Furthermore, a temporal decrease in the double labeling of [beta]-galactosidase-labeled cells with [3H]thymidine was observed, indicating that only a subpopulation of the migratory subependymal-derived cells continue to actively proliferate en route to the olfactory

bulb. These two events may contribute to the lack of a significant increase in the total number of retrovirally labeled subependymal cells during rostral migration. Evidence from separately published studies suggests that cell death is also an important regulator of the size of the constitutively proliferating subependymal population. In summary, in vivo studies utilizing retroviral reporter gene labeling demonstrate that constitutively proliferating subependymal cells born in the lateral ventricle migrate rostrally to the olfactory bulb. Loss of proliferation potential and retroviral reporter gene down-regulation contribute to the lack of any significant increase in the total number of labeled cells recovered in the olfactory bulb.

Daadi, M., M. Y. Arcellana-Panlilio, et al. (1998). "Activin co-operates with fibroblast growth factor 2 to regulate tyrosine hydroxylase expression in the basal forebrain ventricular zone progenitors." Neuroscience **86**(3): 867.

<http://www.sciencedirect.com/science/article/B6T0F-3TN4YT0-H/2/c2b7d4ba6ebae5d94fb2929631f2482>

Activin and its cognate receptors are expressed during embryogenesis in the rapidly dividing cells of the basal forebrain ventricular zone. This finding prompted us to study the role of activin in regulating neurotransmitter phenotype expression and other aspects of the ventricular zone-derived progenitor cell differentiation. Although virtually ineffective alone, activin co-operated with fibroblast growth factor 2 to induce a rapid tyrosine hydroxylase-immunoreactivity in cultured ventricular zone progenitors. Northern analysis indicated that the increase in tyrosine hydroxylase-immunoreactivity was associated with increased tyrosine hydroxylase gene expression. Activin and fibroblast growth factor 2 action was specific to tyrosine hydroxylase, as it did not induce the expression of choline acetyltransferase, nor enhance the expression of glutamate decarboxylase. Cultures treated with the DNA replication marker bromodeoxyuridine revealed that both proliferating ventricular zone progenitors and their post-mitotic progeny were induced to express tyrosine hydroxylase. In these cultures, activin acted to reduce fibroblast growth factor 2 stimulated mitotic activity. Furthermore, activin permitted neuronal differentiation and survival of the ventricular zone progenitors after three days in vitro. Together these data demonstrate a novel role of activin and fibroblast growth factor 2 in regulating the fate of the embryonic basal forebrain ventricular zone progenitors.

Elliott, J. M., N. R. Newberry, et al. (1995). "Characterization of the 5-hydroxytryptamine_{2A} receptor-activated cascade in rat c6 glioma cells." Neuroscience **69**(4): 1119.

<http://www.sciencedirect.com/science/article/B6T0F-4007BN5-G/2/6ae26b062832279ed9285c3d5c3fab79>

We have investigated the identity and intracellular cascade of responses resulting from activation of the endogenous 5-hydroxytryptamine receptor in the C6 rat glioma cell line. Sequence analysis of reverse transcription-polymerase chain reaction products derived from C6 glioma cell messenger RNA revealed complete homology with a portion of the rat 5-hydroxytryptamine_{2A} receptor. The binding of [³H]ketanserin to cell membranes demonstrated a significant correlation with the 5-hydroxytryptamine_{2A} receptor in rat frontal cortex. On intact cells, 5-hydroxytryptamine stimulated a concentration-dependent increase in phosphatidyl inositide turnover and intracellular [Ca²⁺] mediated by 5-hydroxytryptamine_{2A} receptors. In whole-cell patch-clamp recordings, 5-hydroxytryptamine induced an outward current mediated predominantly by K⁺ ions (reversal POTENTIAL = -80 mV). Using caged molecules containing Ca²⁺ or inositol 1,4,5-trisphosphate in the patch electrode solution, we found that rapid photolytic release of Ca²⁺ and particularly inositol 1,4,5-trisphosphate within the cytosol induced an outward current with characteristics similar to those seen after application of 5-hydroxytryptamine. Comparison between differentiated

and undifferentiated cells revealed significantly higher receptor density and maximal phosphoinositide response to 5-hydroxytryptamine in undifferentiated cells but the associated rise in $[Ca^{2+}]_i$ and activation of an outward current was observed more frequently in differentiated cells. Prolonged exposure of the cells to 5-hydroxytryptamine led to a decrease in all responses and to the down-regulation of receptor number. We conclude that the rat C6 glioma cell expresses a 5-hydroxytryptamine_{2A} receptor identical to that found in rat brain and that stimulation of the receptor in C6 cells leads to the activation of Ca^{2+} activated K^+ channels via phosphoinositide hydrolysis and subsequent rise in cytosolic Ca^{2+} ion concentration. However, the contrasting effects of differentiation on receptor number and phosphoinositide response to 5-hydroxytryptamine compared to Ca^{2+} release and conductance change indicate that a complex relationship exists between the component parts of the receptor-activated cascade.

Facchinetti, F., M. Sasaki, et al. (1999). "Lack of involvement of neuronal nitric oxide synthase in the pathogenesis of a transgenic mouse model of familial amyotrophic lateral sclerosis." *Neuroscience* **90**(4): 1483.

<http://www.sciencedirect.com/science/article/B6T0F-3W4B16B-13/2/41990c02b42ccd082785d0f33462458e>

A subset of familial cases of amyotrophic lateral sclerosis are linked to missense mutations in copper/zinc superoxide dismutase type 1. Patients with missense mutations in copper/zinc superoxide dismutase type 1 develop a paralytic disease indistinguishable from sporadic amyotrophic lateral sclerosis through an unknown toxic gain of function. Nitric oxide reacts with the superoxide anion to form the strong oxidant, peroxynitrite, which participates in neuronal injury in a variety of model systems. Peroxynitrite is an alternate substrate for copper/zinc superoxide dismutase type 1, causing catalytic nitration of tyrosine residues in other proteins. Mutations in copper/zinc superoxide dismutase type 1 may disrupt the active site of the enzyme and permit greater access of peroxynitrite to copper, leading to increased nitration by peroxynitrite of critical cellular targets. To investigate whether neuronal-derived nitric oxide plays a role in the pathogenesis of familial amyotrophic lateral sclerosis, we examined the effects of three different nitric oxide synthase inhibitors: a non-selective nitric oxide synthase inhibitor, nitro-arginine methyl ester; a relatively selective inhibitor of neuronal nitric oxide synthase, 7-nitroindazole; and a novel highly selective neuronal nitric oxide synthase inhibitor, AR-R 17,477, in transgenic mice expressing a familial amyotrophic lateral sclerosis-linked mutant human copper/zinc superoxide dismutase type 1 (Gly->Ala at position 93; G93A) containing a high transgene copy number and a low transgene copy number. AR-R 17,477, but not nitro-arginine methyl ester or 7-nitroindazole, significantly prolonged survival in both the high and low transgene transgenic mice. To determine whether neuronal nitric oxide synthase is involved in the pathogenesis resulting from the familial amyotrophic lateral sclerosis copper/zinc superoxide dismutase type 1 mutation, we produced mice with the copper/zinc superoxide dismutase type 1 mutation which lack the neuronal nitric oxide synthase gene. The transgenic mice expressing a familial amyotrophic lateral sclerosis-linked mutant human copper/zinc superoxide dismutase type 1 on neuronal nitric oxide synthase null background do not live significantly longer than transgenic mice expressing a familial amyotrophic lateral sclerosis-linked mutant human copper/zinc superoxide dismutase type 1. Western blot analysis indicates the presence of two neuronal nitric oxide synthase-like immunoreactive bands in spinal cord homogenates of the neuronal nitric oxide synthase null mice, and residual neuronal nitric oxide synthase catalytic activity (>7%) is detected in the spinal cord of the transgenic mice expressing a familial amyotrophic lateral sclerosis-linked mutant human copper/zinc superoxide dismutase type 1 on neuronal nitric oxide synthase null background. This amount of residual activity probably does not account for lack of protection afforded by the disrupted neuronal nitric oxide synthase gene in the familial amyotrophic lateral sclerosis-linked mutant human copper/zinc superoxide dismutase type 1 mice. Immunological nitric oxide synthase is not detected in the copper/zinc superoxide dismutase type 1 mutant mice at several different ages, thus excluding immunological nitric oxide

synthase as a contributor to the pathogenesis of familial amyotrophic lateral sclerosis. Levels of neuronal nitric oxide synthase as well as Ca²⁺-dependent nitric oxide synthase catalytic activity in the copper/zinc superoxide dismutase type 1 mutant mice do not differ from wild type mice. Endothelial nitric oxide synthase levels may be decreased in the copper/zinc superoxide dismutase type 1 mutant mice. Together, these results do not support a significant role for neuronal-derived nitric oxide in the pathogenesis of familial amyotrophic lateral sclerosis transgenic mice.

Franke, H., J. Grosche, et al. (2001). "P2X receptor expression on astrocytes in the nucleus accumbens of rats." Neuroscience **108**(3): 421.

<http://www.sciencedirect.com/science/article/B6T0F-44SHC0J-7/2/6c4cc8de5b80ab05383c962971f620b8>

Astrocytes express a variety of neurotransmitter receptors which render them capable of responding to extracellular stimuli, like ATP. Release of ATP, e.g. after brain injury, may initiate reactive gliosis via stimulation of purinergic P2X and P2Y receptors. In the present study, the expression and cellular localization of P2X receptor subtypes on astrocytes in the nucleus accumbens of rats under normal physiological conditions and after stab wound were investigated. Reverse transcription-polymerase chain reaction (RT-PCR) with specific P2X1-7 primers, and double immunofluorescence with antibodies to glial fibrillary acidic protein (GFAP, a specific marker of fibrous astrocytes) and to different P2X receptor subtypes (P2X1-4, P2X7) were used. The RT-PCR of tissue extracts of the nucleus accumbens of untreated rats revealed the presence of all seven currently known P2X receptor subtype mRNAs indicating the presence of these receptors in this region. A double immunofluorescence approach with confocal laser scanning microscopy showed the localization of P2X2-4 receptor subtypes on GFAP-labelled astrocytes in untreated rats. Labelling for P2X1 and P2X7 receptor subtypes was not found. After mechanical damage all P2X receptor subtypes studied (P2X1-4, P2X7) were observed on the GFAP-labelled reactive astrocytes. A characteristic distribution of the P2X receptors on astrocytic processes and cell bodies as well as an up-regulation of the P2X-immunofluorescence was found. In conclusion, the data show the presence of P2X receptors on rat nucleus accumbens astrocytes and suggest that astrogliosis in vivo is associated with an up-regulation of distinct P2X receptor subtypes.

Goldstein, L. B., M. P. Vitek, et al. (2000). "Expression of the Apolipoprotein E Gene Does Not Affect Motor Recovery After Sensorimotor Cortex Injury in the Mouse." Neuroscience **99**(4): 705.

<http://www.sciencedirect.com/science/article/B6T0F-414N4JJ-D/2/029a87c3d9ea9c46f660112dea8761b9>

Motor recovery after unilateral sensorimotor cortex ablation or sham-injury was measured in apolipoprotein E knockout and wild-type mice by testing their abilities to traverse a narrow beam. All mice trained without difficulty. Sham-operated mice performed perfectly regardless of genotype throughout testing. There was no difference in motor scores between lesioned apolipoprotein E knockout and wild-type mice on a first trial 24 h after injury ($P > 0.05$). There was a significant overall effect of lesion on motor performance (two-way repeated measures analysis of variance $F_{1,42} = 304$, $PF_{17,714} = 58$, $PF_{17,714} = 58$, $PF_{17,714} = 0.33$, $P = 1.00$) and no effect of genotype on the final level of motor performance 12 days after the lesion (Kruskal-Wallis $H = 5.79$, $P = 0.12$). These data suggest that motor recovery after unilateral injury to the sensorimotor cortex does not vary with apolipoprotein E genotype.

Harrison, D. C., J. Roberts*, et al. (2000). "TR3 death receptor expression in the normal and ischaemic brain." Neuroscience **96**(1): 147.

<http://www.sciencedirect.com/science/article/B6T0F-3YKKDB8-M/2/ac446dce4f1d01fe987a8864f0facab9>

Members of the death receptor family may play a prominent role in developmental and pathological neuronal cell death. We report the expression of the TR3 and TR7 death receptors in the adult human and rat central nervous system. Whereas expression of TR3 appears to be high in the human cerebellum, with lower levels in other brain regions, robust expression is observed in many regions of the rat brain. We also analysed modulation of death receptor expression in an in vivo rat model of acute stroke. In contrast to tumour necrosis factor receptor 1, Fas and p75NGFR, which all show up-regulation specifically in lesioned cortex of the permanent middle cerebral artery occlusion model of stroke, TR3 shows a rapid global increase in both lesioned and unlesioned brain. In comparison, the recently described death receptor TR7 shows no change in this model. These data indicate that the death receptors show clear differences in patterns of expression in response to ischaemic injury.

Hervieu, G. J., J. E. Cluderay, et al. (2001). "Gene expression and protein distribution of the orexin-1 receptor in the rat brain and spinal cord." Neuroscience **103**(3): 777.

<http://www.sciencedirect.com/science/article/B6T0F-4320M92-H/2/5575cda6eec1b9898d491a61826269b9>

Orexins-A and -B are neuropeptides derived from a single precursor prepro-orexin. The mature peptides are mainly expressed in the lateral hypothalamic and perifornical areas. The orexins have been implicated in the control of arousal and appear to be important messengers in the regulation of food intake. Two receptors for orexins have been characterised so far: orexin-1 and -2 receptors. To gain a further understanding of the biology of orexins, we studied the distribution of the orexin-1 receptor messenger RNA and protein in the rat nervous system. We first assessed the expression profile of the orexin-1 receptor gene (ox-r1) in different regions by using quantitative reverse transcription followed by polymerase chain reaction. Using immunohistochemical techniques, we investigated the distribution of orexin-1 receptor protein in the rat brain using a rabbit affinity-purified polyclonal antiserum raised against an N-terminal peptide. The orexin-1 receptor was widely and strongly expressed in the brain. Thus, immunosignals were observed in the cerebral cortex, basal ganglia, hippocampal formation, and various other subcortical nuclei in the hypothalamus, thalamus, midbrain and reticular formation. In particular, robust immunosignals were present in many hypothalamic and thalamic nuclei, as well as in the locus coeruleus. The distribution of the receptor protein was generally in agreement with the distribution of the receptor messenger RNA in the brain as reported previously by others and confirmed in the present study. In addition, we present in situ hybridisation and immunohistochemical data showing the presence of orexin-1 receptor messenger RNA and protein in the spinal cord and the dorsal root ganglia. Finally, due to the shared anatomical and functional similarities between orexins and melanin-concentrating hormone, we present a comparison between the neuroanatomical distribution of the orexin-1 receptor and melanin-concentrating hormone receptor protein-like immunoreactivities in the rat central nervous system, and discuss some functional implications. In conclusion, our neuroanatomical data are consistent with the biological effects of orexins on food intake and regulation of arousal. In addition, the data suggest other physiological roles for orexins mediated through the orexin-1 receptor.

Hua, X. Y., A. Moore, et al. (2002). "Inhibition of spinal protein kinase C[alpha] expression by an antisense oligonucleotide attenuates morphine infusion-induced tolerance." Neuroscience **113**(1):

<http://www.sciencedirect.com/science/article/B6T0F-4684HBY-B/2/c9729d4c1d1dd125631d8f6ebbb1f38c>

Protein kinase C isoforms including the α isozyme have been implicated in morphine tolerance. In the present study, we examined the effect of intrathecal delivery of an antisense oligonucleotide targeting rat protein kinase C α mRNA on the expression of spinal protein kinase C α isozyme and spinal morphine tolerance. Continuous intrathecal infusion of rats with morphine produced an increase in paw withdrawal threshold to thermal stimulation on day 1, which disappeared by day 5. On day 6, a bolus intrathecal injection of morphine (a probe dose) produced significantly less analgesia in morphine-infused rats than in saline-infused rats, suggesting tolerance. Intrathecal treatment with the protein kinase C α antisense concurrent with spinal morphine infusion not only maintained the analgesic effect of morphine during the 5-day infusion, it also significantly increased responsiveness to the probe morphine dose on day 6. In comparison, the missense used in the same treatment paradigm had no effect. The inhibitory effect of protein kinase C α antisense on spinal morphine tolerance was dose-dependent, and reversible. Intrathecal treatment with the antisense, but not the missense, in rats decreased expression of spinal protein kinase C α mRNA and protein, as revealed by real-time quantitative reverse transcription-polymerase chain reaction and western blots. Expression of the γ isozyme was not affected by the oligonucleotides. The antisense also attenuated protein kinase C-mediated phosphorylation in spinal cord. These results demonstrate that selective reduction in the expression of the spinal protein kinase C α isozyme followed by a decrease of local protein kinase C-mediated phosphorylation will reverse spinal morphine infusion-induced tolerance. This finding is consistent with the view that tolerance produced by morphine infusion is dependent upon an increase in phosphorylation by protein kinase C, and also it emphasizes that the protein kinase C α isozyme and its activation in spinal cord may specifically participate in the phenomenon of opiate tolerance.

Imboden, M., V. Devignot, et al. (2001). "Regional distribution of glycine receptor messenger RNA in the central nervous system of zebrafish." *Neuroscience* **103**(3): 811.

<http://www.sciencedirect.com/science/article/B6T0F-4320M92-K/2/77823ae56668aa81f640edb2cb7e3295>

We report the cloning of the zebrafish β subunit of the glycine receptor and compare the anatomical distribution of three glycine receptor subunit constituents in adult zebrafish brain (α 1, α 2 and β) to the expression pattern of homologous receptor subunits (α 1, α 2 and β) in the mammalian adult CNS. Non-radioactive hybridization was used to map the distribution of the α 1, α 2 and β glycine receptor subunit messenger RNAs in the adult zebrafish brain. The anterior-posterior expression gradient found in adult zebrafish brain was similar to that reported in mammalian CNS. However, the glycine receptor transcripts, notably the α 1 subunit, were more widely distributed in the anterior regions of the zebrafish than in the adult mammalian brain. The isoform-specific distribution pattern was less regionalized in zebrafish than in the rat mammalian CNS. Nevertheless, there was some regionalization of α 1, α 2 and β transcripts in the diencephalic and mesencephalic nuclei where different sensory and motor centers express either α 1/ β or α 2 subunits. In contrast to the widespread distribution of the β subunit in adult mammalian brain, α 2 messenger RNA presented the widest expression territory of all three glycine receptor subunits tested. α 2 messenger RNA was expressed in the absence of α 1 and β messenger RNA in the outer nuclear layer of the retina, the inferior olive and the raphe of the medulla oblongata, as well as in the nucleus of Cajal of the medulla spinalis. In contrast, an identified central neuron of the reticular formation, the Mauthner cell, expresses all three glycine receptor subunits (α 1, α 2 and β). This report extends the already

described glycine receptor expression in the vertebrate CNS and confirms the importance of glycine-mediated inhibition in spinal cord and brainstem.

Itoh, K., M. Watanabe, et al. (2004). "Magnetic resonance and biochemical studies during pentylenetetrazole-kindling development: The relationship between nitric oxide, neuronal nitric oxide synthase and seizures." *Neuroscience* **129**(3): 757.

<http://www.sciencedirect.com/science/article/B6T0F-4DN9GV6-6/2/1697f473a08f6b77b351a6f734fbe94c>

The major aim of this study was to elucidate the role of nitric oxide (NO) in the development of pentylenetetrazole (PTZ)-kindling as an animal model of primary generalized epilepsy. The daily administration of PTZ is associated with an increase in the amount of neuronal nitric oxide synthase (nNOS). NO generation was measured directly by in vivo and ex vivo electron paramagnetic resonance on rodents undergoing progressive convulsions. We found that primary generalized epilepsy is caused by NO induction during the persistent up-regulation of nNOS expression, but that NO induction is not associated with severe generalized seizures following long-term kindling phenomena after PTZ withdrawal. Morphological changes in the brain structure of rats were measured by magnetic resonance imaging during epileptic convulsions induced by repetitive administration of PTZ. Cerebellum volume for kindled rats decreased 20% but not in rats treated with the nNOS inhibitor, 3Br-7NI, suggesting that generation of NO in the cerebellum is related to decrease in cerebellum volume following PTZ-kindling.

Kanayama, H., O. Yasuhara, et al. (2003). "Expression of a splice variant of choline acetyltransferase in magnocellular neurons of the tuberomammillary nucleus of rat." *Neuroscience* **118**(1): 243.

<http://www.sciencedirect.com/science/article/B6T0F-485P6CP-4/2/927ab77d13d8a8866758f6cd993a6239>

A splice variant of choline acetyltransferase mRNA has recently been identified in the pterygopalatine ganglion of rat. An antibody against this variant protein (designated pChAT) was demonstrated to immunolabel peripheral cholinergic neurons. In the present study, we investigated the expression of pChAT in rat brain. Amongst the brain regions examined, magnocellular neurons in the tuberomammillary nucleus of the posterior hypothalamus were immunohistochemically labelled with anti-pChAT antibody, whilst no immunolabelling was detected in cholinergic neurons in the basal forebrain or striatum. RT-PCR analysis confirmed the expression of pChAT mRNA in the posterior hypothalamus. The distribution of pChAT-positive neurons in the tuberomammillary nucleus was compared with that of neurons positive for adenosine deaminase, which is contained in all neurons of this nucleus. After colchicine treatment to inhibit axonal transport of enzyme, virtually all pChAT-positive cells contained adenosine deaminase. Conversely, about 85% of adenosine deaminase-positive cells contained pChAT in the ventral area, whilst 19% of adenosine deaminase-positive cells were pChAT-positive in the dorsal area. Long axonal projections of pChAT-positive cells in the tuberomammillary nucleus were shown by retrograde labelling of these cells after injection of cholera-toxin B subunit into the cerebral cortex. This study demonstrates that a splice variant of choline acetyltransferase is expressed in the tuberomammillary nucleus of rat. The results raise the possibility that some of the known diverse projection areas of this nucleus may have a cholinergic component.

Kolk, S. M., A. J. A. Groffen, et al. (2004). "Differential distribution and regulation of expression of synaptosomal-associated protein of 25 kDa isoforms in the *Xenopus* pituitary gland and brain." *Neuroscience* **128**(3): 531.

<http://www.sciencedirect.com/science/article/B6T0F-4D75JXJ-9/2/f8a4db4f4a3c7bf59625feb124ba465>

Synaptosomal-associated protein of 25 kDa (SNAP-25) regulates various membrane fusion processes including exocytosis by endocrine and neural cells. To increase our understanding of the occurrence and regulation of SNAP-25 isoforms, we identified and characterized SNAP-25a and SNAP-25b mRNAs in the pituitary gland and brain of the amphibian *Xenopus laevis*. The proteins are strongly conserved and are resistant to botulinum neurotoxin A but not to botulinum neurotoxin E, as shown by Western blotting. The spatial distribution of the two SNAP-25 isoforms was assessed with in situ hybridization. Both SNAP-25a mRNA and SNAP-25b mRNA reside in cells in the pituitary distal lobe and, particularly, in the endocrine melanotrope cells in the pituitary intermediate lobe. The melanotrope cells are involved in the background adaptation process of the skin by releasing [alpha]-melanophore-stimulating hormone. Quantitation of the respective in situ hybridization signals in the *Xenopus* pars intermedia indicated a differential response, SNAP-25b mRNA being more highly expressed in black-adapted animals than SNAP-25a mRNA, and more than in white-adapted toads. This differential upregulation was also studied by real-time reverse transcriptase polymerase chain reaction, showing that in the intermediate pituitary lobe, both isoforms are physiologically controlled by the background light intensity stimulus, but with different intensities; in black-adapted animals SNAP-25b mRNA is upregulated by 3.33 times compared with white-adapted animals, but SNAP-25a only by 1.96 times. As to neural tissue, in situ hybridization showed that both isoforms coexist throughout the brain, sometimes with similar strengths, but in various areas either SNAP-25a mRNA or SNAP-25b mRNA expression is prevalent. It is speculated that each of the SNAP-25 isoforms in the *Xenopus* pituitary and brain has a distinct function in cellular fusion processes including secretion, and that their occurrence and regulation depend on the type of secreted neurotransmitter/hormone and/or the activity state of the cell.

Korutla, L., P. J. Wang, et al. (2002). "Differences in expression, actions and cocaine regulation of two isoforms for the brain transcriptional regulator NAC1." *Neuroscience* **110**(3): 421.

<http://www.sciencedirect.com/science/article/B6T0F-44J6BDD-2/2/632ba88c0273a727351b19db899ff5ef>

BTB/POZ proteins can influence the cell cycle and contribute to oncogenesis. Many family members are present in the mammalian CNS. Previous work demonstrated elevated NAC1 mRNA levels in the rat nucleus accumbens in response to cocaine. NAC1 acts like other BTB/POZ proteins that regulate transcription but is unusual because of the absence of identifiable DNA binding domains. cDNAs were isolated encoding two NAC1 isoforms differing by only 27 amino acids (the longer isoform contains 514 amino acids). The mRNAs for both isoforms were simultaneously expressed throughout the rat brain and peripheral tissues. Semi-quantitative reverse transcription-polymerase chain reaction analysis revealed that the mRNA of the longer isoform was more abundant than the mRNA of the shorter isoform. Western blot analysis demonstrated a similar unequal distribution between the isoforms in the CNS. The longer isoform was the more abundant of the two NAC1 proteins and the ratio between them differed throughout the rat brain. The shorter isoform was not detected in most of the examined peripheral tissues, suggesting differences from the CNS in post-transcriptional processing. Both isoforms repressed transcription in H293T cells using a Gal4-luciferase reporter system. However, the shorter isoform did not repress transcription as effectively as the longer isoform. Transfection of different ratios for both isoforms, in order to replicate the relative amounts observed throughout the CNS, supported an interaction between the isoforms. The net effect on transcriptional repression was

determined by the ratio of the two NAC1 isoforms. Each isoform exhibited the subnuclear localization that is characteristic of many BTB/POZ proteins. A rapid and transient increase in the level of the shorter isoform occurred in the nucleus accumbens 2 h following a single i.p. cocaine injection. We conclude that the two isoforms of NAC1 may differentially affect neuronal functions, including the regulation of cocaine-induced locomotion.

Kucenas, S., Z. Li, et al. (2003). "Molecular characterization of the zebrafish P2X receptor subunit gene family." Neuroscience **121**(4): 935.

<http://www.sciencedirect.com/science/article/B6T0F-49NRJH5-3/2/ca40b8b33a378cc096a7ea8a34b3ebb0>

P2X receptors are non-selective cation channels gated by extracellular ATP and are encoded by a family of seven subunit genes in mammals. These receptors exhibit high permeabilities to calcium and in the mammalian nervous system they have been linked to modulation of neurotransmitter release. Previously, three complementary DNAs (cDNAs) encoding members of the zebrafish gene family have been described. We report here the cloning and characterization of an additional six genes of this family. Sequence analysis of all nine genes suggests that six are orthologs of mammalian genes, two are paralogs of previously described zebrafish subunits, and one remains unclassified. All nine subunits were physically mapped onto the zebrafish genome using radiation hybrid analysis. Of the nine gene products, seven give functional homo-oligomeric receptors when recombinantly expressed in human embryonic kidney cell line 293 cells. In addition, these subunits can form hetero-oligomeric receptors with phenotypes distinct from the parent subunits. Analysis of gene expression patterns was carried out using in situ hybridization, and seven of the nine genes were found to be expressed in embryos at 24 and 48 h post-fertilization. Of the seven that were expressed, six were present in the nervous system and four of these demonstrated considerable overlap in cells present in the sensory nervous system. These results suggest that P2X receptors might play a role in the early development and/or function of the sensory nervous system in vertebrates.

Lai, J. P., G. X. Zhan, et al. (2000). "Detection of substance P and its receptor in human fetal microglia." Neuroscience **101**(4): 1137.

<http://www.sciencedirect.com/science/article/B6T0F-41V2NKH-28/2/d57a84725602599a0a8e1c2c317a111e>

Substance P, the most abundant neurokinin in the CNS, is a major modulator of the immune system. We have examined the gene expression of substance P and its receptor in human fetal brain microglia. Using reverse transcription-polymerase chain reaction and Southern blotting assay, the four isoforms of preprotachykinin-A gene transcripts ([alpha], [beta], [gamma] and [delta]) were detected in the microglia. The human fetal microglia produced significantly higher levels of endogenous substance P protein (640-850 pg/106 cells) than did human peripheral blood monocyte-derived macrophages (25-50 pg/106 cells), as determined by an enzyme immunoassay. Using immunohistochemical staining with an anti-substance P antibody, cell membrane substance P immunoreactivity was observed. In addition, we identified the presence of messenger RNA for neurokinin-1 receptor, a primary receptor for substance P in human fetal microglia. From these data, we propose that substance P and its receptor are biologically involved in regulating the functions of microglia, and potentially play an important role in host defense of the central nervous system.

Lips, K. S., U. Pfeil, et al. (2002). "Coexpression of [alpha]9 and [alpha]10 nicotinic acetylcholine receptors in rat dorsal root ganglion neurons." Neuroscience **115**(1): 1.

<http://www.sciencedirect.com/science/article/B6T0F-4712HRS-1/2/72705fa206ee67da603e3718db07e2ee>

Previous binding studies have suggested the presence of a so far unknown nicotinic acetylcholine receptor subunit in dorsal root ganglia (Pugh et al., 1995). Here, we investigated whether the most recently identified subunit, [alpha]10, and its potential interaction partner, [alpha]9 (Elgoyhen et al., 2001), are expressed in these ganglia. All neurons of rat dorsal root ganglia, but no glial cells, expressed both [alpha]9 and [alpha]10 mRNA in in situ hybridization, and exhibited [alpha]10 immunoreactivity using a newly raised antibody. These findings were confirmed by RT-PCR and western blotting. The data show that dorsal root ganglion neurons coexpress [alpha]9 and [alpha]10 nicotinic receptor subunits, thereby providing the first example of neuronal expression of this receptor subunit pair.

Little, A. R., S. A. Benkovic, et al. (2002). "Chemically induced neuronal damage and gliosis: enhanced expression of the proinflammatory chemokine, monocyte chemoattractant protein (MCP)-1, without a corresponding increase in proinflammatory cytokines." Neuroscience **115**(1): 307.

<http://www.sciencedirect.com/science/article/B6T0F-46HWTG6-1/2/7711705bbf10c553e13e8ed63b9e1e2b>

Enhanced expression of proinflammatory cytokines and chemokines has long been linked to neuronal and glial responses to brain injury. Indeed, inflammation in the brain has been associated with damage that stems from conditions as diverse as infection, multiple sclerosis, trauma, and excitotoxicity. In many of these brain injuries, disruption of the blood-brain barrier (BBB) may allow entry of blood-borne factors that contribute to, or serve as the basis of, brain inflammatory responses. Administration of trimethyltin (TMT) to the rat results in loss of hippocampal neurons and an ensuing gliosis without BBB compromise. We used the TMT damage model to discover the proinflammatory cytokines and chemokines that are expressed in response to neuronal injury. TMT caused pyramidal cell damage within 3 days and a substantial loss of these neurons by 21 days post dosing. Marked microglial activation and astrogliosis were evident over the same time period. The BBB remained intact despite the presence of multiple indicators of TMT-induced neuropathology. TMT caused large increases in whole hippocampal-derived monocyte chemoattractant protein (MCP)-1 mRNA (1000%) by day 3 and in MCP-1 (300%) by day 7. The mRNA levels for tumor necrosis factor (TNF)-[alpha], interleukin (IL)-1[beta] and IL-6, cytokines normally expressed during the earliest stage of inflammation, were not increased up to 21 days post dosing. Lipopolysaccharide, used as a positive control, caused large inductions of cytokine mRNA in liver, as well as an increase in IL-1[beta] in hippocampus, but it did not result in the induction of astrogliosis. The data suggest that enhanced expression of the proinflammatory cytokines, TNF-[alpha], IL-1[beta] and IL-6, is not required for neuronal and glial responses to injury and that MCP-1 may serve a signaling function in the damaged CNS that is distinct from its role in proinflammatory events.

Medhurst, A. D., B. Y. Zeng, et al. (2001). "Up-regulation of secretoneurin immunoreactivity and secretogranin II mRNA in rat striatum following 6-hydroxydopamine lesioning and chronic L-DOPA treatment." Neuroscience **105**(2): 353.

<http://www.sciencedirect.com/science/article/B6T0F-43N7F4T-9/2/a854d35e74df55f1979cc2894b3ed13c>

Destruction of the nigro-striatal pathway in Parkinson's disease and treatment with L-DOPA lead to persistent alterations in basal ganglia output pathways that are poorly characterised. Differential display mRNA analysis was used to study the effects of 6-hydroxydopamine-induced lesions of the medial forebrain bundle on gene expression in the rat striatum. One up-regulated cDNA identified in two independent groups of 6-hydroxydopamine-lesioned animals was cloned and sequence analysis showed 97% homology to secretogranin II. Differential up-regulation of secretogranin II following 6-hydroxydopamine lesioning was confirmed in a further group of 6-hydroxydopamine-lesioned rats using TaqMan real time quantitative reverse transcription-polymerase chain reaction. Following chronic L-DOPA treatment of 6-hydroxydopamine-lesioned rats, secretogranin II mRNA was further up-regulated to a similar degree to that observed for preproenkephalin A mRNA expression. Immunohistochemical analysis confirmed the increase in secretogranin II peptide levels in striatal neurones in 6-hydroxydopamine-lesioned rats following chronic L-DOPA treatment. The increase in secretogranin II mRNA occurring following destruction of the nigro-striatal pathway and chronic L-DOPA treatment may result in an increase in secretoneurin levels, which could be important for the regulation of striatal output pathways.

Newton, R. A., S. Bingham, et al. (1999). "Identification of differentially expressed genes in dorsal root ganglia following partial sciatic nerve injury." *Neuroscience* **95**(4): 1111.

<http://www.sciencedirect.com/science/article/B6T0F-3Y6PT6X-N/2/8f931643749c34525eece2c6235b4003>

Partial sciatic nerve injury, a model of neuropathic pain, elicits a variety of neurochemical, electrophysiological and neuroanatomical changes in primary sensory neurons. We have used the technique of messenger RNA differential display to identify genes with altered expression in these neurons which may contribute to the development of aberrant sensation following such peripheral nerve damage. This approach identified 14 distinct complementary DNA clones, representing transcripts with increased ipsilateral expression in L4/5 dorsal root ganglia, two weeks after unilateral partial ligation of the rat sciatic nerve. Both Zucker diabetic fatty rats and their lean counterparts were used in this study but none of the transcripts identified showed an induction that was confined to one of the two groups. The majority of the clones did not show significant sequence similarity to previously reported genes and therefore may represent novel messenger RNA sequences or, alternatively, unknown regions of partially characterised messenger RNAs. Two of the clones represented transcripts for the known proteins muscle LIM protein and acidic epididymal glycoprotein, neither of which had previously been associated with expression in the nervous system. Reverse transcriptase-polymerase chain reaction analysis and in situ hybridization confirmed that the messenger RNA expression of both muscle LIM protein and acidic epididymal glycoprotein was induced in an ipsilateral-specific manner. Their localisations, examined with in situ hybridization in L5 dorsal root ganglia, were limited in each case to a sub-population of neuronal profiles. Those neuronal profiles that demonstrated muscle LIM protein hybridization were distributed across the profile size range, whereas the distribution of acidic epididymal glycoprotein-positive profiles appeared to be skewed towards smaller profiles. The induction of muscle LIM protein and acidic epididymal glycoprotein in dorsal root ganglia may play an important functional role in the adaptive response of primary sensory neurons following partial sciatic nerve injury.

Nomura, M., K. T. Akama, et al. (2005). "Differential distribution of estrogen receptor (ER)-[alpha] and ER-[beta] in the midbrain raphe nuclei and periaqueductal gray in male mouse: Predominant role of ER-[beta] in midbrain serotonergic systems." *Neuroscience* **130**(2): 445.

<http://www.sciencedirect.com/science/article/B6T0F-4DS7V9F-2/2/641718b7a46fbdeab073b3d208626614>

We examined the distribution of estrogen receptor (ER)-[alpha] and ER-[beta] immunoreactive (ir) cells in the dorsal (DRN) and median/paramedian (MPRN) raphe nuclei in male mice. ER-[alpha] ir neurons were scattered across the three subdivisions (ventral, dorsal, and lateral) of the DRN and the MPRN. Robust ER-[beta] ir cells were observed throughout the raphe nuclei, and were particularly abundant in the ventral and dorsal subdivisions of the DRN. Using dual-label immunocytochemistry for ER-[alpha] or ER-[beta] with tryptophan hydroxylase (TPH), the rate-limiting enzyme for 5-hydroxytryptamine (5-HT) synthesis, over 90% of ER-[beta] ir cells exhibited TPH-ir in all DRN subdivisions, whereas only 23% of ER-[alpha] ir cells contained TPH. Comparisons of ER-[alpha] knockout ([alpha]ERKO) as well as ER-[beta] knockout ([beta]ERKO) mice with their respective wild-type (WT) littermates revealed that gene disruption of either ER-[alpha] or ER-[beta] did not affect the other ER subtype expression in the raphe nuclei. In situ hybridization histochemistry revealed that there was a small but statistically significant decrease in TPH mRNA expression in the ventral DRN subdivision in [beta]ERKO mice compared with [beta]WT mice, whereas TPH mRNA levels were not affected in [alpha]ERKO mice. These findings support a hypothesis that ER-[beta] activation may contribute to the estrogenic regulation of neuroendocrine and behavioral functions, in part, by acting directly on 5-HT neurons in the raphe nuclei in male mice.

Powers, J. F., K. H. Schelling, et al. (2001). "Chromaffin cell mitogenesis by neurturin and glial cell line-derived neurotrophic factor." *Neuroscience* **108**(2): 341.

<http://www.sciencedirect.com/science/article/B6T0F-44J73KB-G/2/810874afa3e4e8e045933809ca3d13f7>

Neurturin and glial cell line-derived neurotrophic factor are novel mitogens for normal adult rat chromaffin cells in vitro. These neurotrophic factors differ from the previously described adult chromaffin cell mitogens, nerve growth factor and basic fibroblast growth factor, in that their effects are potentiated by depolarization and activation of protein kinase C. Neurturin and glial cell line-derived neurotrophic factor signal via the receptor tyrosine kinase, ret, but may also act independently of ret. Both depolarization and phorbol esters act synergistically with neurturin to up-regulate ret protein expression in chromaffin cell cultures, suggesting a mechanism for potentiation of mitogenesis. However, a direct role for ret in mitogenesis has not been established. Stimulation by neurturin causes increased phosphorylation of extracellular signal-regulated kinases 1 and 2 in cultured chromaffin cells, and mitogenesis is prevented by inhibitors of their phosphorylation. Inhibitors of phosphatidylinositol 3-kinase also prevent mitogenesis. The present findings suggest the hypothesis that neurotrophic factors and neurally derived signals might cooperatively regulate chromaffin cell proliferation in vivo in the rat. In addition, trans-synaptic stimulation might provide a route by which epigenetic factors could influence the development of adrenal medullary hyperplasia in humans with hereditary multiple endocrine neoplasia syndromes 2A and 2B by affecting expression and/or activation of ret.

Schilling, T., H. Repp, et al. (2002). "Lysophospholipids induce membrane hyperpolarization in microglia by activation of IKCa1 Ca²⁺-dependent K⁺ channels." *Neuroscience* **109**(4): 827.

<http://www.sciencedirect.com/science/article/B6T0F-45F4VFT-J/2/cdbad4d48a55dd5c5e17dcff4a8046c8>

Effects of the lysophospholipids sphingosine-1-phosphate and lysophosphatidic acid were studied in cultured murine microglia using the patch-clamp and video imaging techniques. Both lysophospholipids induced transient membrane hyperpolarization and K⁺ current activation. The lysophospholipid-induced K⁺ current was blocked by charybdotoxin or iberiotoxin, but was unaffected by apamin. In recordings with 1 [mu]M intracellular free Ca²⁺, Ca²⁺-dependent K⁺

currents of microglia showed a similar pharmacological profile to lysophospholipid-induced currents. The Ca^{2+} -dependent K^{+} channels activated in microglia by lysophospholipids are most likely encoded by the IKCa1 channel gene. The presence of IKCa1 mRNA in microglia was demonstrated by reverse transcriptase-polymerase chain reaction studies. Ca^{2+} imaging experiments revealed increases in the intracellular free Ca^{2+} concentration of microglia to a mean value of about 400 nM after application of 1 $[\mu\text{M}]$ sphingosine-1-phosphate or 1 $[\mu\text{M}]$ lysophosphatidic acid. We suggest that the transient membrane hyperpolarization seen in microglia following exposure to sphingosine-1-phosphate or lysophosphatidic acid is caused by activation of IKCa1 Ca^{2+} -dependent K^{+} channels. Increases in the concentration of intracellular free Ca^{2+} evoked by the lysophospholipids are sufficient to activate microglial Ca^{2+} -dependent K^{+} channels.

Simmons, M. A., R. M. Brodbeck, et al. (1997). "Molecular characterization and functional expression of a substance P receptor from the sympathetic ganglion of *Rana catesbeiana*." *Neuroscience* **79**(4): 1219.

<http://www.sciencedirect.com/science/article/B6T0F-3S59W9T-T/2/faa2426def1505d072dc6025680826fc>

Substance P is an important neuropeptide neurotransmitter in the central, autonomic and enteric nervous systems. In sympathetic ganglia, substance P is thought to play a role in modulating synaptic transmission. Release of substance P by neuronal stimulation or direct application of substance P to ganglionic neurons increases neuronal excitability. An amphibian substance P receptor complementary DNA has been cloned and characterized from bullfrog, *Rana catesbeiana*, sympathetic ganglion complementary DNA libraries. The deduced primary structure contains features indicative of a seven transmembrane domain G-protein-coupled receptor. The deduced protein sequence shows 69% identity to previously cloned mammalian substance P receptors. In situ hybridization analysis performed on bullfrog sympathetic ganglia using digoxigenin-labelled complementary RNA probe demonstrated that approximately 75% of the principal neurons displayed reaction product above background levels. Radioligand binding studies were performed on stably transfected cells with $[^{125}\text{I}]\text{Tyr-1-substance P}$ as the ligand. Substance P had an IC_{50} of 16 nM and the agonist potency profile was substance P > neurokinin A >> neurokinin B. The order of potency for three tachykinins to increase intracellular calcium when applied to a stably transfected clonal cell line was substance P > neurokinin A >> neurokinin B. This order of agonist potency also held for inhibition of the M-type potassium current in intact bullfrog sympathetic neurons. The non-peptide substance P antagonists CP-96345 and RP-67580 at concentrations that block mammalian substance P receptors had little or no effect on the responses to substance P at the bullfrog receptor. Overall, these results demonstrate that the cloned sequence has the features consistent with and characteristic of a substance P receptor. The results are discussed with reference to the established pharmacology of the bullfrog substance P receptor and known structure-activity relationships of mammalian tachykinin receptors.

Uwabe, K., Y. Gahara, et al. (1997). "Identification and characterization of a novel gene (neurorep 1) expressed in nerve cells and up-regulated after axotomy." *Neuroscience* **80**(2): 501.

<http://www.sciencedirect.com/science/article/B6T0F-3S8397P-1H/2/e2d2d9a2e202e5aa6b60c879b490a971>

A novel gene, designated neurorep 1, was isolated by differential hybridization screening from a complementary DNA library constructed from the rat facial nucleus whose nerve had been transected seven days before sampling. In situ hybridization revealed that this gene was up-

regulated in the repair stage after axotomy. The deduced protein, Neurorep 1, consists of 293 amino acid residues, and its molecular mass is approximately 34,000. Protein sequence motif search indicates that this protein has an ecto-5'-nucleotidase consensus sequence at the carboxyl terminal region. In vitro studies showed that Neurorep 1 significantly increased the activity of ecto-5'-nucleotidase, which is considered to be involved in regeneration and repair of the central nervous system. Neurorep 1 might play a significant role in the repair process of nerve tissues by its regulation of ecto-5'-nucleotidase activity.

Vemuganti, R. and R. J. Dempsey (2005). "Carotid atherosclerotic plaques from symptomatic stroke patients share the molecular fingerprints to develop in a neoplastic fashion: A microarray analysis study." Neuroscience **131**(2): 359.

<http://www.sciencedirect.com/science/article/B6T0F-4F9SXWD-1/2/60f916b073a653218c6c823ed7606aea>

Identification of genetic mechanisms that promote the onset of stroke and transient cerebral ischemic attack symptoms in carotid atherosclerotic patients would further our understanding of the pathophysiology of this disease and could lead to new pharmacological and molecular therapies. Using Affymetrix Human Genome 230 GeneChip set, the present study evaluated the gene expression differences in geometrically similar carotid artery plaque samples extricated from six symptomatic stroke patients and four asymptomatic patients. There was no significant difference in the degree of stenosis between the two groups. Of the 44,860 transcripts analyzed, 289 (approximately 0.6% of the total transcripts) were differentially expressed between the plaques from the symptomatic and asymptomatic groups (236 were expressed more abundantly and 53 were expressed less abundantly in the symptomatic group). Of the 236 transcripts expressed more abundantly in the symptomatic plaques, 71% (167 transcripts) indicate an active cell proliferation and neoplastic process. These include oncogenes, growth factors, tumor promoters, tumor markers, angiogenesis promoters, transcription factors, RNA splicing factors, RNA processing proteins, signal transduction mediators and those that control the metabolism. Real-time polymerase chain reaction confirmed the increased expression of 63 transcripts in the symptomatic plaques. The other groups of transcripts expressed more abundantly in the symptomatic plaques are those that control ionic homeostasis, those that participate in the progression of degenerative neurological diseases (Alzheimer's disease, amyotrophic lateral sclerosis and Huntington's disease) and epilepsy. This indicates that symptomatic plaques are molecularly and biochemically more active than the asymptomatic plaques, or active plaque growth precipitates stroke symptoms.

Wang, H., H. Sun, et al. (2002). "Chronic neuropathic pain is accompanied by global changes in gene expression and shares pathobiology with neurodegenerative diseases." Neuroscience **114**(3): 529.

<http://www.sciencedirect.com/science/article/B6T0F-46P9MSK-3/2/ad0393314a74c9ad1c00515e81e1e740>

Neuropathic pain is induced by injury or disease of the nervous system. Studies aimed at understanding the molecular pathophysiology of neuropathic pain have so far focused on a few known molecules and signaling pathways in neurons. However, the pathophysiology of neuropathic pain appears to be very complex and remains poorly understood. A global understanding of the molecular mechanisms involved in neuropathic pain is needed for a better understanding of the pathophysiology and treatment of neuropathic pain. Towards this end, we examined global gene expression changes as well as the pathobiology at the cellular level in a spinal nerve ligation neuropathic pain model using DNA microarray, quantitative real-time PCR

and immunohistochemistry. We found that the behavioral hypersensitivity that is manifested in the persistent pain state is accompanied by previously undescribed changes in gene expression. In the DRG, we found regulation of: (1) immediate early genes; (2) genes such as ion channels and signaling molecules that contribute to the excitability of neurons; and (3) genes that are indicative of secondary events such as neuroinflammation. In addition, we studied gene regulation in both injured and uninjured DRG by quantitative PCR, and observed differential gene regulation in these two populations of DRGs. Furthermore, we demonstrated unexpected co-regulation of many genes, especially the activation of neuroinflammation markers in both the PNS and CNS. The results of our study provide a new picture of the molecular mechanisms that underlie the complexity of neuropathic pain and suggest that chronic pain shares common pathobiology with progressive neurodegenerative disease.

Yuan, W., R. T. Matthews, et al. (2002). "Association between protease-specific proteolytic cleavage of brevicin and synaptic loss in the dentate gyrus of kainate-treated rats." *Neuroscience* **114**(4): 1091.

<http://www.sciencedirect.com/science/article/B6T0F-46T45F8-P/2/946922cf5bcb67b6bf1808f136c41d26>

Proteolytic fragments generated by ADAMTS (isintegrin and metalloprotease with thrombospondin motifs)-mediated cleavage of the aggregating chondroitin sulfate proteoglycan, brevicin, have been identified, but not localized in the CNS. The purpose of this study, using kainate-induced CNS lesion, was to examine the spatial and quantitative relationship between ADAMTS1 and 4 mRNA expression and ADAMTS-mediated cleavage of brevicin (as determined by the abundance of the neo-epitope QEAVESE at the C-terminal of the cleaved brevicin G1 domain). In untreated rats, in situ hybridization and reverse transcriptase polymerase chain reaction indicated that ADAMTS4 expression was higher than ADAMTS1 and was localized to hippocampus, temporal lobe and other areas of cortex, striatum and hypothalamus. ADAMTS4 mRNA expression in these regions correlated with the presence of the QEAVESE neo-epitope, which was concentrated in perineuronal nets and in neuropil. In rats that seized after kainate, there was a dramatic elevation in ADAMTS1 and ADAMTS4 transcript that correlated and co-localized with a robust elevation in an extractable, 55-kDa fragment of brevicin in temporal lobe and hippocampus. This fragment consisted, at least in part, of the ADAMTS-cleaved epitope G1-QEAVESE. The kainate-induced elevation in this ADAMTS-cleaved fragment was localized to amygdaloid and thalamic nuclei, hippocampus, caudate-putamen, cingulate cortex, and the outer molecular layer of the dentate gyrus where it was accompanied by a robust elevation in ADAMTS1 and 4 mRNA and a 28% decline in synaptic density 5 days after kainate. Thus, complexes of extracellular matrix proteins that exist in perineuronal nets and in the neuropil are cleaved by specific matrix-degrading proteases at early time points during excitotoxic neurodegeneration. The observed ADAMTS-induced cleavage of brevicin in the dentate outer molecular layer is closely associated with diminished synaptic density, and may, therefore, contribute to synaptic loss and/or reorganization in this region.

Zhan, G., F. Shaheen, et al. (2002). "Single cell laser dissection with molecular beacon polymerase chain reaction identifies 2A as the predominant serotonin receptor subtype in hypoglossal motoneurons." *Neuroscience* **113**(1): 145.

<http://www.sciencedirect.com/science/article/B6T0F-4684HBY-H/2/4b8382fe0f6fb7282080ee262525246c>

We hypothesize that sleep state-dependent withdrawal of serotonin (5-hydroxytryptamine, 5-HT) at upper airway (UAW) dilator motoneurons contributes significantly to sleep-related suppression

of dilator muscle activity in obstructive sleep apnea. Identification of 5-HT receptor subtypes involved in postsynaptic facilitation of UAW motoneuron activity may provide pharmacotherapies for this prevalent disorder. We have adapted two assays to provide semi-quantitative measurements of mRNA copy numbers for 5-HT receptor subtypes in single UAW motoneurons. Specifically, soma of 111 hypoglossal (XII) motoneurons in 10 adult male rats were captured using a laser dissection microscope, and then used individually in single round molecular beacon polymerase chain reaction (PCR) for real-time quantitation of 5-HT_{2A}, 5-HT_{2C}, 5-HT₃, 5-HT₄, 5-HT_{5A}, 5-HT_{5B}, 5-HT₆ or 5-HT₇ receptor. Receptor mRNA copy numbers from single XII motoneurons were compared to control samples from within the XII nucleus and lateral medulla. All 20 motoneuronal soma assayed for the 5-HT_{2A} receptor had measurable copy numbers (7028 $\pm$ 2656 copies/cell). In contrast, copy numbers for the 5-HT_{2A} receptor in XII non-motoneuronal (n=17) and lateral medulla (n=15) samples were 81 $\pm$ 51 copies and 83 $\pm$ 35 copies, respectively, P2C receptor copy numbers of mRNA (287 $\pm$ 112 copies/cell). XII soma had minimal 5-HT₃, 5-HT₄, 5-HT_{5A}, 5-HT_{5B}, 5-HT₆ or 5-HT₇ receptor mRNA. 5-HT_{2A} receptor mRNA presence within XII motoneurons was confirmed with digoxigenin-labeled in situ hybridization. In summary, combined use of laser dissection and molecular beacon PCR revealed 5-HT_{2A} receptor as the predominant 5-HT receptor mRNA in XII motoneurons, and identified small quantities of 5-HT_{2C} receptor. This information will allow a more complete understanding of serotonergic control of respiratory activity.

Neuroscience & Biobehavioral Reviews (1)

Leo, D., E. Sorrentino, et al. (2003). "Altered midbrain dopaminergic neurotransmission during development in an animal model of ADHD." Neuroscience & Biobehavioral Reviews 27(7): 661.

<http://www.sciencedirect.com/science/article/B6T0J-49XPGRD-1/2/e6cb0d08762ca070eba6cbc3da87e199>

To understand the onset and the molecular mechanisms triggering dopaminergic (DA) dysregulation in Attention-Deficit Hyperactivity Disorder (ADHD), we have used the Spontaneously Hypertensive Rats (SHR), the most widely studied animal model for this disease. We have studied the pattern of expression of specific genes involved in DA neuron differentiation, survival and function during postnatal (P) development of the ventral midbrain in SHR males. Our results show that tyrosine hydroxylase and DA transporter gene expression are significantly and transiently reduced in the SHR midbrain during the first month of postnatal development, although with a different kinetic. The other genes analyzed do not show significant variation between SHR and control rats. In addition, high-affinity DA uptake activity is significantly reduced in synaptosomes obtained from the striatum of 1-month-old SHR, when compared to controls. Our data suggest that down-regulation of DA neurotransmission occurs in the midbrain of SHR in a developmentally regulated temporal window during postnatal development, thus strengthening the hypodopaminergic hypothesis in the pathogenesis of ADHD.

Neuroscience Letters (70)

Alquier, T., C. Leloup, et al. (2001). "Altered Glut4 mRNA levels in specific brain areas of hyperglycemic-hyperinsulinemic rats." Neuroscience Letters **308**(2): 75.

<http://www.sciencedirect.com/science/article/B6T0G-43G419D-3/2/0f405388277248866e2855f7e49b0918>

The insulin sensitive glucose transporter Glut4 is expressed in neurons of the brain among which those of hypothalamic nuclei. It has been proposed that this transporter might be involved in the hypothalamic glucose-insulin sensing mechanism and thus in the nervous regulation of metabolism. In order to get further insights into its putative role, Glut4 expression was analyzed by quantitative competitive reverse transcription-polymerase chain reaction, in hypothalamic nuclei of hyperglycemic-hyperinsulinemic (HG-HI) rats, a model characterized by alteration of the autonomic nervous system activity. Glut4 mRNA content was decreased in the lateral hypothalamic area (33%) and arcuate nucleus (27%) but significantly only in the former. It was unchanged in other structures. These results are in favor of an alteration of Glut4 expression by short-term hyperglycemia and hyperinsulinemia that, in turn, could affect autonomic nervous system activity.

Armstrong, J., M. Boada, et al. (2004). "Familial Alzheimer disease associated with A713T mutation in APP." Neuroscience Letters **370**(2-3): 241.

<http://www.sciencedirect.com/science/article/B6T0G-4D99X62-2/2/c95e92003474298f189e2017683c74a6>

Mutations in APP are associated with familial early-onset Alzheimer disease (FAD). Examination of the genomic sequence in one patient with FAD revealed a change located in the exon 17 of the APP gene at position 275329G>A (GenBank accession number: D87675; GI: 2429080); cDNA sequence 2137G>A (GenBank accession number: X06989; GI: 28720). This corresponds to the mutation A713T in APP. AD stage VI of neurofibrillary degeneration and stage C of A[beta]-amyloid burden was found at the post-mortem neuropathological examination. Previous studies have suggested that the mutation A713T in APP is a silent mutation or polymorphism. However, we have not found this change in APP in a control population analyzed by the amplification-refractory mutation system (ARMS). It is concluded that A713T in APP is implicated in the pathogenesis of AD. Since the immunohistochemical study indicates that A713T mutation is not likely to relate with A[beta]-amyloid processing, the causative role of this rare mutation remains to be warranted.

Beischlag, T. V., D. Nam, et al. (1996). "A polymorphic dinucleotide repeat in the human dopamine D5 receptor gene promoter." Neuroscience Letters **205**(3): 173.

<http://www.sciencedirect.com/science/article/B6T0G-3VXH7M3-1K/2/caaec5f636428100a1c7bea84c13cc62>

Previously, we have reported the cloning and characterization of the 5'-flanking region of the human dopamine D5 receptor encoding gene (D5) and that the major transactivation domain was 119-182 by upstream of the transcriptional start site [Beischlag, T V. et al., *Biochemistry*, **34** (1995) 5960-5970]. Within this region existed a small dinucleotide repeat termed (TC)₁₃. In this report, we describe the screening of genomic DNAs from 18 unrelated individuals by single-strand conformation polymorphism (SSCP) analysis. SSCP analysis revealed the existence of two additional alleles, termed (TC)₁₂ and (TC)₁₄. Neither form significantly altered D5 promoter-mediated luciferase activity when compared to that of the wild-type control, suggesting that small

differences in the number of dinucleotide repeats are not likely of any functional consequence for D5 transactivation.

Biervert, C., E. Horvath, et al. (2001). "Semiquantitative expression analysis of ephrine-receptor tyrosine kinase mRNA's in a rat model of traumatic brain injury." Neuroscience Letters **315**(1-2): 25.

<http://www.sciencedirect.com/science/article/B6T0G-44D43DF-7/2/385c1dace510628890a6cd8a32fad423>

The molecular mechanisms involved in recovery of function of the central nervous system (CNS) after injury to the brain are incompletely understood. Here the expression of ephrine (Eph) kinases following traumatic brain injury (subdural haematoma) was analysed in order to find out whether these developmentally regulated genes may be involved in tissue remodelling after brain damage. mRNA was isolated from ipsilateral cortices 7, 18, and 28 days after surgery and semiquantitative reverse transcription-polymerase chain reaction was performed. Most Eph kinases did not show significant regulation at gene expression level during the time course of recovery from acute brain injury but there is some evidence that mRNA of EphB1 might be slightly upregulated.

Bigl, M., J. Apelt, et al. (2000). "Expression of [beta]-secretase mRNA in transgenic Tg2576 mouse brain with Alzheimer plaque pathology." Neuroscience Letters **292**(2): 107.

<http://www.sciencedirect.com/science/article/B6T0G-4177HW3-B/2/983b1c2a51ec90a9864d8836e0d88a44>

On the basis of the recent cloning of the [beta]-secretase, the beta-site amyloid precursor protein (APP)-cleaving enzyme (BACE), (Science, 286 (1999) 735), digoxigenin-labelled riboprobes were generated to localize the cellular expression pattern of BACE mRNA in brain sections of transgenic Tg2576 mice, overexpressing the Swedish mutation of the APP695 isoform. Non-radioactive in situ hybridization in combination with immunohistochemistry to identify the cell types and [beta]-amyloid deposits revealed strong BACE mRNA hybridization signals in neurons of the cerebral cortex, hippocampal formation, thalamus and cholinergic basal forebrain nuclei, while astrocytes did not display any labeling. Neurons surrounding [beta]-amyloid deposits did not demonstrate altered expression level of BACE mRNA as compared to neurons in cortical areas that are free of [beta]-amyloid deposits, and the regional expression pattern of BACE mRNA did not correlate with the distribution of [beta]-amyloid deposits. These data suggest that high level of expression of BACE mRNA is not necessarily related to enhanced deposition of [beta]-amyloid plaques. To elucidate those factors that contribute to [beta]-amyloid plaque deposition in a particular region, the transgenic Tg2576 mouse may represent an appropriate tool.

Brandle, U., H. P. Zenner, et al. (1999). "Gene expression of P2X-receptors in the developing inner ear of the rat." Neuroscience Letters **273**(2): 105.

<http://www.sciencedirect.com/science/article/B6T0G-3XDRYT8-9/2/7f9ff944d396a2bb31efc583593e6e10>

Reverse transcription-polymerase chain reaction (RT-PCR) was used to characterize the expression of P2X receptor subunits (P2X1-P2X7) in different inner ear tissues. The present study revealed the presence of P2X2, P2X3, P2X4 and P2X7-mRNA in rat organ of Corti,

vestibular organ and spiral ganglion at different postnatal developmental stages (PD1-PD16), with slight differences in the onset of expression. Expression of P2X1, P2X5 and P2X6-mRNA was not detectable in the inner ear tissues. In addition, single cell RT-PCR experiments with outer hair cells (OHC) revealed the expression of either the P2X2 or the P2X2-2 splice variant or coexpression of both isoforms in individual cells. Our data suggest that extracellular adenosine-5'-triphosphate (ATP) may play an important role in signal transduction in the inner ear.

Bristulf, J., A. Simoncsits, et al. (1991). "Characterization of a neuronal interleukin-1 receptor and the corresponding mRNA in the mouse anterior pituitary cell line AtT-20." Neuroscience Letters **128**(2): 173.

<http://www.sciencedirect.com/science/article/B6T0G-485RMVR-14T/2/f3b5759369516cabf1c713b997c52732>

Interleukin-1 (IL-1) mediates numerous responses on the mouse anterior pituitary cell line AtT-20. We have studied the ligand binding properties of the IL-1 receptors (IL-1R) of the AtT-20 cells and found that they possess 1.5×10^3 receptors/cell with a K_d of 0.15 nM for [125I]IL-1[alpha]. Using oligonucleotide primers, which define a 236 bp region of the cDNA coding for the cytosolic part of the mouse T-cell IL-1R, and cDNA prepared from AtT-20 cells, we obtained by polymerase chain reaction (PCR) a DNA fragment which was shown to possess an identical sequence to that of the corresponding region of the mouse T-cell IL-1R. Thus the AtT-20 pituitary cell line IL-1 receptor appears to be similar to those found on mouse T-cells and fibroblasts.

Chio, C. L., G. L. Alberts, et al. (2002). "Discovery of a protein sequence in the N-terminal region of the human neuronal [alpha]7 nicotinic acetylcholine receptor involved in homomeric interactions." Neuroscience Letters **334**(1): 49.

<http://www.sciencedirect.com/science/article/B6T0G-475BFKS-6/2/76b7a7468f02acb06afad5c5a814ae6e>

The neuronal nicotinic acetylcholine receptor subunit, [alpha]7, can form homopentameric receptor/ion channel complexes. Potential contributions of its N-terminal region to homomeric interactions were investigated, in comparison with the corresponding region of an analogous heteromeric subunit, [alpha]3. Recombinant chimeras were prepared upon engineering the N-terminal [alpha]7 (M1-V224) or [alpha]3 (M1-S232) sequence into the background of another homomeric mouse 5-hydroxytryptamine3 (5-HT)3 receptor. The [alpha]7/5-HT3 chimera, when expressed heterologously in a human epithelial cell line, SH-EP1, robustly expressed [alpha]-bungarotoxin binding sites as homooligomers while the [alpha]3/5-HT3 did not produce epibatidine (non-selective ligand) binding sites, and did not interfere the [alpha]7/5-HT3 phenotype, upon co-expression. Yeast two hybrid assays with the N-terminal regions showed positive responses between [alpha]7:[alpha]7, but not between [alpha]7:[alpha]3 and [alpha]3:[alpha]3. Similar assays with the [alpha]7 N-terminal region and its five smaller fragments (G23-N46, D47-N90, V91-N133, S134-M182 and Q183-V224) revealed that the G23-N46 sequence is involved in homomeric interactions. Replacement of the corresponding region of the [alpha]3/5-HT3 chimera with the [alpha]7 G23-N46 sequence conferred a dominant negative role on the chimera, by abolishing the [alpha]7/5-HT3 phenotype. These results support the view that the G23-N46 portion of the [alpha]7 N-terminal region may contribute to receptor homooligomerizations.

Citron, B. A., S. X. Zhang, et al. (1997). "Apoptotic, injury-induced cell death in cultured mouse murine motor neurons." Neuroscience Letters **230**(1): 25.

<http://www.sciencedirect.com/science/article/B6T0G-3PKTFT0-7/2/f72e5dd57d5988c7051484449ecd9af8>

In order to develop in vitro models of CNS injury, astrocytes have been mechanically injured in culture to study reactive astrogliosis. However, scratch injury models of pure neuronal cultures have not yet been exploited to study programmed cell death (PCD). For this study, we examined model motor neurons (NSC19 cells) in culture and found time-dependent cell death in proximity (within 2.5 mm) to a physical scratch injury. Injury-induced cell death was apoptotic verified by positively-stained nuclei using both the in situ end-labeling (ISEL) procedure and Hoechst 33342. Unexpectedly, cells proximal to the injury site were not affected by the injury until 3 days later suggesting that adjacent motor neuron loss was dependent on a 'death signal' produced by direct injury to sister neurons. 'Executioners' in apoptosis include free radicals, cell cycle kinases and cysteine proteases (caspases). Extracellular serine proteases, such as thrombin and granzyme B, may activate such intracellular pathways and several inhibitors (serpins), such as CrmA, are effective in blocking apoptosis. Since protease nexin I (PNI), a serpin homologous with CrmA, prevents apoptosis of lumbar motor neurons and is increased after nerve injury, we examined mRNA by RT-PCR for PNI expression. Of interest, although we were unable to find significant levels of PNI message in NSC19 cells, we did detect it in the parent neuroblastoma.

Deary, I. J., S. E. Harris, et al. (2005). "KLOTHO genotype and cognitive ability in childhood and old age in the same individuals." Neuroscience Letters **378**(1): 22.

<http://www.sciencedirect.com/science/article/B6T0G-4F9N71H-2/2/f9545657a692e6fe1b8ed6ef8278a385>

This study examined the influence of a variation in the KLOTHO gene on cognitive ability at age 11 and age 79 in 464 people from the Lothian Birth Cohort 1921 (LBC1921), and at age 11 and age 64 in 451 people in the Aberdeen Birth Cohort 1936 (ABC1936). In the LBC1921, people with the KLOTHO V/V genotype had lower verbal reasoning ability at age 11 and age 79, and lower non-verbal reasoning at age 79, than those with the F/F genotype, or heterozygotes. The effect of the KLOTHO polymorphism on cognition at age 79 was non-significant when adjusted for IQ at age 11. In this sample, KLOTHO V allele status accounts for about 2% of the variance in life-long traits related to verbal and non-verbal reasoning, but not to age-related cognitive change. These results were not replicated in the ABC1936 sample. In a combined analysis of the LBC1921 and the ABC1936 cohorts there was a significant KLOTHO X sex interaction: women with the V/V genotype had lower non-verbal reasoning scores at age 79, after adjustment for cognitive ability at age 11. Variation in the KLOTHO gene is a possible contributor to life-long reasoning differences in humans and/or to the ageing of non-verbal reasoning, especially in women.

Edvinsson, L., L. Cantera, et al. (1997). "Expression of calcitonin gene-related peptide1 receptor mRNA in human trigeminal ganglia and cerebral arteries." Neuroscience Letters **229**(3): 209.

<http://www.sciencedirect.com/science/article/B6T0G-3S0MJGM-K/2/10e9918dad20125e44beb6b5b66a7dce>

Reverse transcriptase polymerase chain reaction (RT-PCR) using primers for the recently cloned human CGRP1 receptor detected mRNA expression of CGRP1 receptors in trigeminal ganglia and cerebral vessels, obtained at autopsy or during neurosurgical tumor resections. An RT-PCR

product of the expected size (339 bp) was seen in cerebral arteries, both in the presence and in the absence of endothelium and in trigeminal ganglia. Sequence analysis of the RT-PCR product of the published sequence showed 100% homology with the human CGRP1 receptor. The presence of the CGRP1 receptor mRNA in human trigeminal ganglia and cerebral blood vessels, indicates the occurrence of both prejunctional (trigeminal) and postjunctional location (blood vessels) of the CGRP1 receptor.

Fujii, T., S. Tajima, et al. (1999). "Constitutive expression of mRNA for the same choline acetyltransferase as that in the nervous system, an acetylcholine-synthesizing enzyme, in human leukemic T-cell lines." Neuroscience Letters **259**(2): 71.

<http://www.sciencedirect.com/science/article/B6T0G-3VCMTPS-1Y/2/2024509d65296458a89fafc63aeacc47>

Both muscarinic and nicotinic acetylcholine (ACh) receptors are known to be present on the surface of lymphocytes. We have shown that variable amounts of ACh are detectable in the blood of various mammals including humans, and a major portion of blood ACh is localized in circulating mononuclear leukocytes in humans. In order to investigate which types of blood cell are the source of ACh in human blood, expression of mRNA for choline acetyltransferase (ChAT, EC 2.3.1.6), which catalyzes ACh synthesis, was analyzed using human leukemic cell lines as models of lymphocytes and the reverse transcription-polymerase chain reaction (RT-PCR) method. We observed that mRNA for the same ChAT as that in the nervous system is expressed constitutively in all the T-cell lines tested, but not in B-, pre-lymphoma or monocytic cell lines. Furthermore, only T-cell lines showed high ACh-synthesizing activities and intracellular ACh contents. These results suggest that the major portion of ACh in the circulating blood originates from T-lymphocytes.

Fujinaga, M. and J. C. Scott (1997). "Gene expression of catecholamine synthesizing enzymes and [beta] adrenoceptor subtypes during rat embryogenesis." Neuroscience Letters **231**(2): 108.

<http://www.sciencedirect.com/science/article/B6T0G-3PV1RMM-10/2/f9055252471efa519a8aae8c4f8a9a5b>

Timed-pregnant Sprague-Dawley rats were killed between gestational day (GD) 8 and 10, and embryos were explanted and separated into developmental stages according to a modified Theiler's system. Total RNA from each stage was isolated and subjected to reverse transcription-polymerase chain reaction (RT-PCR) assays to examine gene expression of catecholamine synthesizing enzymes and three subtypes of [beta] adrenoceptors. Expression of these genes was detected at much earlier stages than previously reported, and each enzyme and receptor subtype showed a different pattern of gene expression. For example, mRNA for tyrosine hydroxylase, the rate-limiting enzyme for catecholamine synthesis, was detected as early as stage 10a, late GD 8, before the neural crest cells appear (stage 12, mid GD 10). This contradicts the common belief that catecholamines are produced only in the cells of sympathoadrenal lineage which originate from the neural crest cells and the cells of central nervous system. Results from the present study indicate that catecholamine synthesis is not limited to the cells of sympathoadrenal lineage.

Garbay, B., F. Boiron-Sargueil, et al. (1995). "Expression of the exon 1A-containing PMP22 transcript is altered in the trembler mouse." Neuroscience Letters **198**(3): 157.

<http://www.sciencedirect.com/science/article/B6T0G-3YB9Y46-S/2/daf8d69aa9575f1891d7dcbb64b4a188>

The trembler mouse suffers from a dominantly inherited mutation of the peripheral myelin protein 22 (PMP22) gene which results in an abnormal myelination of its peripheral nervous system. The recent identification of two different PMP22 mRNA differing in their 5' non-translated region led us to monitor their respective levels of expression in the trembler peripheral nervous system (PNS) during the myelination period. We showed that the steady-state levels of the exon 1A-containing transcript, which is thought to be involved in the myelination process, were greatly reduced in heterozygous and homozygous trembler mice when compared to the normal animals. Such a difference was not observed for the exon 1B-containing transcript. Therefore, our results support the idea that the two alternatively used promoters of the PMP22 gene are under different regulation control, and that the up-regulation of the exon 1A-transcript is necessary for the normal myelination of the mouse PNS.

Ginham, R., D. C. Harrison, et al. (2001). "Upregulation of death pathway molecules in rat cerebellar granule neurons undergoing apoptosis." *Neuroscience Letters* **302**(2-3): 113.

<http://www.sciencedirect.com/science/article/B6T0G-42P51GH-F/2/baa6904cfd640c11fb7fee88586129bf>

Cerebellar granule neurons can be maintained in culture in a medium containing high serum and depolarising levels of KCl. When serum is removed and the KCl levels lowered from 25 to 5 mM, the cells undergo apoptosis. Apoptosis can be prevented by inhibitors of transcription or translation, suggesting a need for macromolecular synthesis in the apoptotic process. Using quantitative reverse transcription-polymerase chain reaction the levels of mRNA for a range of genes postulated to be important in apoptosis have been examined. Elevated levels of caspase 3, c-Jun, and Fas ligand were found, in addition to a corresponding increase in c-Jun protein and activation of caspase-3. These results suggest that cerebellar granule neurons upregulate components of both death receptor-mediated and the mitochondrial-mediated death pathways.

Guo, S., Y. Shi, et al. (2004). "No genetic association between polymorphisms in the AMPA receptor subunit GluR4 gene (GRIA4) and schizophrenia in the Chinese population." *Neuroscience Letters* **369**(2): 168.

<http://www.sciencedirect.com/science/article/B6T0G-4D9DF7J-1/2/c248abc18176ba6011382cdb443c40ee>

The glutamatergic dysfunction hypothesis of schizophrenia suggests genes involved in glutamatergic transmission as candidates for schizophrenia-susceptibility genes. It has recently been reported that some haplotypes in the AMPA receptor subunit GluR4 Gene (GRIA4), which is located on chromosome 11q22, are positively associated with schizophrenia in the Japanese population. In order to assess the role of GRIA4 in schizophrenia, we examined three reported positive SNPs (single nucleotide polymorphisms): rs609239, rs641574 and rs659840 at the GRIA4 locus in schizophrenic cases (n = 372) and controls (n = 392) of the Chinese population. Although we had observed similar allele and genotype frequencies compared with that in the Japanese population, no evidence was found for association with the disease in the analysis of either single nucleotide polymorphisms (all P-values > 0.300) or haplotype relative risk (all P-values > 0.088). Our results suggest that the three SNPs of GRIA4 are unlikely to play a major role in the susceptibility to schizophrenia in the Chinese population.

Haas, J., U. Meyding-Lamade, et al. (1999). "Acyclovir treatment of experimentally induced herpes simplex virus encephalitis: monitoring the changes in immunologic NO synthase expression and viral load within brain tissue of SJL mice." Neuroscience Letters **264**(1-3): 129.

<http://www.sciencedirect.com/science/article/B6T0G-3W788DW-15/2/f18eb33a7368297ae9bc735ffbfb85f>

The effect of acyclovir treatment on viral burden and the expression of immunologic nitric oxide synthase (iNOS) within brains of 42 HSV-1F infected mice was studied by using a titration PCR assay for HSV-1 DNA and a semiquantitative RT-PCR for iNOS mRNA. iNOS mediated NO-production may possibly be involved in secondary mechanisms of brain injury following virus infection, which may account for treatment failures in human herpes simplex virus encephalitis (HSVE). Following infection, a parallel increase of iNOS mRNA and HSV-1F-DNA occurred with peaks after 7 days that were both significantly lower under acyclovir treatment. Six months post infection viral load had declined, but iNOS mRNA expression in both treated and untreated mice was still enhanced as compared with mock infected controls. This suggests that acyclovir decreases iNOS expression via inhibition of viral replication shortly after infection but fails to influence elevated iNOS within the brain late in the course of experimental HSVE.

Haas, J., B. Storch-Hagenlocher, et al. (2002). "Inducible nitric oxide synthase and argininosuccinate synthetase: co-induction in brain tissue of patients with Alzheimer's dementia and following stimulation with [beta]-amyloid 1-42 in vitro." Neuroscience Letters **322**(2): 121.

<http://www.sciencedirect.com/science/article/B6T0G-452WHMW-2/2/85051b0b00a8588e88fa3546d81e3b8f>

In Alzheimer's disease (AD), amyloid plaques within the brain are surrounded by activated glial cells (microglia and astrocytes). The mechanisms of glial activation and its effect on disease progression are not fully understood. Growing evidence suggests that beta-amyloid (A[beta]) peptide, a major constituent of the amyloid plaque, is critically involved in the induction of an inflammatory response. The goal of this study was to examine the role of A[beta] in the pathogenesis of local inflammation and neuronal cell death. We found increased mRNA levels of inducible nitric oxide synthase (iNOS) and the arginine regenerating enzyme argininosuccinate synthetase (ASS) within cortices of AD patients suggesting high output NO production. In vitro, synthetic A[beta]1-42 and to a lesser extent A[beta]1-40 induced iNOS and ASS transcription with consecutive NO overproduction in mixed rat neuronal-glial cultures. Furthermore, A[beta]-stimulation lead to an increased release of inflammatory cytokines interleukin (IL)-1[beta], IL-6 and tumor necrosis factor-[alpha]. Again, A[beta]1-42 had a much more pronounced effect as compared to A[beta]1-40. Our data suggest that A[beta]1-42 is a key mediator of glial activation and via the induction of inflammatory mediators may be a critical component of the neurodegenerative process in AD.

Haberberger, R., M. Henrich, et al. (1999). "Muscarinic M2-receptors in rat thoracic dorsal root ganglia." Neuroscience Letters **266**(3): 177.

<http://www.sciencedirect.com/science/article/B6T0G-3XPDB8W-7/2/fd251f68ecdbe1931e971b3fd90db72b>

The occurrence and distribution of the muscarinic M2-receptor subtype (M2R) was investigated in

rat thoracic dorsal root ganglia (DRG). Messenger RNA for M2R was demonstrated by RT-PCR in total RNA from DRG. Immunoreactivity to M2R-protein was localized to 26% of sensory neurons, the majority of them (85%) belonging to the size class of 25-40 μm in diameter. Double-labeling (immuno)histochemistry revealed that all M2R-immunoreactive neurons bind the lectin, I-B4, whereas they are generally devoid of substance P-immunoreactivity. These data show the presence of M2R on a subpopulation of presumably nociceptive primary afferent neurons, thereby extending previous pharmacological and electrophysiological studies that indicated a role of M2R and/or M4R in inhibition of calcium channel currents in rat sensory neurons (Wanke, E., Bianchi, L., Mantegazza, M., Guatteo, E., Macinelli, E. and Ferroni, A., Muscarinic regulation of Ca^{2+} currents in rat sensory neurons: channel and receptor types, dose-response relationships and cross-talk pathways. *Eur. J. Neurosci.*, 6 (1994) 381-391).

Hashimoto, N., H. Yamanaka, et al. (2003). "Increased expression of 3[beta]-hydroxysteroid dehydrogenase mRNA in dorsal root ganglion neurons of adult rats following peripheral nerve injury." *Neuroscience Letters* **340**(1): 45.

<http://www.sciencedirect.com/science/article/B6T0G-483SX2P-9/2/35a784b583ec9b9cfebbb8b1fd883512>

3[beta]-hydroxysteroid dehydrogenase (3[beta]-HSD) is an enzyme that converts pregnenolone to progesterone. It has been believed that 3[beta]-HSD is simply a converting enzyme of female steroid hormone. Recently, 3[beta]-HSD expressing cells were identified in the spinal cord. Steroid synthesis in the nervous system may indicate that steroid plays a role in the nervous system. We report here the increased expression of 3[beta]-HSD mRNA in the dorsal root ganglion (DRG) after peripheral nerve injury using reverse transcription-polymerase chain reaction and in situ hybridization histochemistry techniques. We detected only a few 3[beta]-HSD signals in the naive DRG, and found that 3[beta]-HSD mRNA expression increased 3 days after injury and this increase was still observed at 14 days. Our results suggest that progesterone may have a role in the process against neuronal injury or in regeneration in the peripheral nervous system.

Hou, M., R. Uddman, et al. (2002). "Capsaicin receptor immunoreactivity in the human trigeminal ganglion." *Neuroscience Letters* **330**(3): 223.

<http://www.sciencedirect.com/science/article/B6T0G-46SK5FH-4/2/ed048e31ed56fda06cc837a6aa3b4d80>

The cloned capsaicin receptor, also known as vanilloid receptor subtype 1 (VR1) receptor, has been demonstrated to be an integral membrane protein with homology to a family of putative store-operated calcium channels. The VR1 receptor is activated not only by capsaicin but also by noxious heat and protons, and therefore it is suggested as a molecular integrator of chemical and physical stimuli that elicit pain. In the present study, indirect immunofluorescence detected a small number of neurons that are VR1 receptor immunoreactive (ir) (171 versus 1038 or 16% of all neuronal cell bodies) in the human trigeminal ganglion (TG). In addition, RT-PCR confirmed the presence of VR1 mRNA in the human TG. It has been hypothesized that TG neuronal cell bodies are the source of capsaicin-stimulated release of calcitonin gene-related peptide (CGRP), and hence co-localization experiments were performed. Around 10% of the VR1 receptor-ir is expressed on neurons that contain CGRP-ir (ten among 74) in the human TG, indicating that capsaicin may act through the VR1 receptor to modulate the release of CGRP and in turn to modulate pain. We observed that 8% of the VR1 receptor-ir neuronal cell bodies contain substance P-ir and 5% nitric oxide synthase. Capsaicin can release nitric oxide, CGRP and substance P from sensory nerves and contribute to central sensitization.

Itokawa, M., K. Yamada, et al. (2003). "Genetic analysis of a functional GRIN2A promoter (GT)_n repeat in bipolar disorder pedigrees in humans." Neuroscience Letters **345**(1): 53.

<http://www.sciencedirect.com/science/article/B6T0G-48NXD5P-F/2/1f8e4f7e541f4bb717b65bccf445cb0e>

Hypofunction of glutamatergic neurotransmission has been hypothesized to underlie the pathophysiology of bipolar affective disorder, as well as schizophrenia. We examined the role of the N-methyl--aspartate receptor 2A subunit (GRIN2A) gene on 16p13.3, a region thought to be linked to bipolar disorder, (1) because in a prior study we identified a functional and polymorphic (GT)_n repeat in the 5' regulatory region of the gene, with longer alleles showing lower transcriptional activity and an over representation in schizophrenia, and (2) because of the suggestion of a genetic overlap between affective disorder and schizophrenia. Family-based association tests detected a nominally significant preferential transmission of longer alleles in a panel of 96 multiplex bipolar pedigrees. These results support the hypothesis that a hypoglutamatergic state is involved in the pathogenesis of bipolar affective disorder.

Iwayama-Shigeno, Y., K. Yamada, et al. (2005). "Extended analyses support the association of a functional (GT)_n polymorphism in the GRIN2A promoter with Japanese schizophrenia." Neuroscience Letters **378**(2): 102.

<http://www.sciencedirect.com/science/article/B6T0G-4F662KF-5/2/6c599fb523146d134ce7ee96a74383bb>

Dysfunction of the N-methyl-d-aspartate (NMDA) type glutamate receptor has been proposed as a mechanism in the etiology of schizophrenia. Recently, we identified a variable (GT)_n repeat in the promoter region of the NMDA NR2A subunit gene (GRIN2A), and showed its association with schizophrenia in a case-control study, together with a correlation between the length of the repeat and severity of chronic outcome. In this study, we extended our analyses, by increasing the number of case-control samples to a total of 672 schizophrenics and 686 controls, and excluded potential sample stratification effects. We confirmed the significant allelic association between the repeat polymorphism and disease ($P = 0.011$), and as in the previous study, we observed an over-representation of longer alleles in schizophrenia. These results suggest a probable genetic effect for the GRIN2A promoter (GT)_n variation on the predisposition to schizophrenia in Japanese cohorts.

Kahlem, P. and P. Djian (2000). "The expanded CAG repeat associated with juvenile Huntington disease shows a common origin of most or all neurons and glia in human cerebrum." Neuroscience Letters **286**(3): 203.

<http://www.sciencedirect.com/science/article/B6T0G-40BPR2M-F/2/c3e6ffeb12a98d04ab8b28edb8194b39>

We have analyzed the size of the expanded poly(CAG) associated with juvenile Huntington disease in the cerebrum and the cerebella of five patients. The expanded poly(CAG) was always longer in the cerebrum than in the cerebellum, but the difference in size varied from patient to patient. Except for one patient who possessed an unusually large expansion, very little heterogeneity of size was detected within the cerebrum or within the cerebellum. The larger size of the expanded poly(CAG) in cerebrum must therefore have resulted from a single expansion

event that took place early in cerebral development. In both cerebrum and cerebellum, the size of the expanded allele of gray matter was identical to that of white matter. We conclude that most if not all neurons and glia of cerebrum are descended from a common bipotent precursor, which segregated early in neurogenesis from the lineage leading to cerebellar neurons and glia.

Kamboh, M. I., C. E. Aston, et al. (1999). "A novel mutation in the apolipoprotein E gene (APOE*4 Pittsburgh) is associated with the risk of late-onset Alzheimer's disease." Neuroscience Letters **263**(2-3): 129.

<http://www.sciencedirect.com/science/article/B6T0G-3W4XHF1-G/2/8a804ab054c32a209c722c34b9e53825>

Using a combination of polymerase chain reaction (PCR), single-strand conformation polymorphism (SSCP) and DNA sequencing techniques, we identified a unique missense mutation (T->C) in exon 3 of the APOE gene which resulted in the substitution of pro-28 for leu-28. We screened 1118 White cases of late-onset (>60 years) Alzheimer's disease (AD) from three independent centers (Pittsburgh=489, Indiana=319, Mayo Clinic Rochester=310) and 1123 controls (607 clinically assessed and 516 individuals randomly ascertained from the general population). Two of the 1123 control subjects had the pro-28 mutation (0.18%). However, this mutation was observed in heterozygous state in 2.66, 2.51 and 1.94% of the AD cases from Pittsburgh, Indiana and Mayo Clinic Rochester, respectively, with an overall frequency of 2.42%. All individuals with this mutation were carriers of the APOE*4 allele and hence the mutation was denoted as APOE*4 Pittsburgh (APOE*4P). Compared with the non-E*4P carriers, the E*4P carriers were associated with an increased risk of AD (odds ratio (OR) 13.2) and this risk remained significant even after adjusting for the known effect of APOE*4 (OR 5.4). The risk associated with the E*4P/E*4 combination was about five times (OR 29.1) the risk attributed to APOE*4 carriers alone (OR 5.7). Our data indicates that the new mutation most likely exists in cis-orientation with APOE*4 and is associated with increased risk of developing AD.

Kamino, K., S. Sato, et al. (1996). "Three different mutations of presenilin 1 gene in early-onset Alzheimer's disease families." Neuroscience Letters **208**(3): 195.

<http://www.sciencedirect.com/science/article/B6T0G-3VXJJY5-F/2/35112c05f86a1d27e3ea73dc968bc8ab>

Presenilin-1 (PS-1) gene of three Japanese pedigrees with early-onset familial Alzheimer's disease (FAD) disclosed two novel missense mutations resulting in Va196Phe and Ile213Thr, and one mutation resulting in His163Arg. The mean age at onset in a family with His163Arg mutation was similar to those reported in other families with His163Arg. Our results suggested the existence of a variety of PS-1 mutations, and that early-onset FAD with PS-1 mutations is highly penetrant and is only rarely subject to modulation by genetic or environmental modifying factors.

Kanenishi, K., M. Ueno, et al. (2004). "Prepro-orexin mRNA expression in the rat brain is increased during pregnancy." Neuroscience Letters **368**(1): 73.

<http://www.sciencedirect.com/science/article/B6T0G-4D3B3V4-4/2/69caf10c4820e608ee6102a01be4e8ca>

The purpose of this study was to investigate whether orexin expression in the rat brain was

changed during pregnancy. Brain samples were obtained from 5 nonpregnant rats and 10 pregnant rats (5; day 10 of gestation, and 5; day 20 of gestation). Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis was performed to investigate the expression of prepro-orexin mRNA and the housekeeping gene in the rat brain. The signals were quantified by the densitometric analysis. The distribution and expression of orexin-A and orexin-B were determined using immunohistochemistry. The ratio of the prepro-orexin mRNA expressions to the housekeeping gene expression in pregnant rat brain were significantly higher than that in nonpregnant control. There was no significant difference between prepro-orexin mRNA levels of day 10 and day 20 of gestation. Immunohistochemical staining for orexin-A and orexin-B was present in neurons within and around the lateral and posterior hypothalamic areas in both nonpregnant and pregnant rats. These results suggest that increased prepro-orexin mRNA levels at early gestational age in the maternal rat has a role on energy metabolism during pregnancy.

Kawakami, T., Y. Wakabayashi, et al. (2002). "Expression of neurotrophin messenger RNAs during rat urinary bladder development." Neuroscience Letters **329**(1): 77.

<http://www.sciencedirect.com/science/article/B6T0G-46F6ST6-5/2/9a59d1eec4678f058d9f93c3e45b1210>

The family of neurotrophins, encompassing nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5), is important in the regulation of neuronal development and function. We examined the expression of neurotrophin messenger RNAs (mRNAs) in the rat urinary bladder during pre- and postnatal development using competitive reverse transcription-polymerase chain reaction. The mRNA levels showed a biphasic pattern of expression; one peak was at prenatal ages (embryonic day (E)15-E18) and the other peak was at postnatal ages (postnatal day (P)14-P28). NT-4/5, BDNF and NGF mRNA levels were greatest at E15, E16 and E18, respectively. In contrast, NT-3 mRNA levels were significantly highest at P14. These data suggest that neurotrophins are involved in the mechanisms of bladder nerve growth for the prenatal period and reorganization of bladder reflex pathways during the second to the fourth postnatal week.

Ke, C., W. S. Poon, et al. (2001). "Heterogeneous responses of aquaporin-4 in oedema formation in a replicated severe traumatic brain injury model in rats." Neuroscience Letters **301**(1): 21.

<http://www.sciencedirect.com/science/article/B6T0G-42FS95B-6/2/ff4c014a14a8468aa237e061a5c641a0>

Aquaporin-4 (AQP4) is the most abundant water channel in the rat brain. In this study, the distribution pattern and mRNA expression levels of AQP4 were examined in a severe traumatic brain injury model by immunohistochemistry and reverse transcription-polymerase chain reaction. Oedema formation and blood-brain barrier (BBB) integrity were assessed by wet-dry weight measurements and immunostaining of endogenous IgG respectively. In the oedematous contusional cortex with impaired BBB integrity, negative immunostaining of AQP4 and down-regulation of its mRNA level were identified ($P < 0.05$) at 1 day post-injury, while in other oedematous regions of the injured brain where BBB was intact, there was no significant change in the AQP4 expression level. This heterogeneous pattern of AQP4 responses can be interpreted as follows: focal brain injury (such as a contusion) with impaired BBB resulting in vasogenic oedema is associated with reduction of AQP4 expression, whereas, in cytotoxic oedema, associated with diffuse brain injury with intact BBB, changes in AQP4 expression are not significant. This study provides basic information for investigating new treatments for traumatic brain oedema.

Kolko, M., E. B. R. de Turco, et al. (2003). "Neuronal damage by secretory phospholipase A2: modulation by cytosolic phospholipase A2, platelet-activating factor, and cyclooxygenase-2 in neuronal cells in culture." Neuroscience Letters **338**(2): 164.

<http://www.sciencedirect.com/science/article/B6T0G-47S6YPN-K/2/72b52f3e2f227699e4e9b30e465c24d8>

Activation of cytosolic phospholipase A2 (cPLA2) is an early event in brain injury, which leads to the formation and accumulation of bioactive lipids: platelet-activating factor (PAF), free arachidonic acid, and eicosanoids. A cross-talk between secretory PLA2 (sPLA2) and cPLA2 in neural signal transduction has previously been suggested (J Biol Chem 271:32722; 1996). Here we show, using neuronal cell cultures, an up-regulation of cPLA2 expression and an inhibition by the selective cPLA2 inhibitor AACOCF3 after exposure to neurotoxic concentrations of sPLA2-OS2. Pretreatment of neuronal cultures with recombinant PAF acetylhydrolase (rPAF-AH) or the presynaptic PAF receptor antagonist, BN52021, partially blocked neuronal cell death induced by sPLA2-OS2. Furthermore, selective COX-2 inhibitors ameliorated sPLA2-OS2-induced neurotoxicity. We conclude that sPLA2-OS2 activates a neuronal signaling cascade that includes activation of cPLA2, arachidonic acid release, PAF production, and induction of COX-2.

Kubota, Y., C. Ito, et al. (1999). "Transient increases of histamine H1 and H2 receptor mRNA levels in the rat striatum after the chronic administration of methamphetamine." Neuroscience Letters **275**(1): 37.

<http://www.sciencedirect.com/science/article/B6T0G-3XP0GK9-B/2/e29eb9c7d050121ced8b7857b03079ab>

In our previous studies, we found that behavioral sensitization evoked by repeated administration of methamphetamine (METH) was suppressed by the activation of the histaminergic neuron system in the brain. In continuation of these studies, we measured the levels of H1 and H2 receptor mRNAs in the rat striatum by semi-quantitative reverse transcription-polymerase chain reaction. Seven days after the 21 consecutive administrations of METH (4 mg/kg, i.p.), the levels of both H1 and H2 receptor mRNAs in the rat striatum increased significantly. However, 1 and 14 days after the last administration, there were no significant changes in levels of either H1 or H2 receptor mRNA in the rat striatum. These transient increases of H1 and H2 receptor mRNAs may have some relation to chronic METH abuse and its withdrawal.

Kummer, W., R. Shigemoto, et al. (1999). "Smooth muscle cells are the site of neurokinin-1 receptor localization in the arterial supply of the rat sciatic nerve." Neuroscience Letters **259**(2): 119.

<http://www.sciencedirect.com/science/article/B6T0G-3VCMTPS-2C/2/7cb2b2eab62d30d90b0f74d58686ce5d>

The occurrence and distribution of the preferred receptor for the neuropeptide, substance P (SP), the neurokinin-1 receptor (NK1R) was investigated in the vascular supply of the rat sciatic nerve. Messenger RNA for NK1R was demonstrated by RT-PCR in the epineurial layer where the majority of small arteries and arterioles feeding the endoneurial vasculature are located. Immunoreactivity to NK1R-protein was localized on the smooth muscle cells of these arterial vessels by means of immunofluorescence using a polyclonal NK1R antiserum. This muscular localization of NK1R explains the previously reported [Zochodne, D.W. and Ho, L.T., J. Physiol.,

444 (1991) 615-630] moderate vasoconstrictor rather than vasodilator effects of SP in this vascular bed.

Lattuada, E., R. Cavallaro, et al. (2004). "Genetic dissection of drug effects in clinical practice: CLOCK gene and clozapine-induced diurnal sleepiness." Neuroscience Letters **367**(2): 152.

<http://www.sciencedirect.com/science/article/B6T0G-4D09M7P-1/2/515fa9ee5b79f26fbd7fae5c5113506f>

Psychotic patients treated with clozapine often experience persistent daytime sleepiness. This is a frequent side effect of clozapine that may reduce patient compliance. We hypothesized that clozapine might interfere with the circadian rhythms regulated by the biological clock. In 171 patients with major psychosis, we investigated the association between hypersomnolence during clozapine therapy and a CLOCK gene polymorphism (3111 T/C substitution). Forty-six patients showed persistent daytime sleepiness and were classified as "sleepy". "Sleepy" patients were significantly more likely to have a mutated allele compared to both "non sleepy" patients and healthy subjects ($[\chi^2] = 20.36$, d.f. = 1, $P = 0.000007$, and $[\chi^2] = 13.91$, d.f. = 1, $P = 0.0002$, respectively). We conclude that an interaction between clozapine and the CLOCK gene polymorphism 3111 T/C substitution could explain persistent daytime sleepiness in a significant proportion of patients treated with clozapine.

Leitner, B., C. Schneitler, et al. (1998). "Formation and sequence analysis of secretoneurin, a neuropeptide derived from secretogranin II, in mammalian, bird, reptile, amphibian and fish brains." Neuroscience Letters **248**(2): 105.

<http://www.sciencedirect.com/science/article/B6T0G-3T11VR4-9/2/84527275253272edc8755a00be75ca79>

Secretoneurin is a recently-characterized neuropeptide derived from secretogranin II, a protein belonging to the class of chromogranins. We investigated the phylogeny of this peptide by immunoblotting and gel-filtration high performance liquid chromatography followed by radioimmunoassay of brain extracts of various species including chicken, lizard, frog and fish. In addition the amino acid sequence of secretoneurin from pig, hamster, rabbit, guinea-pig and chicken was established by reverse transcriptase polymerase chain reaction. Secretoneurin is strongly conserved during evolution, it is not only expressed in various mammalian species but found also in the brain of birds, reptiles, amphibians and fish. In all these species a significant or near complete processing of secretogranin II to secretoneurin was observed. These data provide significant evidence for the neuropeptide nature of the novel functional peptide.

Li, N., C.-Y. Fang, et al. (2004). "Expression of calcitonin gene-related peptide type 1 receptor mRNA and their activity-modifying proteins in the rat nucleus accumbens." Neuroscience Letters **362**(2): 146.

<http://www.sciencedirect.com/science/article/B6T0G-4C5PXN1-D/2/9b1f85c8eb2a657726dc7507ea15ef35>

The calcitonin receptor-like receptor (CRLR) and the orphan receptor RDC-1 have been proposed to be calcitonin gene-related peptide type 1 (CGRP1) receptors, and receptor activity-modifying proteins (RAMPs) determine the ligand specificity of CRLR. Coexpression of RAMP1 and CRLR resulted in functional CGRP1 receptors; the complex of RAMP2 or RAMP3 and CRLR

created functional adrenomedullin receptor. Although high levels of CGRP binding sites in the nucleus accumbens have been reported, little is known about the expression of these novel CGRP receptors. In the present study, we used real-time quantitative RT-PCR to detect and quantitate the relative expression of CGRP, CRLR, RAMP1-3 and RDC-1 in the nucleus accumbens of intact rats and rats with inflammation. Our results demonstrate that CGRP, CRLR, RAMP1 and RAMP2 exist in the nucleus accumbens of intact rats, and that they were significantly upregulated in rats with inflammation. In contrast, no expression was detected for RDC-1 and RAMP3. These findings indicated a functional role for CGRP and its receptors in inflammation and pain modulation.

Li, X. and P. B. DePetrillo (2002). "Corticosterone increases serotonin type-3 receptor mRNA in rat pheochromocytoma-12 cells." Neuroscience Letters **331**(2): 143.

<http://www.sciencedirect.com/science/article/B6T0G-46VJ9X0-N/2/3c375b6ac4b101ff8a8953539b412363>

Serotonin type-3 (5-HT₃) receptors are cation permeable membrane receptors which are involved in modulation of calcium entry in neuronal cells. Along with other ion-channels such as the N-methyl--aspartate receptor, it appears to be a target for the actions of ethanol and has been the focus of considerable work in this regard. Since in animals, ethanol exposure results in elevations of corticosteroids in both acute and chronic conditions, we studied the effects of both ethanol and corticosteroid exposure on 5-HT₃ gene expression in an in situ pheochromocytoma-12 (PC12) cell model. We found that ethanol exposure alone (80 mM x 4 days) did not significantly alter target gene expression. Corticosterone (CORT) (50, 150, and 300 ng/ml) resulted in significant increases in 5-HT₃ expression which were attenuated by mifepristone (50 ng/ml). Ethanol in combination with CORT did not significantly alter the increase in 5-HT₃ mRNA seen with CORT alone. We conclude that in PC12 cells, exposure to CORT at physiologically relevant concentrations increases 5-HT₃ gene expression.

Liang, F.-Q., R. Walline, et al. (1998). "Circadian rhythm of brain-derived neurotrophic factor in the rat suprachiasmatic nucleus." Neuroscience Letters **242**(2): 89.

<http://www.sciencedirect.com/science/article/B6T0G-3S3DP74-8/2/ca6768ed2cf8871df4bb6ed6f3a416d6>

This study was conducted to determine whether the rat suprachiasmatic nucleus (SCN) is characterized by circadian expression of brain-derived neurotrophic factor (BDNF). In constant darkness, SCN content of both BDNF mRNA and protein oscillated in a circadian fashion. BDNF mRNA and protein levels in the SCN reached peak values during the early subjective day and during the subjective night, respectively. In contrast, the hippocampus showed no sign of circadian rhythmicity in its expression of BDNF mRNA and protein. Since BDNF enhances synaptic transmission in other brain regions, the coincidence between peak expression of BDNF protein in the SCN and the known interval of circadian pacemaker sensitivity to the phase-shifting effects of light may have some implications for the role of BDNF in the circadian regulation of the SCN pacemaker by photic signals from the retinohypothalamic tract.

Lin, H., N. Hikawa, et al. (2000). "Interleukin-12 promotes neurite outgrowth in mouse sympathetic superior cervical ganglion neurons." Neuroscience Letters **278**(3): 129.

<http://www.sciencedirect.com/science/article/B6T0G-3Y6PTPB-2/2/56294a334b631069b368d9d79c849f97>

To determine the role of cytokines in the nervous system, we examined the effect of interleukin-12(IL-12) on the nerve regeneration of mouse superior cervical ganglion cells (SCG). IL-12 enhanced the neurite outgrowth in a concentration-dependent manner. Immunocytochemical studies demonstrated the expression of IL-12 receptors in neuronal bodies and neurites. The mRNA expression of IL-12 receptors in SCG cells was confirmed by reverse transcription-polymerase chain reaction. Our data demonstrated the presence of IL-12 receptors in sympathetic neurons and suggest that IL-12 plays an important role in neuronal regeneration.

Liu, X., G. He, et al. (2004). "Association of DAAO with schizophrenia in the Chinese population." Neuroscience Letters **369**(3): 228.

<http://www.sciencedirect.com/science/article/B6T0G-4DBKH8W-5/2/ee862af9abb42edcbaa371ac209139ee>

Recently, the gene called DAAO was reported to be associated with schizophrenia in the French Canadian populations. Here, we report a result obtained in the study of our large collection of 547 schizophrenia cases and 536 controls in the Chinese population. Six single-nucleotide polymorphisms (SNPs) were genotyped at and around the DAAO locus, covering a 10-kb region entirely encompassing the complementary DNA sequences of DAAO. We found statistically significant differences in allele distributions on one marker: SNP rs3741775 ($P = 0.0000001$). In the haplotype analysis based on the information of linkage-disequilibrium block across this gene locus, we demonstrated a highly significant association between schizophrenia and a DAAO haplotype ($P = 2.0173 \times 10^{-21}$), which therefore provides an independent statistical support for association of the DAAO gene with schizophrenia and indicates that the DAAO gene may play a significant role in the etiology of schizophrenia in the Han Chinese.

Martinez-Torres, F. J., S. Wagner, et al. (2004). "Increased presence of matrix metalloproteinases 2 and 9 in short- and long-term experimental herpes simplex virus encephalitis." Neuroscience Letters **368**(3): 274.

<http://www.sciencedirect.com/science/article/B6T0G-4DC6590-1/2/d2cd2fa0cf634de335ef9cf1aeaa3dfc>

Herpes simplex virus encephalitis (HSVE) causes elevated morbidity and mortality despite antiviral treatment. Virus-independent mechanisms may perpetuate brain damage. Matrix metalloproteinases (MMPs) target extracellular matrix components. This study describes the protein and mRNA expression of MMP2 and MMP9 in experimental HSVE in the short and long term. Ten SJL-NBOM mice were infected with neurovirulent HSV-1 and compared with nine controls. The presence of MMP2 and MMP9 in brain tissue was analyzed with sodium-dodecyl-sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gelatin zymography and mRNA expression of MMP2 and MMP9 with quantitative real-time PCR at days 7, 21 and 180 post-inoculation. Infected animals had a significantly elevated gelatinolytic activity of MMP2 at all time points, and of MMP9 at days 21 and 180. Increased presence of MMP2 and MMP9 in chronic HSVE may contribute to ongoing damage. Inhibition of MMP2 and MMP9 might be a suitable target for therapeutic intervention.

Meyding-Lamade, U., J. Haas, et al. (1998). "Herpes simplex virus encephalitis: long-term comparative study of viral load and the expression of immunologic nitric oxide synthase in mouse brain tissue." Neuroscience Letters **244**(1): 9.

<http://www.sciencedirect.com/science/article/B6T0G-3S83B02-3/2/525255694a13783826e3572a2ba6f42d>

In the brain tissue of 21 mice infected with herpes simplex virus type 1 (HSV-1) strain F we determined the expression of immunologic nitric oxide synthase (iNOS) as a potential mediator of neuronal injury with a semiquantitative reverse transcription polymerase chain reaction. Viral burden in brain tissue was quantitated with a dilutional polymerase chain reaction assay. Viral burden and iNOS-expression peaked at day 7 following infection. Thereafter viral burden declined to a low baseline value at 6 months following infection, whereas iNOS-expression was still 4-fold increased compared to baseline levels. In experimental herpes simplex virus encephalitis iNOS, as one potent mediator of neuronal injury, is upregulated in the acute and chronic disease. In future, in addition to antiviral treatment, inhibitors of iNOS might offer new therapeutic strategies in herpes simplex virus encephalitis.

Meyding-Lamade, U., S. Seyfer, et al. (2002). "Experimental herpes simplex virus encephalitis: inhibition of the expression of inducible nitric oxide synthase in mouse brain tissue." Neuroscience Letters **318**(1): 21.

<http://www.sciencedirect.com/science/article/B6T0G-44J3V24-5/2/739393acb1d9466f1bb0b1d1c504ca7a>

In the brain tissue of 36 mice infected with herpes simplex virus type 1, strain F, we determined the expression of inducible nitric oxide synthase (iNOS) with semiquantitative reverse transcription polymerase chain reaction. The viral burden was quantitated by polymerase chain reaction. Nitric oxide, induced by iNOS, may contribute to neuronal cell damage following virus infection. As the experimental therapeutic strategy in herpes simplex virus encephalitis (HSVE), we used: N-nitro--arginin (L-NA), a selective inhibitor of iNOS; and combination therapies of either methylprednisolone/acyclovir or L-NA/acyclovir. The viral burden peaked in acute disease, and then returned to a low baseline value, except in untreated controls. The expression of iNOS mRNA was suppressed by L-NA and by acyclovir/corticosteroids. INOS inhibition may provide an additional therapeutic strategy targeted specifically to suppress iNOS expression as a potential secondary mechanism of tissue damage in acute and chronic HSVE.

Mika, J., Y. Li, et al. (2003). "Relationship of pronociceptin/orphanin FQ and the nociceptin receptor ORL1 with substance P and calcitonin gene-related peptide expression in dorsal root ganglion of the rat." Neuroscience Letters **348**(3): 190.

<http://www.sciencedirect.com/science/article/B6T0G-49621GK-6/2/b09967500e581df276c4b191d5e66a93>

Recent evidence suggests a role of prepronociceptin/orphanin FQ (preproN/OFQ) derived neuropeptides in nociceptive signaling. Here, we examined the expression of preproN/OFQ and the nociceptin receptor ORL1 (opioid receptor like receptor 1) in the dorsal root ganglion (DRG) of the rat in relation to that of substance P (SP) and calcitonin gene-related peptide (CGRP). Double labeling in situ hybridization revealed a constitutive expression of preproN/OFQ in a distinct minor subpopulation of very small DRG neurons with no evidence for coexpression with either SP or CGRP. However, a major subpopulation of the preproN/OFQ-positive neurons showed direct

juxtaposition to SP and CGRP containing neurons. ORL1 was abundantly expressed with a high degree of coexpression with SP (72%) and CGRP (82%) suggesting that N/OFQ may presynaptically modulate primary sensory nociceptive signaling. The DRG cell line F11 was found to express preproN/OFQ, but not ORL1, and, therefore, is well suited to study the mechanisms of N/OFQ gene regulation in vitro.

Minami, M., H. Yamakuni, et al. (2001). "Leukemia inhibitory factor induces nociceptin mRNA in cultured rat cortical neurons." Neuroscience Letters **311**(1): 17.

<http://www.sciencedirect.com/science/article/B6T0G-43X77PX-5/2/82c99925de751c320916c8ca40006990>

The effects of leukemia inhibitory factor (LIF) on the expression of neurotransmitter synthetase and neuropeptide mRNAs in cultured rat cortical neurons were examined by reverse transcription-polymerase chain reaction. Nociceptin mRNA expression was increased by treatment with 20 or 80 ng/ml LIF for 24 h, but choline acetyl transferase, glutamic acid decarboxylase, enkephalin, dynorphin, substance P, somatostatin and galanin mRNA expression were not altered by LIF. These observations indicated a specific effect of LIF on nociceptin gene regulation in cultured cortical neurons.

Miyazaki, I., C. A. Sogawa, et al. (2000). "Expression of metallothionein-III mRNA and its regulation by levodopa in the basal ganglia of hemi-parkinsonian rats." Neuroscience Letters **293**(1): 65.

<http://www.sciencedirect.com/science/article/B6T0G-41BV6TT-K/2/b78c9ec58dea8e60f10a18c2f7699a09>

In the brain, metallothionein (MT)-III exhibits a free radical scavenging activity. Here we examined the expression of MT-III mRNA in the basal ganglia of 6-hydroxydopamine (6-OHDA)-lesioned hemi-parkinsonian rats and its regulation by levodopa. The level of MT-III mRNA was significantly decreased in the striatum of 6-OHDA-lesioned side. Levodopa treatment significantly increased the expression of striatal MT-III mRNA in the non-lesioned side, but showed no significant effect in the 6-OHDA-lesioned side. These results suggest that the regulation of MT-III mRNA may be related to the progressive degeneration in parkinsonism.

Nagai, M., K. Abe, et al. (1998). "Identification of alternative splicing forms of GLT-1 mRNA in the spinal cord of amyotrophic lateral sclerosis patients." Neuroscience Letters **244**(3): 165.

<http://www.sciencedirect.com/science/article/B6T0G-3SBVRX6-D/2/81aa544f0b50bbd97935c03d7d23adb1>

The glutamate transporter plays an essential role in regulating glutamate levels in the synaptic cleft. It has been postulated that the dysfunction of GLT-1, one subtype of glutamate transporter, may be etiologically related to amyotrophic lateral sclerosis (ALS). Two alternative splicing forms of GLT-1 messenger RNA (mRNA) were found in the cervical spinal cord of five ALS patients and three controls. Analysis with reverse transcription-polymerase chain reaction (RT-PCR) showed that the shorter mRNA was a result of exon 8 skipping. A truncated transcript containing an intronic sequence at the 3' end of exon 7 was also demonstrated. However, the incidence of both alternative mRNAs was not different between the five ALS patients and three controls. Interestingly, the mRNA were also found in the cerebral cortex of a control subject. These results

suggest that alternative splicing forms of GLT-1 mRNAs do not play a pathogenetic role in ALS but rather a physiological one in the normal spinal cord and brain.

Nakayama, J., K. Hamano, et al. (2003). "Failure to find causal mutations in the GABAA-receptor [gamma]2 subunit (GABRG2) gene in Japanese febrile seizure patients." Neuroscience Letters **343**(2): 117.

<http://www.sciencedirect.com/science/article/B6T0G-48BV2B1-G/2/94900ff4b087fd00f8befb77fdc8ba30>

Recently, mutations in the GABAA-receptor [gamma]2 subunit (GABRG2) gene were identified in two families with generalized epilepsy with febrile seizures plus (GEFS+) and two families with childhood absence epilepsy (CAE) and febrile seizures (FS). We tested the hypothesis that genetic variations in the GABRG2 gene confer susceptibility to FS in the Japanese population. We performed a systematic search for mutations in 94 unrelated Japanese patients with FS and detected six variants (-158C>T, 315C>T, 588T>C, IVS5-55C>T, IVS7+20G>A, and IVS7-141T>A). No non-synonymous mutation was detected. We genotyped three exonic polymorphisms and performed a case control study and a transmission disequilibrium test using 55 independent complete trios with FS and 106 control subjects. None of these polymorphic alleles were significantly associated with FS. Our results indicate that genomic variations of GABRG2 are not likely to be substantially involved in the etiology of FS in the Japanese population.

Nilsson, T., L. Cantera, et al. (1996). "Presence of neuropeptide Y Y1 receptor mediating vasoconstriction in human cerebral arteries." Neuroscience Letters **204**(3): 145.

<http://www.sciencedirect.com/science/article/B6T0G-3VXH1J6-1/2/d9db40a93d871ad241dcff624e0500cd>

The neuropeptide Y-induced vasoconstriction of human cerebral arteries is mediated by the neuropeptide Y Y1 receptor. We conclude this on the basis of our results from: (1) in vitro studies on neuropeptide Y agonists. Neuropeptide Y and pro34NPY caused potent and long-lasting contractions of human cerebral arteries, while NPY 13-36 had no contractile effect at all on the vessels tested; (2) in vitro studies using the selective Y1 receptor antagonist BIBP3226 which in increasing concentrations (10⁻⁹-10⁻⁶M) caused a parallel shift to the right of the neuropeptide Y concentration-response curve without change of the maximum contractile response (pA₂ value 8.38±0.10); and (3) with reverse transcriptase-polymerase chain reaction (RT-PCR) we detected specific mRNA for a neuropeptide Y Y1 receptor in human pial and human middle cerebral arteries using three forward primers and one reverse primer.

Park, K., J. H. Kim, et al. (2000). "Vascular endothelial growth factor expression under ischemic stress in human meningiomas." Neuroscience Letters **283**(1): 45.

<http://www.sciencedirect.com/science/article/B6T0G-3YVDN5F-D/2/e22c9d5ac6e3dc1f3fce6d88db2e5efb>

Vascular endothelial growth factor (VEGF) is an endothelial cell-specific antigen and angiogenic factor that plays a role in angiogenesis. We analyzed the expression of four VEGF mRNA isoforms in meningiomas. Among 35 meningiomas, 11 came from patients who underwent

complete (n=4) or partial (n=7) preoperative embolization. Northern blotting revealed markedly elevated expression of total VEGF mRNA in meningiomas compared with normal brain tissues. Semiquantitative reverse transcription-polymerase chain reaction revealed that the four isoforms are expressed with relative levels of VEGF121>165>206=189 in all samples. However, the VEGF121 and 165 isoforms were significantly upregulated in samples from patients who underwent partial preoperative embolization. The diffusible VEGF121 isoform may be important for vascularity and edema formation in meningiomas.

Poduslo, S. E. and X. Yin (2001). "Chromosome 12 and late-onset Alzheimer's disease." Neuroscience Letters **310**(2-3): 188.

<http://www.sciencedirect.com/science/article/B6T0G-43W0VH8-11/2/5f9f9b0622974ab8f760f9bbf768a624>

Alzheimer's disease is a complex neurodegenerative disorder, characterized by cognitive decline and distinctive neuropathology. Using large extended families with multiple affected, we found that three markers on chromosome 12 were linked with late-onset Alzheimer's disease. These markers were downstream from the gene for alpha-2 macroglobulin. It is likely that multiple genes will be identified either as risk factors or as causative agents for late-onset Alzheimer's disease.

Pola, R., A. Flex, et al. (2002). "Association between intercellular adhesion molecule-1 E/K gene polymorphism and probable vascular dementia in humans." Neuroscience Letters **326**(3): 171.

<http://www.sciencedirect.com/science/article/B6T0G-45MDW6Y-2/2/2e1c34d0e559e4967d76d53ba9bb6c57>

Intercellular adhesion molecule-1 (ICAM-1) is implicated in the pathogenesis of ischemic cardiovascular disorders, including cerebral ischemia. A common polymorphism of the ICAM-1 gene (K469E) has been recently reported. In this case-control study, we evaluated the association between this polymorphism and vascular dementia (VD) by studying 107 patients affected by probable VD and 115 age- and sex-matched controls. The frequency of the EE genotype was significantly higher in VD patients than controls (P=0.009). Logistic regression analysis indicated that the presence of the EE genotype significantly increased the risk of VD (odds ratio 3.25, P=0.024). Our findings support the hypothesis that ICAM-1 plays a role in the physiopathology of ischemic cerebrovascular disorders and suggest that genetic polymorphisms of ICAM-1 might be clinically important in the development and progression of neurodegenerative diseases.

Pregenzer, J. F., G. L. Alberts, et al. (1997). "Characterization of ligand binding properties of the 5-HT1D receptors cloned from chimpanzee, gorilla and rhesus monkey in comparison with those from the human and guinea pig receptors." Neuroscience Letters **235**(3): 117.

<http://www.sciencedirect.com/science/article/B6T0G-3TJC7K5-V/2/be3e465042be2a2f4aface5df02daa6e>

The 5-HT1D receptor is a potential target of anti-migraine drugs, and here its genes were cloned from chimpanzee, gorilla and rhesus monkey, via polymerase chain reactions with their genomic DNAs and the primers designed from the 5' and 3' untranslated regions of the human receptor. Direct sequencing of the polymerase chain reaction (PCR) products revealed high degrees of

identity between their deduced amino acid sequences (the chimpanzee, gorilla and rhesus monkey) and that of human, differing by two, four and 11 residues, respectively. The binding properties of the receptors, as expressed in human embryonic kidney 293 cells, were compared to those obtained with the human and guinea pig receptors, the latter differing by 33 residues from the human receptor. Standard serotonergic ligands including several indoles, ergots and methiothepin bound all the cloned primate and guinea pig receptors with comparable, low nanomolar affinities, leading to high correlation coefficients among their K_i values. R(+)-8-Hydroxydipropylaminotetralin, on the other hand, bound the human receptor with the affinity higher than those for the primates and guinea pig receptors. This indicates that certain chemical templates may differentiate the molecular divergences among the 5-HT_{1D} receptors of various animal species, and the use of the non-human primates will be beneficial for pharmacological characterizations, more relevant to the human receptor, of future novel ligands for the 5-HT_{1D} receptor, which are potential anti-migraine drugs.

Pun, S. and K. W. K. Tsim (1995). "Truncated form of pro-acetylcholine receptor-inducing activity (ARIA) induces AChR [alpha]-subunit but not AChE transcripts in cultured chick myotubes." Neuroscience Letters **198**(2): 107.

<http://www.sciencedirect.com/science/article/B6T0G-3YB9Y52-1N/2/712daefdf2e9c7d9cb0557419f6f0554>

Acetylcholine receptor-inducing activity (ARIA) is a glycoprotein initially purified from chick brain based on its ability to increase the synthesis of acetylcholine receptor (AChR). We used reverse transcription-polymerase chain reaction (RT PCR) to obtain a partial pro-ARIA cDNA clone from methionine-1 to serine-358 including the full functional sequence of ARIA. Northern blot analysis of mRNAs from the embryonic chick brain and muscle showed a transcript with a size of ~7.5 kb. The cloned cDNA was subcloned into an eukaryotic expression vector and stably transfected into human embryonic kidney 293 cells. The conditioned medium of the transfected cells was found to increase the level of transcript encoding for the [alpha]-subunit of AChR by ~4.4-fold, but not for acetylcholinesterase (ACNE), in the cultured chick myotubes.

Qin, W., J. Gao, et al. (2005). "A family-based association study of PLP1 and schizophrenia." Neuroscience Letters **375**(3): 207.

<http://www.sciencedirect.com/science/article/B6T0G-4DXWMBT-4/2/9589ed5318dfa311401f83d831a54732>

Recently, proteolipid protein 1 (PLP1) has been identified as downregulated in schizophrenia by quantitative PCR and other technologies. In this work we attempted to investigate the role of PLP1 in the etiology of schizophrenia using a family based association study in 487 Chinese Han family trios. The TDT for allelic association demonstrated that, in male, a weak association was detected in SNP rs475827 with $p = 0.0294$, suggesting that the genetic polymorphisms within PLP1 in male are likely to confer an increased susceptibility to schizophrenia in the Chinese population.

Saito, S., M. Ikeda, et al. (2005). "No association was found between a functional SNP in ZDHHC8 and schizophrenia in a Japanese case-control population." Neuroscience Letters **374**(1): 21.

<http://www.sciencedirect.com/science/article/B6T0G-4DPCB36->

2/2/580b439ad197837eee306cf467e0291d

ZDHHC8 is a new and attractive candidate for a schizophrenia-susceptibility factor. First, several lines of linkage studies showed that 22q11, on which ZDHHC8 is located, is a "hot" region. Second, fine linkage disequilibrium mapping revealed a significant association around ZDHHC8. Moreover, a very recent study reported that one single nucleotide polymorphism (SNP: rs175174) in ZDHHC8 might affect the splicing process, the ZDHHC8 knock-out mice showed the gender-specific phenotype, and the transmission disequilibrium test (TDT) using this SNP also showed significant association with human female schizophrenia. Thus, we attempted a replication study of this SNP using relatively large Japanese case-control samples (561 schizophrenics and 529 controls). No association was found between schizophrenia and controls even after dividing samples by gender. Because our sample size provided quite high power, ZDHHC8 may not play a major role in Japanese schizophrenia. And our results did not support the gender-specific effect of this SNP.

Sakamoto, K. and N. Ishida (1998). "Molecular cloning of serotonin N-acetyltransferase gene from the mouse and its daily expression in the retina." *Neuroscience Letters* **250**(3): 181.

<http://www.sciencedirect.com/science/article/B6T0G-3TJC7K5-1N/2/332a3a6659e3b1703168546c681cdac0>

The primary structure of serotonin N-acetyltransferase (arylalkylamine N-acetyltransferase, AA-NAT: the rate-limiting enzyme in melatonin synthesis) in the mouse retina was deduced from the cDNA nucleotide sequence. The deduced protein consisted of 205 amino-acid residues with sequences highly conserved in AA-NATs of vertebrates, and was 96% identical to rat AA-NAT. Northern blot analysis of mouse retinal mRNA showed two obvious bands, of 1.5 kb and 4.5 kb in length. The levels of both transcripts were low at day and high at night, but the night-to-day ratios were <2. These findings suggest that the expression mechanism of AA-NAT transcripts in the mouse retina may be different from those in other mammals, where a single transcript of AA-NAT is normally observed in Northern blots.

Salsano, E., B. Pollo, et al. (2004). "Expression of MATH1, a marker of cerebellar granule cell progenitors, identifies different medulloblastoma sub-types." *Neuroscience Letters* **370**(2-3): 180.

<http://www.sciencedirect.com/science/article/B6T0G-4DBKH8W-D/2/006ddc8c8cfc20c1f85dde2f3d631cbc>

In order to look for genetic markers helpful for the biological risk stratification of medulloblastomas (MBs) we assayed by real-time PCR expression levels of the following genes: MATH1, encoding a critical transcription factor for the differentiation of cerebellar granular cells (CGCs); PEDF, that encodes a trophic factor for CGCs and is located in a region of frequent allelic imbalance in MBs; and BIRC5, encoding the antiapoptotic protein survivin, usually overexpressed in malignancies. Expression levels of TRKC, higher in MBs with a more favorable prognosis, were also studied. Twenty-three patients were considered: MATH1 expression was strong in 14/23 and undetectable in the others. PEDF was up-regulated in 8/23, TRKC in 9/23, and BIRC5 in 23/23. MATH1 expression was significantly correlated with adult age (p p PEDF and TRKC up-regulation (p p MATH1 is selectively expressed in the external germinal layer (EGL) of the cerebellum. Thus, MATH1 expression identifies a subgroup of MBs that derive from the EGL and arise during adult age into cerebellar hemispheres. MATH1 mRNA-positive MBs express high levels of PEDF and show a trend towards longer survival, in agreement with increased expression of TRKC. BIRC5 expression, which is strong in all MBs and absent in normal cerebellum, lacks any prognostic

value but could be explored for selective targeting of therapeutic factors to MBs.

Sato, K. Z., T. Fujii, et al. (1999). "Diversity of mRNA expression for muscarinic acetylcholine receptor subtypes and neuronal nicotinic acetylcholine receptor subunits in human mononuclear leukocytes and leukemic cell lines." Neuroscience Letters **266**(1): 17.

<http://www.sciencedirect.com/science/article/B6T0G-3WRJPMH-5/2/e03d1448f010ed14e942ecfa2b609358>

Previously, we reported that various levels of acetylcholine (ACh), currently known as a neurotransmitter, are detectable in the blood of several mammals including humans and that most blood ACh originates from T-lymphocytes. To investigate whether ACh in the blood acts on lymphocytes and participates in the modulation of immune responses, we have analyzed the expression of mRNA for muscarinic (Ms) ACh receptor subtypes and nicotinic (Nc) ACh receptor subunits using reverse transcription-polymerase chain reaction (RT-PCR) methods. The cells tested were human peripheral mononuclear leukocytes (MNLs) from seven healthy donors and eight leukemic cell lines, as models of lymphocytes. We detected mRNA expression for various neuronal Nc receptor subunits and Ms receptor subtypes in all of the MNL samples and in all of the cell lines tested. However, the expression pattern of mRNA for neuronal Nc receptor subunits ([alpha]2-[alpha]7 and [beta]2-[beta]4) and Ms receptor subtypes (m1-m5) varied among the individuals and cell lines. No expression of mRNA for three muscle-type Nc receptor subunits ([alpha]1, [beta]1 and [epsilon]) was observed in the MNLs and cell lines. These results indicate that both neuronal-type Nc and Ms ACh receptors are present on the surface of lymphocytes.

Sellner, J., F. Dvorak, et al. (2005). "Acute and long-term alteration of chemokine mRNA expression after anti-viral and anti-inflammatory treatment in herpes simplex virus encephalitis." Neuroscience Letters **374**(3): 197.

<http://www.sciencedirect.com/science/article/B6T0G-4DXBTX8-2/2/7a80ead9f3f76fddba5090c40466bcec>

Mortality and morbidity rates remain high among patients with herpes simplex virus encephalitis (HSVE). Chemokine-mediated recruitment and activation of leukocytes to focal areas of viral CNS infection are crucial steps in antiviral response and clearance. However, the inflammatory reaction and cellular antiviral response may enhance collateral damage to neurons and account for chronic progressive brain damage. We identified a specific mRNA expression of the interferon-gamma-inducible chemokines (CXCL9, CXCL10 and CXCL11), and RANTES (CCL5) in the acute course and long-term of experimental HSVE. This pattern was substantially altered by anti-viral and anti-inflammatory treatment. Our findings indicate a pivotal role of these chemokines in the immunopathogenesis of HSVE.

Small, D. L., M. O. Poulter, et al. (1997). "Alteration in NMDA receptor subunit mRNA expression in vulnerable and resistant regions of in vitro ischemic rat hippocampal slices." Neuroscience Letters **232**(2): 87.

<http://www.sciencedirect.com/science/article/B6T0G-3PV1RMM-2F/2/3bba2b59bb2ff4c6662137009064e72a>

Brain insults, including cerebral ischemia, can alter glutamate receptor subunit expression in

vulnerable neurons. Understanding these post-ischemic changes in glutamate receptors could enhance our ability to identify specific, novel neuroprotective compounds. Reverse transcription-polymerase chain reaction (RT-PCR) amplification was used to quantify the altered expression of the N-methyl-D-aspartate (NMDA) NR2A, NR2B and NR2C subunits relative to one another in rat hippocampal slices in resistant and vulnerable regions following in vitro oxygen-glucose deprivation. Ninety minutes after re-oxygenation and return to 10 mM glucose, there was a significant increase in the expression of NR2C relative to NR2B and NR2A in the slice as a whole, as well as in the selectively vulnerable CA1 region and the resistant CA3 and dentate gyrus regions.

Suzuki, M., T. Yonezawa, et al. (2001). "Induction of granulin precursor gene expression by estrogen treatment in neonatal rat hypothalamus." Neuroscience Letters **297**(3): 199.

<http://www.sciencedirect.com/science/article/B6T0G-41Y838R-G/2/809a79402f7590076b4d3386721ff44a>

Our previous research has demonstrated that androgen treatment during the perinatal period increases granulin (grn) precursor mRNA levels in the neonatal rat hypothalamus. To elucidate whether exogenous estrogen increases grn mRNA in the neonatal hypothalamus, expression of grn gene in the neonatal hypothalamus was studied by the competitive reverse transcription-polymerase chain reaction method. At 6 and 10 days of age, grn gene expression was significantly increased in the hypothalamus of pups whose dam has been dietarily administered ethinyl estradiol from day 15 of gestation to the day of sampling. The subcutaneous injection of estradiol benzoate to neonatal rats at 2 days of age significantly increased grn gene expression on day 10. It was shown that estrogen, as well as androgen, was able to induce grn gene expression in the neonatal hypothalamus.

Sylvester, J. B., J. Mwanjewe, et al. (2001). "Transient receptor potential protein mRNA expression in rat substantia nigra." Neuroscience Letters **300**(2): 83.

<http://www.sciencedirect.com/science/article/B6T0G-42C08XP-6/2/94138605e1dcbd29806f6099e685a5f7>

Receptor gated Ca²⁺ entry has been associated with transient receptor potential (TRP) proteins encoded by several different genes. Here, we compare expression of mRNA for TRP isoforms encoded by genes TRP1-6 in the rat substantia nigra and whole brain. The substantia nigra and the whole brain expressed mRNA predominantly for TRP3 and TRP6. The levels of TRP1, 2, 4 and 5 were very low in both. The TRP6 mRNA levels in substantia nigra and the whole brain were comparable while those for TRP3 were significantly lower in substantia nigra than in the whole brain. Thus substantia nigra differs from the whole brain in its TRP expression.

Tajti, J., S. Moller, et al. (1999). "The human superior cervical ganglion: neuropeptides and peptide receptors." Neuroscience Letters **263**(2-3): 121.

<http://www.sciencedirect.com/science/article/B6T0G-3W4XHF1-D/2/c23e29a749e7851f051df9c4218af1ac>

Noradrenaline (NA)- and neuropeptide Y (NPY)- containing cell bodies were found to occur in high numbers (>75% of all cells were positive) in the human superior cervical ganglion and

distributed homogeneously throughout the ganglion and showed co-localisation. A few cell bodies were VIP-immunoreactive (-ir) less than 5% but none of them showed NOS-, CGRP- or SP-ir. Receptor mRNA expression was studied with RT-PCR. Total RNA from the superior cervical ganglion was successfully extracted. By using appropriate sense and antisense oligonucleotides designed from the published human sequences, we could show the presence of mRNA for the human NPY Y1, NPY Y2 and VPAC1 receptors but not CGRP1 receptor mRNA.

Thornwall-Le Greves, M., Q. Zhou, et al. (2001). "Morphine decreases the levels of the gene transcripts of growth hormone receptor and growth hormone binding protein in the male rat hippocampus and spinal cord." Neuroscience Letters **304**(1-2): 69.

<http://www.sciencedirect.com/science/article/B6T0G-42WX572-M/2/0b1572e5d8ca6d627dc7ae4926c12584>

In this study we have characterized the nucleotide sequence of the cDNA for the growth hormone receptor (GHR) and examined the effects of morphine on the gene transcripts for GHR as well as GH binding protein (GHBP) in the male rat hippocampus and spinal cord. Using reverse transcription-polymerase chain reaction followed by cloning and sequencing, we found that the entire coding region of the GHR mRNA in the spinal cord is identical to that previously described in liver. A similar observation was made for the partially sequenced GHR cDNA from hippocampus. Northern blot analysis showed that in both tissues the levels of the transcripts for both GHR and GHBP were significantly decreased 4 h after a single dose of morphine. After 24 h the level of both transcripts did not significantly differ from that of control animals. This result indicates that the opiate does not only affect the receptor protein as shown earlier by binding studies, but also reduces the expression or turnover of the GHR as well as GHBP at the transcription level.

Urai, Y., O. Jinnouchi, et al. (2002). "Gene expression of D-amino acid oxidase in cultured rat astrocytes: regional and cell type specific expression." Neuroscience Letters **324**(2): 101.

<http://www.sciencedirect.com/science/article/B6T0G-45CNG1F-3/2/2a38260078fe6383c07657847702fb11>

Neuromodulative free D-serine is present in mammalian brain, and localized to type-2 astrocytes in culture. D-amino acid oxidase (DAO) is a flavoenzyme that catalyzes D-amino acids. We examined the DAO gene expression in cultured rat astrocytes by reverse transcriptase-polymerase chain reaction. We established a method to prepare highly purified culture of type-1 and type-2 astrocytes from any brain region. This method utilizes combination of cell type specific separation by shaking and subsequent purification by immunopanning or treatment with cytosine arabinoside. We detected higher DAO gene expression in type-1 astrocyte cultures from cerebellum than that from cerebral cortex. In cerebellum, we observed higher DAO expression in type-1 astrocyte cultures than that in type-2. We also revealed that DAO expression in C6, corresponding to type-1 astrocyte, was higher than that in CG-4 derived type-2 astrocytes.

Volicer, L., J. M. Wells, et al. (1999). "Enhanced inhibition of free radical-induced deoxyribose breakdown by Alzheimer brain homogenates." Neuroscience Letters **270**(3): 169.

<http://www.sciencedirect.com/science/article/B6T0G-3WXP07W-C/2/3fd899e50c17809d3a78ca9559f0e7cb>

The ability of homogenates from Alzheimer and control brains to inhibit formation of thiobarbituric acid reactive products (TBAR) induced by free radicals was compared. The assay for TBAR was modified by adding 1% sodium dodecyl sulfate (SDS) to prevent chromogen adsorption by biological matrices, and by extending the incubation time. The inhibitory activities required smaller equivalents of Alzheimer brain homogenates than control homogenates. Similar inhibitory activities were seen in homogenates from amygdala, temporal cortex and cerebellum. The inhibitory activities were similar in brain homogenates from individuals with different apolipoprotein E status. These results indicate that Alzheimer brain tissue has either increased content of free radical scavengers or is more sensitive to free radical attack than control brains.

Wragg, M. A., C. J. Talbot, et al. (1995). "No association found between Alzheimer's disease and a mitochondrial tRNA glutamine gene variant." *Neuroscience Letters* **201**(2): 107.

<http://www.sciencedirect.com/science/article/B6T0G-3YCF4WM-T/2/5b41af3f483b8876e1d88cea10cfe90d>

We have screened a large sample of patients with sporadic late-onset dementia of the Alzheimer type (DAT) and age-matched controls for a mitochondrial tRNA^{Gln} variant previously reported to be associated with increased risk of developing Alzheimer's disease (AD). The frequency of an Ava II site gain was determined by restriction analysis of a PCR-amplified mitochondrial DNA product. One of 155 DAT cases and four of 105 age-matched controls carried the variant. Both the affected and control frequencies are statistically different from those previously reported. The mitochondrial lineage of those individuals harboring the variant was determined by sequencing a short region of the hypervariable mitochondrial D-loop. The affected individual and three of the four controls carrying the Ava II variant belong to the same mitochondrial lineage previously reported to be associated with AD.

Xi, Z. R., W. Qin, et al. (2004). "Transmission disequilibrium analysis of the GSN gene in a cohort of family trios with schizophrenia." *Neuroscience Letters* **372**(3): 200.

<http://www.sciencedirect.com/science/article/B6T0G-4DKKYV4-5/2/90e2f71c5217e64da22435e1beed292b>

Apoptosis is thought to play a role in neuronal pathology in schizophrenia. Recently, the GSN gene was reported to have anti-apoptotic properties. In a genome-wide expression analysis on schizophrenia, GSN was also found to be significantly down-regulated in schizophrenia. All the hints suggest that GSN is a novel candidate gene in occurrence of schizophrenia. In this work, we genotyped 3 SNPs around the GSN locus in 493 sets of the Han Chinese trio sample using allele-specific PCR. A weak association or a marginally positive result was detected (0.05 for P-value of the overtransmitted haplotype and 0.02 for a global P-value).

Yang, Y., Z. Xiao, et al. (2004). "Tumor suppressor gene TP53 is genetically associated with schizophrenia in the Chinese population." *Neuroscience Letters* **369**(2): 126.

<http://www.sciencedirect.com/science/article/B6T0G-4D985TS-3/2/bf5025eac2308e684dd2bcb479557425>

Schizophrenia is a debilitating mental disorder. The TP53 tumor suppressor gene, encoding a phosphoprotein, is a key element in maintaining genomic stability and cell apoptosis. Recently,

reduced risk of cancer in patients of schizophrenia has been reported. Some evidence also suggests the possible implication of TP53 in neurodevelopment. In order to examine the role of the TP53 gene in the pathogenesis of schizophrenic disorders, we investigated the genetic association between a functional polymorphism rs1042522 and schizophrenia by sequencing the fragment covering 72Pro> Arg in 701 cases and 695 controls in this work. In addition, we studied two other SNPs rs2078486 and rs8064946 by allele-specific PCR in the same samples. Though rs1042522 and rs8064946 did not show positive association with schizophrenia, we did observe statistically significant differences on SNP rs2078486 (P-value = 0.029; OR = 1.21; 95% CI 1.02-1.42) and on haplotype CAC (P-value = 0.0068; OR = 1.36; 95% CI 1.09-1.70). These results demonstrated that TP53 might play a role in susceptibility to schizophrenia.

Zamostiano, R., A. Pinhasov, et al. (1999). "A femtomolar-acting neuroprotective peptide induces increased levels of heat shock protein 60 in rat cortical neurons: a potential neuroprotective mechanism." Neuroscience Letters **264**(1-3): 9.

<http://www.sciencedirect.com/science/article/B6T0G-3W788DW-3/2/157033e68d7a5849e58e85f085bff0a0>

Activity-dependent neurotrophic factor (ADNF) was recently isolated from conditioned media of astrocytes stimulated with vasoactive intestinal peptide (VIP). ADNF provided neuroprotection at femtomolar concentration against a wide variety of toxic insults. A nine amino acid peptide (ADNF-9) captured with even greater potency the neuroprotective activity exhibited by the parent protein. Utilizing Northern and Western blot analyses, it was now shown that ADNF-9 increased the expression of heat shock protein 60 (hsp60) in rat cerebral cortical cultures. In contrast, treatment with the Alzheimer's toxin, the [beta]-amyloid peptide, reduced the amount of intracellular hsp60. Treatment with ADNF-9 prevented the reduction in hsp60 produced by the [beta]-amyloid peptide. The protection against the [beta]-amyloid peptide-associated cell death provided by ADNF-9 may be mediated in part by intracellular increases in hsp60.

Neuroscience Research (9)

Gearhart, D. A., P. F. Toole, et al. (2002). "Identification of brain proteins that interact with 2-methylnorharman: An analog of the parkinsonian-inducing toxin, MPP+." Neuroscience Research **44**(3): 255.

<http://www.sciencedirect.com/science/article/B6T0H-472KXRS-5/2/ebb773b94c534e5abfedf73cd6943012>

N-Methylated [beta]-carbolines, including 2-methylnorharman, are structural and functional analogs of the parkinsonian-inducing toxin, MPP+. We are investigating N-methylated [beta]-carbolines, including 2-methylnorharman, as possible etiologic factors in the pathogenesis of Parkinson's disease. The cellular targets of N-methylated [beta]-carbolines-mediated cytotoxicity are unknown; therefore, we used the T7Select(R) Phage Display System in a novel approach to identify brain proteins that bind to 2-methylnorharman. We incubated (biopanned) immobilized 2-methylnorharman with a phage display cDNA library that expressed a library of human brain proteins on the surface of bacteriophage T7. We washed off unbound phage, amplified the phage that were bound to 2-methylnorharman, and enriched for toxin-interacting phage by repeating the

biopanning and amplification steps. The cDNA sequences from the toxin-interacting phage were used to derive the amino acid sequences of the phage-displayed proteins. Five of the six 2-methylnorharman-interacting proteins may have relevance to Parkinson's disease: [alpha]-tubulin, paraoxonase, dorfin, fatty acid binding protein, and platelet-activating factor acetylhydrolase. Dorfin has sequence homology with parkin, which is interesting because mutations in the parkin gene associate with early-onset Parkinson's disease. Our findings are the basis for future studies aimed at determining whether 2-methylnorharman affects the function of these specific proteins in vitro and in vivo.

Kim, D. K., I. J. Kim, et al. (2004). "System L-amino acid transporters are differently expressed in rat astrocyte and C6 glioma cells." Neuroscience Research **50**(4): 437.

<http://www.sciencedirect.com/science/article/B6T0H-4DBKHBV-2/2/91d972056b554492493e726e3bddabbf>

The system L-amino acid transporter is a major nutrient transport system that is responsible for Na⁺-independent transport of neutral amino acids including several essential amino acids. We have compared and examined the expressions and functions of the system L-amino acid transporters in both rat astrocyte cultures and C6 glioma cells. The rat astrocyte cultures expressed the L-type amino acid transporter 2 (LAT2) with its subunit 4F2hc, whereas the L-type amino acid transporter 1 (LAT1) was not expressed in these cells. The C6 glioma cells expressed LAT1 but not LAT2 with 4F2hc. The [14C]L-leucine uptakes by the rat astrocyte cultures and C6 glioma cells were Na⁺-independent and were completely inhibited by the system L selective inhibitor, BCH. These results suggest that the transport of neutral amino acids including several essential amino acids into rat astrocyte cultures and C6 glioma cells are for the most part mediated by LAT2 and LAT1, respectively. Therefore, the rat astrocyte cultures and C6 glioma cells are excellent tools for examining the properties of LAT2 and LAT1, respectively. Moreover, the specific inhibition of LAT1 in cancer cells might be a new rationale for anti-cancer therapy.

Lu, H., M. Nishi, et al. (2004). "Estrogen reduces the neurite growth of serotonergic cells expressing estrogen receptors." Neuroscience Research **50**(1): 23.

<http://www.sciencedirect.com/science/article/B6T0H-4CWSWST-1/2/1229c0f97a9f847ebf7526fa315a5ed0>

Serotonergic innervation of the central nervous system has a sexual dimorphism. The serotonin level in the hypothalamus was modulated by estrogen, and the formation of sexual dimorphism of serotonergic fiber innervation in the hypothalamus has been shown by the effect of sexual hormone during the critical perinatal period. In this study, we examined the direct effect of estrogen on the neurite growth of serotonergic neurons in primary culture from embryonic day 14 (E14) of rat mesencephalon. The total neurite length of serotonin-immunoreactive (IR) cells was significantly decreased by estradiol benzoate (E2, 10⁻⁸ M) treatment for 7 days, compared with the case of no treatment. Moreover, the presence of estrogen receptor (ER) [alpha] and ER[beta] mRNA in the E14 mesencephalon with reverse transcription-polymerase chain reaction (RT-PCR), and the ER[alpha] or ER[beta] protein in the cultured serotonin-IR cells with double fluorescence immunohistochemistry were also demonstrated. Our results suggest that the inhibitory effects of E2 on the neurite growth of serotonergic cells expressing ER[alpha] or ER[beta] might be involved in the formation of the sexual dimorphic distribution of serotonergic innervation.

Masumura, M., R. Hata, et al. (2001). "Oligodendroglial cell death with DNA fragmentation in the white matter under chronic cerebral hypoperfusion: comparison between normotensive and spontaneously hypertensive rats." Neuroscience Research **39**(4): 401.

<http://www.sciencedirect.com/science/article/B6T0H-42M78PT-6/2/51b8933e1c91ae0bc976e522d9ca3dda>

We investigated the neuropathological and biochemical changes in the white matter of normotensive Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR) after bilateral carotid artery ligation (BCAL). One week after BCAL, both WKY and SHR showed white matter rarefaction and vacuolation with reduced oligodendrocytes, but there was no difference between WKY and SHR. On the other hand, vacuoles formed by oligodendroglial cell death were increased significantly from 2 to 4 weeks in the optic tract and fimbria fornix of hypoperfused SHR. Furthermore, terminal deoxynucleotidyl transferase-mediated dUTP in situ nick end labeling (TUNEL)-positive cells and lectin-positive microglia increased in number and intensities of staining more markedly in SHR than in WKY. In situ cell death detection ELISA supported these results quantitatively. RT-PCR represented the expression of TNF-[alpha], TNF receptor 1 (p55), caspase-2 (Ich-1) and -3 (CPP32) mRNAs in both WKY and SHR brains after BCAL. Immunohistochemical analyses revealed that TNF-[alpha], TNF receptor 1 (p55), Ich-1 and CPP32 immunoreactive cells could also be detected in the white matter regions of hypoperfused SHR. These results suggested that local production of TNF-[alpha] by the activated microglia might selectively induce oligodendroglial cell death through the death domain-containing TNF receptor 1 (p55), caspase-2 or -3 activation, resulting in white matter changes as a primary pathological feature.

Miyazaki, I., M. Asanuma, et al. (2002). "Age-related changes in expression of metallothionein-III in rat brain." Neuroscience Research **43**(4): 323.

<http://www.sciencedirect.com/science/article/B6T0H-45WGH47-3/2/efc9d5d0fbb8d8a206b1d1d914006ec3>

Metallothionein (MT)-III is a metal binding protein, called growth inhibitory factor, and is mainly expressed in the central nervous system. Since MT-III decreases in the brain of Alzheimer's disease (AD), a growing interest has been focused on its relationship to neurodegenerative diseases. To clarify age-related changes in the MT-III expression and its inducibility against oxidative stress, we analyzed the expression of MT-III and its mRNA in the brain of lipopolysaccharide (LPS)-treated aged rats. In the frontal cortex, basal expression of MT-III mRNA was significantly increased with aging, while it was observed no induction of MT-III mRNA against LPS administration in the aged rat brain. MT-III immunopositive cells were increased in the frontal, parietal and piriform cortices, hypothalamus and amygdaloid nucleus with aging. The LPS treatment induced MT-III expression in the brain of young-adult rats, but not in the aged rat brain. Furthermore, the MT-III induction with LPS treatment was mainly observed in oligodendrocyte and microglia. In the present study, we showed that inducibility of brain MT-III against oxidative stress may be reduced with aging. Since it has been reported that MT-III has neuroprotective roles as an antioxidant, present results suggest that MT-III is closely related to the neurodegeneration in the aged animals.

Suzuki, Y., A. Wanaka, et al. (1995). "Identification of differentially expressed mRNAs during neuronal differentiation of P19 embryonal carcinoma cells." Neuroscience Research **23**(1): 65.

<http://www.sciencedirect.com/science/article/B6T0H-482WRWV->

8/2/d9e854130387b48d2d7ce61e25e5c55a

To understand the basic mechanisms underlying neuronal differentiation, we have attempted to isolate differentially expressed genes, which may play a key role in this complex process, from neuronal differentiating P19 embryonal carcinoma cells. RNA fingerprinting by the arbitrarily primed PCR (RAP) method was adapted to detect such differentially expressed genes during P19 neuronal differentiation. Using this method with some modifications, we successfully cloned seven cDNA fragments which were expressed differentially within the first 48 h after 1 [mu]M retinoic acid (RA) treatment, which ultimately induces neuronal differentiation. Comparison of the partial nucleotide sequences of these clones with sequences in DNA databases indicated that one of these clones was identical to a region of the mouse Oct-3 gene, which has been shown to be dramatically repressed by RA. Two clones were highly homologous to the human profilinII and leucine-rich protein genes. The other four clones were not closely related to any sequences in the databases. Except for the Oct-3 gene, the other six genes isolated here have not been reported previously as RA-regulated genes. RAP is, thus, a promising method for identification of novel and potentially important genes which are differentially regulated during neuronal differentiation.

Tabuchi, A., M. Koizumi, et al. (2001). "Involvement of endogenous PACAP expression in the activity-dependent survival of mouse cerebellar granule cells." Neuroscience Research **39**(1): 85.

<http://www.sciencedirect.com/science/article/B6T0H-424KJSF-9/2/cea83877c406f17f33795f45a5e3f4dc>

Membrane depolarization causes Ca²⁺ influx through L-type voltage-dependent calcium channels (L-VDCC), which promotes the activity-dependent survival of mouse cerebellar granule cells (CGCs). Although exogenously added pituitary adenylate cyclase activating polypeptide (PACAP) is effective in promoting the survival of CGCs, it is unknown whether PACAP is synthesized in CGCs and involved in the activity-dependent survival of CGCs. In this study, we found that the PACAP gene was activated in depolarized CGCs cultured at 25 mM KCl (high K⁺), independently of de novo protein synthesis. In addition, the PACAP immunoreactivity increased through the activation of L-VDCC in depolarized CGCs, indicating that PACAP is concomitantly produced with PACAP mRNA in an activity-dependent manner. Exogenously added PACAP attenuated the apoptosis of CGCs through a specific interaction with PACAP receptors. Furthermore, a PACAP receptor antagonist, PACAP(6-38), reduced the survival of CGCs at high K⁺. These findings indicate that endogenous PACAP production induced by Ca²⁺ signals exerts a survival effect on CGCs via PACAP receptors, which, at least in part, accounts for the activity-dependent survival of CGCs.

Taniguchi, M., N. Tani, et al. (1999). "High expression of alternative transcript of granzyme M in the mouse retina." Neuroscience Research **34**(2): 115.

<http://www.sciencedirect.com/science/article/B6T0H-3X6JGP9-7/2/7aef5d381ee8e0581d695e4fa9ba19ef>

We have isolated cDNAs to two transcripts, granzyme M and alternative granzyme M mRNA from the mouse eye. Analysis of genomic DNA revealed these transcripts were derived from alternative transcription initiations. Northern blot analysis and reverse transcription-polymerase chain reactions revealed that both transcripts were expressed in the eye, though the alternative form was the major type. In situ hybridization studies demonstrated that alternative granzyme M mRNA localized exclusively in the photoreceptor cells in the retina and expressed only after the opening of the eye, suggesting that these transcripts are related to the maintenance of the retinal

structure or functions of matured photoreceptor cells rather than the development or differentiation of retinal cells.

Zemo, D. A. and J. T. McCabe (2002). "Transcriptional responses of the rat vasopressin gene to acute and repeated acute osmotic stress." Neuroscience Research **44**(1): 45.

<http://www.sciencedirect.com/science/article/B6T0H-460WN7B-2/2/7899368d43f8cc1fad9f855f71adc25c>

To determine the impact of hypertonic saline administration upon rat arginine vasopressin (AVP) gene transcription in supraoptic nucleus neurons, a probe complementary to the first intron (AVP1) of AVP was used to measure changes in AVP heteronuclear RNA (hnRNA) levels. Animals that received hypertonic saline had increases in AVP1 after 15 and 30 min, with a return to baseline levels by 180 min. In a double injection paradigm, animals were given an injection of normal or hypertonic saline followed 180 min later by a second injection of normal or hypertonic saline and sacrificed 30 min later. When both injections were hypertonic saline (H-H), AVP1 levels were greater than levels seen after a single hypertonic saline injection, or after an injection of normal saline followed by a second injection of hypertonic saline (N-H). This study shows acute, repeated exposure to hypertonic saline causes a robust increase in vasopressin gene transcription. Since a second hyperosmotic stimulus is known to increase neuronal firing rate and activity, our results suggest that a correlation exists with intracellular mechanisms regulating vasopressin gene transcription.

NeuroToxicology (1)

Svensson, I., L. Waara, et al. (2001). "Soman-Induced Interleukin-1[beta] mRNA and Protein in Rat Brain." NeuroToxicology **22**(3): 355.

<http://www.sciencedirect.com/science/article/B6W81-436VY9B-5/2/a5310718b2f180b1ecac6b97089e7ef5>

Exposure to high doses of the toxic organophosphate compound soman, also known as a chemical warfare agent, causes a progression of toxic symptoms including hyper-secretions, convulsions, respiratory depression, and finally death. In previous studies, we have demonstrated pronounced effects following soman intoxication in dopaminergic, GABAergic, and cholinergic systems in rat brain. The aim of this study was to investigate the effects on the pro-inflammatory cytokine interleukin-1[beta] (IL-1[beta]), indicated as mRNA and protein production, at different time intervals following soman intoxication. The peak levels of mRNA was observed 30 min following soman exposure, while a significant increase in the protein was observed at 6 h. Immunohistochemistry analysis revealed the presence of IL-1[beta] protein in astrocytes and endothelial cells. In addition to the previously observed effects of soman, there is an induction of IL-1[beta] in the brain. This effect, which is highly correlated to convulsions, implicates IL-1[beta] as a possible mediator for long-term brain damage observed after soman intoxication.

Nitric Oxide (3)

Andrade, M. A., M. Siles-Lucas, et al. (2004). "Echinococcus multilocularis laminated-layer components and the E14t 14-3-3 recombinant protein decrease NO production by activated rat macrophages in vitro." Nitric Oxide **10**(3): 150.

<http://www.sciencedirect.com/science/article/B6WNT-4CBDC8M-1/2/ef492737c8a436451b82cca6449f9180>

Echinococcus multilocularis and *Echinococcus granulosus* cause alveolar and cystic (unilocular) echinococcosis, respectively, in humans and animals. It is known that these parasites can affect, among other molecules, nitric oxide (NO) production by periparasitic host cells. Nevertheless, detailed dissection of parasite components specifically affecting cell NO production has not been done to date. We compare the effect of *E. granulosus* and *E. multilocularis* defined metacestode structural (laminated-layer associated) and metabolic (14-3-3 protein, potentially related with *E. multilocularis* metacestode tumor-like growth) components on the NO production by rat alveolar macrophages in vitro. Our results showed that none of these antigens could stimulate macrophage NO production in vitro. However, a reversed effect of some *Echinococcus* antigens on NO in vitro production was found when cells were previously exposed to LPS stimulation. This inhibitory effect was found when *E. multilocularis* laminated-layer (LL) or cyst wall (CW) soluble components from both species were used. Pre-stimulation of cells with LPS also resulted in a strong, dose-dependent reduction of NO and iNOS mRNA production after incubation of cells with the E14t protein. Thus, the *E. multilocularis* 14-3-3 protein appears to be one of the components accounting for the suppressive effect of the CW and LL metacestode extracts.

Ghilardi, G., M. L. Biondi, et al. (2003). "Vascular invasion in human breast cancer is correlated to T->786C polymorphism of NOS3 gene." Nitric Oxide **9**(2): 118.

<http://www.sciencedirect.com/science/article/B6WNT-49RCFTH-2/2/299fc4d5e9636de6128d0125971be938>

Background. Nitric oxide (NO) is a free radical known to be a major regulator of vascular tonus, to inhibit cell proliferation, induce apoptosis, and be a mediator of macrophage cytostatic and cytotoxic effects. Recently, NO synthesis has been reported to be elevated in different cancers and is expected to promote metastasis by maintaining a vasodilator tone in blood vessels in and around the tumour. Two different common genetic polymorphisms were found on endothelial NO synthase (NOS3) gene: Glu298Asp on exon 7 and T->786C in the promoter region. **Purpose.** To evaluate the impact of the NOS3 polymorphisms on vascular invasion and metastasis in breast cancer patients. **Design.** Two NOS3 gene polymorphisms (Glu298Asp and T->786C) were genotyped in 71 patients operated for breast cancer and followed for 6-30 months (median 21). A control population of 91 age and sex matched tumour-free subjects was also genotyped for the same polymorphisms. **Results.** The distribution of both polymorphisms was not different between cases and controls. In patients without vascular invasion, T allele frequency was significantly lower than in patients with vascular invasion ($p=0.033$). At the end of the follow-up, T allele frequency was found to be less frequent in the metastasis free group than normal population (0.51 vs 0.64; $p=0.047$). **Conclusion.** Our results suggest that T allele reduction at the NOS3 promoter region may reduce vascular invasion in breast cancer and consequently reduce metastatic spread and be a favorable prognostic factor. These results need further validation in larger studies.

Zamora, R., Y. Vodovotz, et al. (2002). "A DNA microarray study of nitric oxide-induced genes in mouse hepatocytes: implications for hepatic heme oxygenase-1 expression in ischemia/reperfusion." Nitric Oxide **7**(3): 165.

<http://www.sciencedirect.com/science/article/B6WNT-46XHFMS-3/2/a59898196c06b5704ea6f2cc614e3209>

Nuclear Medicine and Biology (1)

Wu, F., G. Lendvai, et al. (2004). "Hybridisation of [⁷⁶Br]-labelled antisense oligonucleotides to Chromogranin A mRNA verified by RT-PCR." Nuclear Medicine and Biology **31**(8): 1073.

<http://www.sciencedirect.com/science/article/B6T9Y-4F1HS1N-C/2/19830bcec83b98622ad02b86e54e50ab>

Methods have been developed to label oligonucleotides (ODNs) in the 5'-position with ⁷⁶Br via a prosthetic group on a hexylamino-linker. The purpose of the study was to explore whether the labelling procedure would prevent specific hybridisation by using reverse transcription-polymerase chain reaction (RT-PCR) followed by sequencing of the PCR product. Antisense ODNs (30 mer, specific for rat Chromogranin A [CgA] mRNA) with phosphodiester (O-ODN) or phosphothioate (S-ODN) backbone, either unlabelled or labelled with ⁷⁶Br, served as one of the primers in individual PCR reactions. Using O-ODN as a primer, irrespective of being labelled or not, a selected 225-bp PCR fragment was successfully amplified. However, no amplification was obtained using S-ODN as a primer. The proper PCR products were only detected in the sample prepared from the adrenal gland, but not in that from the heart, liver or kidney. Autoradiographic recording of the gel, after gel electrophoresis, revealed radioactive signals corresponding to the amplified PCR products. The sequence of the PCR product matched the rat CgA mRNA sequence obtained from the EMBL database. RT-PCR is an attractive method to identify the selective binding of modified ODNs to target mRNA. This method confirmed that the labelling with ⁷⁶Br did not change the hybridisation ability of antisense O-ODN.

Nucleic Acids Res. (77)

Araki, K., M. Araki, et al. (2002). "Site-directed integration of the cre gene mediated by Cre recombinase using a combination of mutant lox sites." Nucleic Acids Res. **30**(19): e103-.

<http://nar.oupjournals.org/cgi/content/abstract/30/19/e103>

The Cre-lox system is an important tool for genetic manipulation. To promote integrative reactions, two strategies using mutant lox sites have been developed. One is the left element/right element (LE/RE)-mutant strategy and the other is the cassette exchange strategy using heterospecific lox sites such as lox511 or lox2272. We compared the recombination efficiencies using these mutant lox sites in embryonic stem (ES) cells, and found that the

combination of the LE/RE mutant and lox2272 showed high recombination efficiency and stability of the recombined structure. Taking advantage of this stability, we successfully integrated the cre gene into the mutant lox sites by Cre-mediated recombination. Germ line chimeric mice were produced from the cre-integrated ES cell clones, and Cre-expressing mouse lines were established. The inserted cre gene was stably maintained through the generations. This cre knock-in system using the LE/RE-lox2272 combination should be useful for the production of various cre mice for gene targeting or gene trapping.

Barbot, W., A. Dupressoir, et al. (2002). "Epigenetic regulation of an IAP retrotransposon in the aging mouse: progressive demethylation and de-silencing of the element by its repetitive induction." Nucleic Acids Res. **30**(11): 2365-2373.

<http://nar.oupjournals.org/cgi/content/abstract/30/11/2365>

The recent insertion of a murine intracisternal A-particle (IAP) retrotransposon within one of the introns of a housekeeping gene, the circadian m.nocturnin gene, revealed a singular expression profile, both throughout the daytime and the mouse life span. Measurement of the levels of transcripts from this element by quantitative real-time RT-PCR, in organs of 1-24-month-old mice, disclosed that the inserted element--which is part of a large family of otherwise severely repressed mobile elements--becomes active upon aging, specifically in the liver where the m.nocturnin housekeeping gene is expressed in a circadian manner and induces a circadian expression of the IAP sequence. This age-dependent induction is cell-autonomous, as it persists in hepatocytes in primary culture. We further show, using methylation-sensitive enzymes, a correlation between the life-time kinetics of this process and a liver-specific demethylation of the IAP promoter. These results strongly support a model whereby the progressive demethylation and turning on of the IAP sequence is the sole result of the transient, daily activation--throughout the mouse life span--of its promoter. This phenomenon, which develops on a timescale of months to years in the aging mouse, might reveal a general epigenetic--and stochastic--process, which could account for a large series of events associated with cell and animal aging.

Bentley, J., C. P. Diggle, et al. (2004). "DNA double strand break repair in human bladder cancer is error prone and involves microhomology-associated end-joining." Nucleic Acids Res. **32**(17): 5249-5259.

<http://nar.oupjournals.org/cgi/content/abstract/32/17/5249>

In human cells DNA double strand breaks (DSBs) can be repaired by the non-homologous end-joining (NHEJ) pathway. In a background of NHEJ deficiency, DSBs with mismatched ends can be joined by an error-prone mechanism involving joining between regions of nucleotide microhomology. The majority of joins formed from a DSB with partially incompatible 3' overhangs by cell-free extracts from human glioblastoma (MO59K) and urothelial (NHU) cell lines were accurate and produced by the overlap/fill-in of mismatched termini by NHEJ. However, repair of DSBs by extracts using tissue from four high-grade bladder carcinomas resulted in no accurate join formation. Junctions were formed by the non-random deletion of terminal nucleotides and showed a preference for annealing at a microhomology of 8 nt buried within the DNA substrate; this process was not dependent on functional Ku70, DNA-PK or XRCC4. Junctions were repaired in the same manner in MO59K extracts in which accurate NHEJ was inactivated by inhibition of Ku70 or DNA-PKcs. These data indicate that bladder tumour extracts are unable to perform accurate NHEJ such that error-prone joining predominates. Therefore, in high-grade tumours mismatched DSBs are repaired by a highly mutagenic, microhomology-mediated, alternative end-joining pathway, a process that may contribute to genomic instability observed in bladder cancer.

Blair, P. L., A. Witney, et al. (2002). "Transcripts of developmentally regulated *Plasmodium falciparum* genes quantified by real-time RT-PCR." *Nucleic Acids Res.* **30**(10): 2224-2231.

<http://nar.oupjournals.org/cgi/content/abstract/30/10/2224>

Plasmodium falciparum intraerythrocytic development is a complex process. Development proceeds rapidly from the trophozoite phase of nutrient acquisition and growth through to the synthetic and reproductive schizont phase, which ends with production of new invasive merozoites. During this process, the malaria parasite must express a series of different gene products, depending on its metabolic and synthetic needs. We are particularly interested in the development of the merozoite's organelles in the apical complex, which form during the later schizont stages. We have used quantitative real-time RT-PCR fluorogenic 5' nuclease assays (TaqMan(R)) for the first time on malaria parasites for analysis of erythrocytic stage-specific gene expression. We analyzed transcripts of the *P. falciparum* eba-175 and other erythrocyte binding-like (eb l) family genes in temperature-synchronized parasites and found ebl genes have tightly controlled, stage-specific transcription. As expected, eba-175 transcripts were abundant only at the end of schizont development in a pattern most common among ebl, including baebl, pebl and jesebl. The maebl transcript pattern was unique, peaking at mid-late trophozoite stage, but absent in late-stage schizonts. ebl-1 demonstrated another pattern of expression, which peaked during mid-schizont stage and then significantly diminished in late-stage schizonts. Our analysis demonstrates that using real-time RT-PCR fluorogenic 5' nuclease assays is a sensitive, quantitative method for analysis of *Plasmodium* transcripts.

Blondal, T., S. H. Hjorleifsdottir, et al. (2003). "Discovery and characterization of a thermostable bacteriophage RNA ligase homologous to T4 RNA ligase 1." *Nucleic Acids Res.* **31**(24): 7247-7254.

<http://nar.oupjournals.org/cgi/content/abstract/31/24/7247>

Thermophilic viruses represent a novel source of genetic material and enzymes with great potential for use in biotechnology. We have isolated a number of thermophilic viruses from geothermal areas in Iceland, and by combining high throughput genome sequencing and state of the art bioinformatics we have identified a number of genes with potential use in biotechnology. We have also demonstrated the existence of thermostable counterparts of previously known bacteriophage enzymes. Here we describe a thermostable RNA ligase 1 from the thermophilic bacteriophage RM378 that infects the thermophilic eubacterium *Rhodothermus marinus*. The RM378 RNA ligase 1 has a temperature optimum of 60-64{degrees}C and it ligates both RNA and single-stranded DNA. Its thermostability and ability to work under conditions of high temperature where nucleic acid secondary structures are removed makes it an ideal enzyme for RNA ligase-mediated rapid amplification of cDNA ends (RLM-RACE), and other RNA and DNA ligation applications.

Blondal, T., A. Thorisdottir, et al. (2005). "Isolation and characterization of a thermostable RNA ligase 1 from a *Thermus scotoductus* bacteriophage TS2126 with good single-stranded DNA ligation properties." *Nucleic Acids Res.* **33**(1): 135-142.

<http://nar.oupjournals.org/cgi/content/abstract/33/1/135>

We have recently sequenced the genome of a novel thermophilic bacteriophage designated as

TS2126 that infects the thermophilic eubacterium *Thermus scotoductus*. One of the annotated open reading frames (ORFs) shows homology to T4 RNA ligase 1, an enzyme of great importance in molecular biology, owing to its ability to ligate single-stranded nucleic acids. The ORF was cloned, and recombinant protein was expressed, purified and characterized. The recombinant enzyme ligates single-stranded nucleic acids in an ATP-dependent manner and is moderately thermostable. The recombinant enzyme exhibits extremely high activity and high ligation efficiency. It can be used for various molecular biology applications including RNA ligase-mediated rapid amplification of cDNA ends (RLM-RACE). The TS2126 RNA ligase catalyzed both inter- and intra-molecular single-stranded DNA ligation to >50% completion in a matter of hours at an elevated temperature, although favoring intra-molecular ligation on RNA and single-stranded DNA substrates. The properties of TS2126 RNA ligase 1 makes it very attractive for processes like adaptor ligation, and single-stranded solid phase gene synthesis.

Blondal, T., B. G. Waage, et al. (2003). "A novel MALDI-TOF based methodology for genotyping single nucleotide polymorphisms." Nucleic Acids Res. **31**(24): e155-.

<http://nar.oupjournals.org/cgi/content/abstract/31/24/e155>

A new MALDI-TOF based detection assay was developed for analysis of single nucleotide polymorphisms (SNPs). It is a significant modification on the classic three-step minisequencing method, which includes a polymerase chain reaction (PCR), removal of excess nucleotides and primers, followed by primer extension in the presence of dideoxynucleotides using modified thermostable DNA polymerase. The key feature of this novel assay is reliance upon deoxynucleotide mixes, lacking one of the nucleotides at the polymorphic position. During primer extension in the presence of depleted nucleotide mixes, standard thermostable DNA polymerases dissociate from the template at positions requiring a depleted nucleotide; this principal was harnessed to create a genotyping assay. The assay design requires a primer- extension primer having its 3'-end one nucleotide upstream from the interrogated site. The assay further utilizes the same DNA polymerase in both PCR and the primer extension step. This not only simplifies the assay but also greatly reduces the cost per genotype compared to minisequencing methodology. We demonstrate accurate genotyping using this methodology for two SNPs run in both singleplex and duplex reactions. We term this assay nucleotide depletion genotyping (NUDGE). Nucleotide depletion genotyping could be extended to other genotyping assays based on primer extension such as detection by gel or capillary electrophoresis.

Boot, H. J. and S. B. E. Pritz-Verschuren (2004). "Modifications of the 3'-UTR stem-loop of infectious bursal disease virus are allowed without influencing replication or virulence." Nucleic Acids Res. **32**(1): 211-222.

<http://nar.oupjournals.org/cgi/content/abstract/32/1/211>

Many questions regarding the initiation of replication and translation of the segmented, double-stranded RNA genome of infectious bursal disease virus (IBDV) remain to be solved. Computer analysis shows that the non-polyadenylated extreme 3'-untranslated regions (UTRs) of the coding strand of both genomic segments are able to fold into a single stem-loop structure. To assess the determinants for a functional 3'-UTR, we mutagenized the 3'-UTR stem-loop structure of the B-segment. Rescue of infectious virus from mutagenized cDNA plasmids was impaired in all cases. However, after one passage, the replication kinetics of these viruses were restored. Sequence analysis revealed that additional mutations had been acquired in most of the stem-loop structures, which compensated the introduced ones. A rescued virus with a modified stem-loop structure containing four nucleotide substitutions, but preserving its overall secondary structure, was phenotypically indistinguishable from wild-type virus, both in vitro (cell culture) and in vivo

(chickens, natural host). Sequence analysis showed that the modified stem-loop structure of this virus was fully preserved after four serial passages. Apparently, it is the stem-loop structure and not the primary sequence that is the functional determinant in the 3'-UTRs of IBDV.

Brohede, J., C. R. Primmer, et al. (2002). "Heterogeneity in the rate and pattern of germline mutation at individual microsatellite loci." Nucleic Acids Res. **30**(9): 1997-2003.

<http://nar.oupjournals.org/cgi/content/abstract/30/9/1997>

There is a lack of information on how individual microsatellite loci differ with respect to their mutation properties. Such variation will have an important bearing on our understanding of the ubiquitous occurrence of simple repeat sequences in eukaryotic genomes and on deriving proper mutation models that can be incorporated into genetic distance estimates. We genotyped [~]100 families of the bird barn swallow (*Hirundo rustica*) for two hypervariable (heterozygosity >95%) microsatellite markers: HrU6, an (AAAG)_n tetranucleotide repeat, and HrU10, an (AAGAG)_n pentanucleotide repeat. A total of 27 germline mutation events were documented, corresponding to mutation rates of 0.57% (HrU6) and 1.56% (HrU10). The mutation rate increased with allele size, at [~]0.1% per repeat unit over the observed range of allele sizes ([~]10-100 repeat units). Single repeat unit changes dominated, with 21/27 mutations representing the gain or loss of one repeat unit. There was no clear difference in the number of gains versus losses nor was there an effect of allele size on the magnitude or direction of mutation. Unexpectedly, the mutation rate of females (maternally transmitted mutations) was 2.5-5 times higher than that of males. Contrasting these observations with mutation data from other microsatellite loci reveals differences not only in the mutation rate, but also in the magnitude, direction and effect of sex on mutation. Thus, microsatellite mutation and evolution may be viewed as a dynamic and variable process.

Calin-Jageman, I. and A. W. Nicholson (2003). "RNA structure-dependent uncoupling of substrate recognition and cleavage by *Escherichia coli* ribonuclease III." Nucleic Acids Res. **31**(9): 2381-2392.

<http://nar.oupjournals.org/cgi/content/abstract/31/9/2381>

Members of the ribonuclease III superfamily of double-strand-specific endoribonucleases participate in diverse RNA maturation and decay pathways. Ribonuclease III of the gram-negative bacterium *Escherichia coli* processes rRNA and mRNA precursors, and its catalytic action can regulate gene expression by controlling mRNA translation and stability. It has been proposed that *E. coli* RNase III can function in a non-catalytic manner, by binding RNA without cleaving phosphodiester bonds. However, there has been no direct evidence for this mode of action. We describe here an RNA, derived from the T7 phage R1.1 RNase III substrate, that is resistant to cleavage in vitro by *E. coli* RNase III but retains comparable binding affinity. R1.1[CL3B] RNA is recognized by RNase III in the same manner as R1.1 RNA, as revealed by the similar inhibitory effects of a specific mutation in both substrates. Structure-probing assays and Mfold analysis indicate that R1.1[CL3B] RNA possesses a bulge-helix-bulge motif in place of the R1.1 asymmetric internal loop. The presence of both bulges is required for uncoupling. The bulge-helix-bulge motif acts as a catalytic antiterminal, which is distinct from recognition antiterminals, which inhibit RNase III binding.

Cardoso, J., L. Molenaar, et al. (2004). "Genomic profiling by DNA amplification of laser capture microdissected tissues and array CGH." Nucleic Acids Res. **32**(19): e146-.

<http://nar.oupjournals.org/cgi/content/abstract/32/19/e146>

Comparative genomic hybridization by means of BAC microarrays (array CGH) allows high-resolution profiling of copy-number aberrations in tumor DNA. However, specific genetic lesions associated with small but clinically relevant tumor areas may pass undetected due to intra-tumor heterogeneity and/or the presence of contaminating normal cells. Here, we show that the combination of laser capture microdissection, ϕ 29 DNA polymerase-mediated isothermal genomic DNA amplification, and array CGH allows genomic profiling of very limited numbers of cells. Moreover, by means of simple statistical models, we were able to bypass the exclusion of amplification distortions and variability prone areas, and to detect tumor-specific chromosomal gains and losses. We applied this new combined experimental and analytical approach to the genomic profiling of colorectal adenomatous polyps and demonstrated our ability to accurately detect single copy gains and losses affecting either whole chromosomes or small genomic regions from as little as 2 ng of DNA or 1000 microdissected cells.

Cawthon, R. M. (2002). "Telomere measurement by quantitative PCR." *Nucleic Acids Res.* **30**(10): e47-.

<http://nar.oupjournals.org/cgi/content/abstract/30/10/e47>

It has long been presumed impossible to measure telomeres in vertebrate DNA by PCR amplification with oligonucleotide primers designed to hybridize to the TTAGGG and CCCTAA repeats, because only primer dimer-derived products are expected. Here we present a primer pair that eliminates this problem, allowing simple and rapid measurement of telomeres in a closed tube, fluorescence-based assay. This assay will facilitate investigations of the biology of telomeres and the roles they play in the molecular pathophysiology of diseases and aging.

Chakraborty, S., B. Sarmah, et al. (2002). "Premature termination of RNA polymerase II mediated transcription of a seed protein gene in *Schizosaccharomyces pombe*." *Nucleic Acids Res.* **30**(13): 2940-2949.

<http://nar.oupjournals.org/cgi/content/abstract/30/13/2940>

The poly(A) signal and downstream elements with transcriptional pausing activity play an important role in termination of RNA polymerase II transcription. We show that an intronic sequence derived from the plant seed protein gene (AmA1) specifically acts as a transcriptional terminator in the fission yeast, *Schizosaccharomyces pombe*. The 3'-end points of mRNA encoded by the AmA1 gene were mapped at different positions in *S. pombe* and in native cells of *Amaranthus hypochondriacus*. Deletion analyses of the AmA1 intronic sequence revealed that multiple elements essential for proper transcriptional termination in *S. pombe* include two site-determining elements (SDEs) and three downstream sequence elements. RT-PCR analyses detected transcripts up to the second SDE. This is the first report showing that the highly conserved mammalian poly(A) signal, AAUAAA, is also functional in *S. pombe*. The poly(A) site was determined as Y(A) both in native and heterologous systems but at different positions. Deletion of these cis-elements abolished 3'-end processing in *S. pombe* and a single point mutation in this motif reduced the activity by 70% while enhancing activity at downstream SDE. These results indicate that the bipartite sequence elements as signals for 3'-end processing in fission yeast act in tandem with other cis-acting elements. A comparison of these elements in the AmA1 intron that function as a transcriptional terminator in fission yeast with that of its native genes showed that both require an AT-rich distal and proximal upstream element. However, these sequences are not identical. Transcription run-on analysis indicates that elongating RNA polymerase II molecules accumulate over these pause signals, maximal at 611-949 nt.

Furthermore, we demonstrate that the AmA1 intronic terminator sequence acts in a position-independent manner when placed within another gene.

Chalaya, T., E. Gogvadze, et al. (2004). "Improving specificity of DNA hybridization-based methods." Nucleic Acids Res. **32**(16): e130-.

<http://nar.oupjournals.org/cgi/content/abstract/32/16/e130>

Methods based on DNA reassociation in solution with the subsequent PCR amplification of certain hybrid molecules, such as coincidence cloning and subtractive hybridization, all suffer from a common imperfection: cross-hybridization between various types of paralogous repetitive DNA fragments. Although the situation can be slightly improved by the addition of repeat-specific competitor DNA into the hybridization mixture, the cross-hybridization outcome is a significant number of background chimeric clones in resulting DNA libraries. In order to overcome this challenge, we developed a technique called mispaired DNA rejection (MDR), which utilizes a treatment of resulting reassociated DNA with mismatch-specific nucleases. We examined the MDR efficiency using cross-hybridization of complex, whole genomic mixtures derived from human and chimpanzee genomes, digested with frequent-cutter restriction enzyme. We show here that both single-stranded DNA-specific and mismatched double-stranded DNA-specific nucleases can be used for MDR separately or in combination, reducing the background level from 60 to 4% or lower. The technique presented here is of universal usefulness and can be applied to both cDNA and genomic DNA subtractions of very complex DNA mixtures. MDR is also useful for the genome-wide recovery of highly conserved DNA sequences, as we demonstrate by comparing human and pygmy marmoset genomes.

Chen, J., M. Sun, et al. (2004). "Over 20% of human transcripts might form sense-antisense pairs." Nucleic Acids Res. **32**(16): 4812-4820.

<http://nar.oupjournals.org/cgi/content/abstract/32/16/4812>

The major challenge to identifying natural sense- antisense (SA) transcripts from public databases is how to determine the correct orientation for an expressed sequence, especially an expressed sequence tag sequence. In this study, we established a set of very stringent criteria to identify the correct orientation of each human transcript. We used these orientation-reliable transcripts to create 26 741 transcription clusters in the human genome. Our analysis shows that 22% (5880) of the human transcription clusters form SA pairs, higher than any previous estimates. Our orientation-specific RT-PCR results along with the comparison of experimental data from previous studies confirm that our SA data set is reliable. This study not only demonstrates that our criteria for the prediction of SA transcripts are efficient, but also provides additional convincing data to support the view that antisense transcription is quite pervasive in the human genome. In-depth analyses show that SA transcripts have some significant differences compared with other types of transcripts, with regard to chromosomal distribution and Gene Ontology-annotated categories of physiological roles, functions and spatial localizations of gene products.

Choi, K., J. H. Kim, et al. (2004). "Identification of cellular proteins enhancing activities of internal ribosomal entry sites by competition with oligodeoxynucleotides." Nucleic Acids Res. **32**(4): 1308-1317.

<http://nar.oupjournals.org/cgi/content/abstract/32/4/1308>

The translation of numerous eukaryotic mRNAs is mediated by internal ribosomal entry sites (IRESs). IRES-dependent translation requires both canonical translation initiation factors and IRES-specific trans-acting factors (ITAFs). Here we report a strategy to identify and characterize ITAFs required for IRES-dependent translation. This process involves steps for identifying oligodeoxynucleotides affecting IRES-dependent translation, purifying proteins interacting with the inhibitory DNA, identifying the specific proteins with matrix-assisted laser desorption ionization/time-of-flight mass spectrometry, and confirming the roles of these proteins in IRES-dependent translation by depletion and repletion of proteins from an in vitro translation system. Using this strategy, we show that poly(rC)-binding proteins 1 and 2 enhance translation through polioviral and rhinoviral IRES elements.

Clepet, C., I. Le Clainche, et al. (2004). "Improved full-length cDNA production based on RNA tagging by T4 DNA ligase." Nucleic Acids Res. **32**(1): e6-.

<http://nar.oupjournals.org/cgi/content/abstract/32/1/e6>

Second-strand cDNA priming is a central problem for full-length characterization of transcripts. A new strategy using bacteriophage T4 DNA ligase and partially degenerate adapters is proposed for grafting a sequence tag to the end of polyribonucleotides. Based on this RNA tagging system and previously described protocols, a new method for full-length cDNA production has been implemented. Validation of the method is shown in *Arabidopsis thaliana* by the construction of a full-length cDNA library and the analysis of 154 clones and by 5'-RACE-PCR run on a documented experimental system.

Cottrell, S. E., J. Distler, et al. (2004). "A real-time PCR assay for DNA-methylation using methylation-specific blockers." Nucleic Acids Res. **32**(1): e10-.

<http://nar.oupjournals.org/cgi/content/abstract/32/1/e10>

DNA methylation-based biomarkers have been discovered that could potentially be used for the diagnosis of cancer by detection of circulating, tumor-derived DNA in bodily fluids. Any methylation detection assay that would be applied to these samples must be capable of detecting small amounts of tumor DNA in the presence of background normal DNA. We have developed a real-time PCR assay, called HeavyMethyl, that is well suited for this application. HeavyMethyl uses methylation-specific oligonucleotide blockers and a methylation-specific probe to achieve methylation-specific amplification and detection. We tested the assays on unmethylated and artificially methylated DNA in order to determine the limit of detection. After careful optimization, our glutathione-S-transferase pi1 and Calcitonin assays can amplify as little as 30 and 60 pg of methylated DNA, respectively, and neither assay amplifies unmethylated DNA. The Calcitonin assay showed a highly significant methylation difference between normal colon and colon adenocarcinomas, and methylation was also detected in serum DNA from colon cancer patients. These assays show that HeavyMethyl technology can be successfully employed for the analysis of very low concentrations of methylated DNA, e.g. in serum of patients with tumors.

Duch, M., M. L. Carrasco, et al. (2004). "An RNA secondary structure bias for non-homologous reverse transcriptase-mediated deletions in vivo." Nucleic Acids Res. **32**(6): 2039-2048.

<http://nar.oupjournals.org/cgi/content/abstract/32/6/2039>

Murine leukemia viruses harboring an internal ribosome entry site (IRES)-directed translational cassette are able to replicate, but undergo loss of heterologous sequences upon continued passage. While complete loss of heterologous sequences is favored when these are flanked by a direct repeat, deletion mutants with junction sites within the heterologous cassette may also be retrieved, in particular from vectors without flanking repeats. Such deletion mutants were here used to investigate determinants of reverse transcriptase-mediated non-homologous recombination. Based upon previous structural analysis the individual recombination sites within the IRES could be assigned to either base-paired or unpaired regions of RNA. This assignment showed a significant bias ($P = 0.000082$) towards recombination within unpaired regions of the IRES. We propose that the events observed in this *in vivo* system result from template switching during first-strand cDNA synthesis and that the choice of acceptor sites for non-homologous recombination are guided by non-paired regions. Our results may have implications for recombination events taking place within structured regions of retroviral RNA genomes, especially in the absence of longer stretches of sequence similarity.

Dumas, E., C. Staedel, et al. (2003). "A promoter activity is present in the DNA sequence corresponding to the hepatitis C virus 5' UTR." *Nucleic Acids Res.* **31**(4): 1275-1281.

<http://nar.oupjournals.org/cgi/content/abstract/31/4/1275>

The hepatitis C virus (HCV) 5' untranslated region (UTR) has been extensively studied with regard to its internal ribosomal entry site (IRES) activity. In this work we present results suggesting the existence of a strong promoter activity carried by the DNA sequence corresponding to the HCV 5' UTR. This activity was not detected when the HCV 5' UTR sequence was replaced by HCV 3' UTR or poliovirus 5' UTR sequences. These results were further confirmed by using bicistronic constructions. We demonstrated the presence of an mRNA initiated in this 5' UTR sequence and located the initiation site by the 5' RACE method at nucleotide 67. Furthermore, northern experiments and flow cytometry analysis showed the unambiguous activity of such a promoter sequence in stably transfected cells. Our results strongly suggest that the data obtained using bicistronic DNA constructs carrying the HCV 5' UTR should be analyzed not only at the translational but also at the transcriptional level.

Farley, A. H., E. T. Luning Prak, et al. (2004). "More active human L1 retrotransposons produce longer insertions." *Nucleic Acids Res.* **32**(2): 502-510.

<http://nar.oupjournals.org/cgi/content/abstract/32/2/502>

The vast majority of L1 insertions are 5' truncated and thus inactive. Yet, the mechanism of 5' truncation is unknown. To examine whether the frequency of L1 retrotransposition is directly correlated with the length of genomic L1 insertions, we used a cell culture assay to measure retrotransposition frequency and a PCR-based assay to measure L1 insertion length. We tested five full-length human L1 elements that retrotranspose at different frequencies: LRE3, L1RP, L1.3, L1.2A and L1.2B. Our data suggest that L1 insertion length correlates with L1 retrotransposition frequency for insertions >1 kb in length. For two elements, L1RP and L1.2A, we found that swapping the reverse transcriptase domains had little effect. Instead, we found that genomic insertion length and retrotransposition frequency are substantially affected by amino acid substitutions at positions 363, 1220 and 1259 in ORF2. We suggest that the region containing residues 1220 and 1259 may be important in the binding of ORF2p to L1 RNA to facilitate reverse transcription.

Forrest, E. C., C. Cogoni, et al. (2004). "The RNA-dependent RNA polymerase, QDE-1, is a rate-limiting factor in post-transcriptional gene silencing in *Neurospora crassa*." Nucleic Acids Res. **32**(7): 2123-2128.

<http://nar.oupjournals.org/cgi/content/abstract/32/7/2123>

The RNA-dependent RNA polymerase (RdRP) qde-1 is an essential component of post-transcriptional gene silencing (PTGS), termed 'quelling' in the fungus *Neurospora crassa*. Here we show that the overexpression of QDE-1 results in a dramatic increase in the efficiency of quelling, with a concomitant net increase in the quantity of al-1 siRNAs. Moreover, in overexpressed strains there is a significant reduction in the number of transgenes required to induce quelling, and an increase in the phenotypic stability despite progressive loss of tandemly repeated transgenes, which normally determines reversion of a silenced phenotype to wild type. These data suggest that the activation and maintenance of silencing in *Neurospora* appear to rely both on the cellular amount of QDE-1 and the amount of transgenic copies producing RNA molecules that act as a substrate for the RdRP, implicating QDE-1 as a rate-limiting factor in PTGS.

Freund, M., C. Asang, et al. (2003). "A novel approach to describe a U1 snRNA binding site." Nucleic Acids Res. **31**(23): 6963-6975.

<http://nar.oupjournals.org/cgi/content/abstract/31/23/6963>

RNA duplex formation between U1 snRNA and a splice donor (SD) site can protect pre-mRNA from degradation prior to splicing and initiates formation of the spliceosome. This process was monitored, using sub-genomic HIV-1 expression vectors, by expression analysis of the glycoprotein env, whose formation critically depends on functional SD4. We systematically derived a hydrogen bond model for the complementarity between the free 5' end of U1 snRNA and 5' splice sites and numerous mutations following transient transfection of HeLa-T4+ cells with 5' splice site mutated vectors. The resulting model takes into account number, interdependence and neighborhood relationships of predicted hydrogen bond formation in a region spanning the three most 3' base pairs of the exon (-3 to -1) and the eight most 5' base pairs of the intron (+1 to +8). The model is represented by an algorithm classifying U1 snRNA binding sites which can or cannot functionally substitute SD4 with respect to Rev-mediated env expression. In a data set of 5' splice site mutations of the human ATM gene we found a significant correlation between the algorithmic classification and exon skipping ($P = 0.018$, χ^2 -test), showing that the applicability of the proposed model reaches far beyond HIV-1 splicing. However, the algorithmic classification must not be taken as an absolute measure of SD usage as it may be modified by upstream sequence elements. Upstream to SD4 we identified a fragment supporting ASF/SF2 binding. Mutating GAR nucleotide repeats within this site decreased the SD4-dependent Rev-mediated env expression, which could be balanced simply by artificially increasing the complementarity of SD4.

Gadal, F., C. Bozic, et al. (2003). "Integrated transcriptome analysis of the cellular mechanisms associated with Ha-ras-dependent malignant transformation of the human breast epithelial MCF7 cell line." Nucleic Acids Res. **31**(19): 5789-5804.

<http://nar.oupjournals.org/cgi/content/abstract/31/19/5789>

To understand the cellular mechanisms of malignant transformation induced by constitutive

activation of the ras oncogene (Ha-ras), we used a subtractive hybridization method (VGIDTM) together with an integrative analytical procedure based upon literature databases in the form of extensive interaction graphs. We found 166 over- and under-expressed genes which, in the human MCF7-ras breast epithelial cell line, are involved in the different aspects of tumoral transformation such as defined signaling pathways, cellular growth, protection against apoptosis, extracellular matrix and cytoskeleton remodeling. Integrative analysis led to the construction of a physiological model defining cross-talk and signaling pathway alterations which explicitly suggested mechanisms directly involved in tumor progression. The model further suggested points and means of intervention which could induce cell death in Ha-ras-transformed cells specifically. These hypotheses were directly tested in vitro and found to be largely correct, hence indicating that these new analytical and technological approaches allow the discovery of pathology-associated cellular mechanisms and physiologically defined targets leading to phenotype-specific pharmacological interventions.

Gardner, A. F. and W. E. Jack (2002). "Acyclic and dideoxy terminator preferences denote divergent sugar recognition by archaeon and Taq DNA polymerases." Nucleic Acids Res. **30**(2): 605-613.

<http://nar.oupjournals.org/cgi/content/abstract/30/2/605>

The nucleotide-binding site in a variety of DNA polymerases was probed by analyzing incorporation of dideoxy and acyclic chain terminators. Family B archaeon DNA polymerases Vent, Deep Vent, 9{degrees}N and Pfu incorporated acyclic in preference to dideoxy terminators, while the Family A DNA polymerases Taq and Klenow preferred dideoxy terminators. These divergent biases suggest that significant differences exist in sugar recognition in these two classes of polymerases. Mutants of Vent (A488L) and Taq (F667Y) that increase incorporation of dideoxy terminators maintained the acyclic/dideoxy bias of the parent enzyme, while more efficiently incorporating both dideoxy and acyclic terminators. The preference of archaeon DNA polymerases for acyclic analogs was exploited in chain terminator DNA sequence and genotype analysis. This technology was additionally aided by identification of specific dye-modified bases that improve terminator incorporation over that of the unmodified terminator. These three enhancing effects, (i) acyclic analogs, (ii) archaeon variants and (iii) specific dyes, appear to act additively and independently to increase terminator incorporation efficiency, collectively enhancing incorporation [~]8000-fold over the wild-type incorporation of dideoxynucleotides. Fluorescent chain terminator DNA sequence traces demonstrate the applicability of these advances in improving terminator incorporation, as required in DNA sequence and genotype determinations.

Giglio, S., P. T. Monis, et al. (2003). "Demonstration of preferential binding of SYBR Green I to specific DNA fragments in real-time multiplex PCR." Nucleic Acids Res. **31**(22): e136-.

<http://nar.oupjournals.org/cgi/content/abstract/31/22/e136>

SYBR Green I (SG) is widely used in real-time PCR applications as an intercalating dye and is included in many commercially available kits at undisclosed concentrations. Binding of SG to double-stranded DNA is non-specific and additional testing, such as DNA melting curve analysis, is required to confirm the generation of a specific amplicon. The use of melt curve analysis eliminates the necessity for agarose gel electrophoresis because the melting temperature (T_m) of the specific amplicon is analogous to the detection of an electrophoretic band. When using SG for real-time PCR multiplex reactions, discrimination of amplicons should be possible, provided the T_m values are sufficiently different. Real-time multiplex assays for *Vibrio cholerae* and *Legionella pneumophila* using commercially available kits and in-house SG mastermixes have highlighted variability in performance characteristics, in particular the detection of only a single product as

assessed by T_m analysis but multiple products as assessed by agarose gel electrophoresis. The detected T_m corresponds to the amplicon with the higher G+C% and larger size, suggesting preferential binding of SG during PCR and resulting in the failure to detect multiple amplicons in multiplex reactions when the amount of SG present is limiting. This has implications for the design and routine application of diagnostic real-time PCR assays employing SG.

Gill, P., J. Curran, et al. (2005). "A graphical simulation model of the entire DNA process associated with the analysis of short tandem repeat loci." Nucleic Acids Res. **33**(2): 632-643.

<http://nar.oupjournals.org/cgi/content/abstract/33/2/632>

The use of expert systems to interpret short tandem repeat DNA profiles in forensic, medical and ancient DNA applications is becoming increasingly prevalent as high-throughput analytical systems generate large amounts of data that are time-consuming to process. With special reference to low copy number (LCN) applications, we use a graphical model to simulate stochastic variation associated with the entire DNA process starting with extraction of sample, followed by the processing associated with the preparation of a PCR reaction mixture and PCR itself. Each part of the process is modelled with input efficiency parameters. Then, the key output parameters that define the characteristics of a DNA profile are derived, namely heterozygote balance (Hb) and the probability of allelic drop-out p(D). The model can be used to estimate the unknown efficiency parameters, such as {pi}extraction. What-if scenarios can be used to improve and optimize the entire process, e.g. by increasing the aliquot forwarded to PCR, the improvement expected to a given DNA profile can be reliably predicted. We demonstrate that Hb and drop-out are mainly a function of stochastic effect of pre-PCR molecular selection. Whole genome amplification is unlikely to give any benefit over conventional PCR for LCN.

Giraldo, P., A. Martinez, et al. (2003). "Functional dissection of the mouse tyrosinase locus control region identifies a new putative boundary activity." Nucleic Acids Res. **31**(21): 6290-6305.

<http://nar.oupjournals.org/cgi/content/abstract/31/21/6290>

Locus control regions (LCRs) are complex high-order chromatin structures harbouring several regulatory elements, including enhancers and boundaries. We have analysed the mouse tyrosinase LCR functions, in vitro, in cell lines and, in vivo, in transgenic mice and flies. The LCR-core (2.1 kb), located at -15 kb and carrying a previously described tissue-specific DNase I hypersensitive site, operates as a transcriptional enhancer that efficiently transactivates heterologous promoters in a cell-specific orientation-independent manner. Furthermore, we have investigated the boundary activity of these sequences in transgenic animals and cells. In mice, the LCR fragment (3.7 kb) rescued a weakly expressed reference construct that displays position effects. In *Drosophila*, the LCR fragment and its core insulated the expression of a white minigene reporter construct from chromosomal position effects. In cells, sequences located 5' from the LCR-core displayed putative boundary activities. We have obtained genomic sequences surrounding the LCR fragment and found a LINE1 repeated element at 5'. In B16 melanoma and L929 fibroblast mouse cells, this element was found heavily methylated, supporting the existence of putative boundary elements that could prevent the spreading of condensed chromatin from the LINE1 sequences into the LCR fragment, experimentally shown to be in an open chromatin structure.

Godthelp, B. C., W. W. Wiegant, et al. (2002). "Mammalian Rad51C contributes to DNA cross-link

resistance, sister chromatid cohesion and genomic stability." Nucleic Acids Res. **30**(10): 2172-2182.

<http://nar.oupjournals.org/cgi/content/abstract/30/10/2172>

The eukaryotic Rad51 protein is a structural and functional homolog of *Escherichia coli* RecA with a role in DNA repair and genetic recombination. Five paralogs of Rad51 have been identified in vertebrates, Rad51B, Rad51C, Rad51D, Xrcc2 and Xrcc3, which are also implicated in recombination and genome stability. Here, we identify a mammalian cell mutant in Rad51C. We show that the Chinese hamster cell mutant, CL-V4B, has a defect in Rad51C. Sequencing of the hamster Rad51C cDNA revealed a 132 bp deletion corresponding to an alternatively spliced transcript with lack of exon 5. CL-V4B was hypersensitive to the interstrand cross-linking agents mitomycin C (MMC) and cisplatin, the alkylating agent methyl methanesulfonate and the topoisomerase I inhibitor camptothecin and showed impaired Rad51 foci formation in response to DNA damage. The defect in Rad51C also resulted in an increase of spontaneous and MMC-induced chromosomal aberrations as well as a lack of induction of sister chromatid exchanges. However, centrosome formation was not affected. Intriguingly, a reduced level of sister chromatid cohesion was found in CL-V4B cells. These results reveal a role for Rad51C that is unique among the Rad51 paralogs.

Hanke, T., M. J. Ramiro, et al. (2003). "Cloning, functional analysis and post-transcriptional regulation of a type II DNA topoisomerase from *Leishmania infantum*. A new potential target for anti-parasite drugs." Nucleic Acids Res. **31**(16): 4917-4928.

<http://nar.oupjournals.org/cgi/content/abstract/31/16/4917>

We identified a type II topoisomerase enzyme from *Leishmania infantum*, a parasite protozoan causing disease in humans. This protein, named Li topo II, which displays a variable C-terminal end, is located in the kinetoplast. The cloned gene encoding Li-TOP2 compensates for the slow growth of topo II-deficient mutants of *Saccharomyces cerevisiae*, resulting in a catalytically active DNA topoisomerase in yeast. Analysis of the specific mRNA levels of the Li-TOP2 gene showed variations throughout the parasite cell cycle in synchronized cells as well as between the distinct forms of the parasite. Thus, the enzyme had higher levels of mRNA expression in the highly infective intracellular form of the parasite, the amastigote, than in the extracellular promastigote form, suggesting a relation with the distinct developmental and infectious phases of the protozoan. In addition, western blot analysis showed differences in protein expression between the proliferative and non-proliferative forms of *L. infantum* promastigotes, which displayed similar levels of mRNA. This indicated possible post-transcriptional regulation mechanisms. The data suggest that Li topo II has a part in DNA decatenation and probably at the initial stages of proliferation in the intracellular form of *L. infantum*, a parasite that has to proliferate into the host macrophage to survive its hostile environment in its first moments of intracellular infection.

Hashimoto, M., Y. He, et al. (2003). "On-line integration of PCR and cycle sequencing in capillaries: from human genomic DNA directly to called bases." Nucleic Acids Res. **31**(8): e41-.

<http://nar.oupjournals.org/cgi/content/abstract/31/8/e41>

A fully integrated system has been developed for genetic analysis based on direct sequencing of polymerase chain reaction (PCR) products. The instrument is based on a serially connected fused-silica capillary assembly. The technique involves the use of microreactors for small-volume PCR and for dye-terminator cycle-sequencing reaction, purification of the sequencing fragments,

and separation of the purified DNA ladder. Four modifications to the normal PCR protocol allow the elimination of post-reaction purification. The use of capillaries as reaction vessels significantly reduced the required reaction time. True reduction in reagent cost is achieved by a novel sample preparation procedure where nanoliter volumes of templates and sequencing reaction reagent are mixed using a micro-syringe pump. The remaining stock solution of sequencing reaction reagent can be reused without contamination. The performance of the whole system is demonstrated by one-step sequencing of a specific 257-bp region in human chromosome DNA. Base calling for the smaller fragments is limited only by the resolving power of the gel. The system is simple, reliable and fast. The entire process from PCR to DNA separation is completed in [~]4 h. Feasibilities for development of a fully automated sequencing system in the high-throughput format and future adaptation of this concept to a microchip are discussed.

Hawkins, M. E. and F. M. Balis (2004). "Use of pteridine nucleoside analogs as hybridization probes." Nucleic Acids Res. **32**(7): e62-.

<http://nar.oupjournals.org/cgi/content/abstract/32/7/e62>

The pteridine nucleoside analog 3-methyl isoxanthopterin (3-MI) is highly fluorescent, with a quantum yield of 0.88, and it can be synthesized as a phosphoramidite and incorporated into oligonucleotides through a deoxyribose linkage. Within an oligonucleotide, 3-MI is intimately associated with native bases and its fluorescence is variably quenched in a sequence-dependent manner. Binding, annealing, binding, digestion or cleavage of fluorophore-containing oligonucleotides can be detected by monitoring changes in fluorescence properties. We developed a single step method for detecting annealing of complementary DNA sequences using 3-MI-containing oligonucleotides as hybridization probes. One of the complementary strands contains the fluorophore as an insertion and when annealing occurs, the fluorophore bulges out from the double strand, resulting in increased fluorescence intensity. We have examined the sequence dependency, optimal strand length and impact of multiple fluorophores per strand in terms of brightness and impact on the annealing process. We describe the application of this technique to the detection of positive PCR products using an HIV-1 detection system. This sequence-dependent hybridization technique can result in fluorescence intensity increases of up to 27-fold. Fluorescence intensity increases are only seen upon specific binding to bulge-generating complements, removing issues of high background from non-specific binding.

He, L., P. F. Chinnery, et al. (2002). "Detection and quantification of mitochondrial DNA deletions in individual cells by real-time PCR." Nucleic Acids Res. **30**(14): e68-.

<http://nar.oupjournals.org/cgi/content/abstract/30/14/e68>

Defects of mitochondrial DNA (mtDNA) are an important cause of disease and play a role in the ageing process. There are multiple copies of the mitochondrial genome in a single cell. In many patients with acquired or inherited mtDNA mutations, there exists a mixture of mutated and wild type genomes (termed heteroplasmy) within individual cells. As a biochemical and clinical defect is only observed when there are high levels of mutated mtDNA, a crucial investigation is to determine the level of heteroplasmic mutations within tissues and individual cells. We have developed an assay to determine the relative amount of deleted mtDNA using real-time fluorescence PCR. This assay detects the vast majority of deleted molecules, thus eliminating the need to develop specific probes. We have demonstrated an excellent correlation with other techniques (Southern blotting and three-primer competitive PCR), and have shown this technique to be sensitive to quantify the level of deleted mtDNA molecules in individual cells. Finally, we have used this assay to investigate patients with mitochondrial disease and shown in individual skeletal muscle fibres that there exist different patterns of abnormalities between

patients with single or multiple mtDNA deletions. We believe that this technique has significant advantages over other methods to quantify deleted mtDNA and, employed alongside our method to sequence the mitochondrial genome from single cells, will further our understanding of the role of mtDNA mutations in human disease and ageing.

Hu, X., S. Hipolito, et al. (2004). "Relative gene-silencing efficiencies of small interfering RNAs targeting sense and antisense transcripts from the same genetic locus." Nucleic Acids Res. **32**(15): 4609-4617.

<http://nar.oupjournals.org/cgi/content/abstract/32/15/4609>

Short interfering RNAs (siRNAs) directed against different regions of genes display marked variation in their potency in mediating mRNA degradation. Various factors have been proposed to affect the efficacy of siRNA. We explored some of the factors by evaluating in cultured human cells 28 randomly selected siRNAs targeting the GPR39 and MGC29643 transcripts derived from the same genetic locus but transcribed in opposite directions. Twenty of the 24 siRNAs targeting the overlapping regions of the transcripts simultaneously reduced the levels of both transcripts. Single nucleotide changes in either of the siRNA strands significantly reduced the gene-silencing efficiency of the siRNA on targeted sense transcript without affecting the antisense transcript. Overall, we observed a greater gene-silencing efficiency on the MGC29643 transcript than on the GPR39 transcript in HeLa cells. Since MGC29643 transcript is more abundant than the GPR39 transcript [0.24 versus 0.008% relative to 100% for glyceraldehyde-3-phosphate dehydrogenase (GAPDH)], the results suggest that the abundance of the mRNA affects the efficiency of silencing. Two additional observations supported this hypothesis. First, GAPDH whose intracellular level is the highest of the three was the most efficiently silenced. Second, a reversal of gene-silencing efficiency was observed in U-138 MG cells in which the relative abundance of the GPR39 and MGC29643 transcripts is also reversed. Our study suggests that low-abundant transcripts are less susceptible to siRNA-mediated degradation than medium- and high-abundant transcripts.

Hultin, E., M. Kaller, et al. (2005). "Competitive enzymatic reaction to control allele-specific extensions." Nucleic Acids Res. **33**(5): e48-.

<http://nar.oupjournals.org/cgi/content/abstract/33/5/e48>

Here, we present a novel method for SNP genotyping based on protease-mediated allele-specific primer extension (PrASE), where the two allele-specific extension primers only differ in their 3'-positions. As reported previously [Ahmadian, A., Gharizadeh, B., O'Meara, D., Odeberg, J. and Lundeberg, J. (2001), *Nucleic Acids Res.*, **29**, e121], the kinetics of perfectly matched primer extension is faster than mismatched primer extension. In this study, we have utilized this difference in kinetics by adding protease, a protein-degrading enzyme, to discriminate between the extension reactions. The competition between the polymerase activity and the enzymatic degradation yields extension of the perfectly matched primer, while the slower extension of mismatched primer is eliminated. To allow multiplex and simultaneous detection of the investigated single nucleotide polymorphisms (SNPs), each extension primer was given a unique signature tag sequence on its 5' end, complementary to a tag on a generic array. A multiplex nested PCR with 13 SNPs was performed in a total of 36 individuals and their alleles were scored. To demonstrate the improvements in scoring SNPs by PrASE, we also genotyped the individuals without inclusion of protease in the extension. We conclude that the developed assay is highly allele-specific, with excellent multiplex SNP capabilities.

Jacobsen, N., J. Bentzen, et al. (2002). "LNA-enhanced detection of single nucleotide polymorphisms in the apolipoprotein E." Nucleic Acids Res. **30**(19): e100-.

<http://nar.oupjournals.org/cgi/content/abstract/30/19/e100>

Genotyping of single nucleotide polymorphisms (SNPs) in large populations presents a great challenge, especially if the SNPs are embedded in GC-rich regions, such as the codon 112 SNP in the human apolipoprotein E (apoE). In the present study, we have used immobilized locked nucleic acid (LNA) capture probes combined with LNA-enhancer oligonucleotides to obtain efficient and specific interrogation of SNPs in the apoE codons 112 and 158, respectively. The results demonstrate the usefulness of LNA oligonucleotide capture probes combined with LNA enhancers in mismatch discrimination. The assay was applied to a panel of patient samples with simultaneous genotyping of the patients by DNA sequencing. The apoE genotyping assays for the codons 112 and 158 SNPs resulted in unambiguous results for all patient samples, concurring with those obtained by DNA sequencing.

Jacobsen, N., P. S. Nielsen, et al. (2004). "Direct isolation of poly(A)+ RNA from 4 M guanidine thiocyanate-lysed cell extracts using locked nucleic acid-oligo(T) capture." Nucleic Acids Res. **32**(7): e64-.

<http://nar.oupjournals.org/cgi/content/abstract/32/7/e64>

LNA oligonucleotides constitute a class of bicyclic RNA analogues having an exceptionally high affinity for their complementary DNA and RNA target molecules. We here report a novel method for highly efficient isolation of intact poly(A)+ RNA using an LNA-substituted oligo(dT) affinity ligand, based on the increased affinity of LNA-T for complementary poly(A) tracts. Poly(A)+ RNA was isolated directly from 4 M guanidine thiocyanate-lysed *Caenorhabditis elegans* worm extracts as well as from lysed human K562 and vincristine-resistant K562/VCR leukemia cells using LNA₂.T oligonucleotide as an affinity probe, in which every second thymidine was substituted by LNA thymidine. In accordance with the significantly increased stability of the LNA₂.T-A duplexes in 4 M GuSCN, we obtained a 30- to 50-fold mRNA yield increase using the LNA-substituted oligo(T) affinity probe compared with DNA-oligo(dT)-selected mRNA samples. The LNA₂.T affinity probe was, furthermore, highly efficient in isolation of poly(A)+ RNA in a low salt concentration range of 50-100 mM NaCl in poly(A) binding buffer, as validated by selecting the mRNA pools from total RNA samples extracted from different *Saccharomyces cerevisiae* strains, followed by northern blot analysis. Finally, we demonstrated the utility of the LNA-oligo(T)-selected mRNA in quantitative real-time PCR by analysing the relative expression levels of the human *mdr1* multidrug resistance gene in the two K562 cell lines employing pre-validated Taqman assays. Successful use of the NH₂-modified LNA₂.T probe in isolation of human mRNA implies that the LNA-oligo(T) method could be automated for streamlined, high throughput expression profiling by real-time PCR by covalently coupling the LNA affinity probe to solid, pre-activated surfaces, such as microtiter plate wells or magnetic particles.

Jansen, L. E. T., A. I. Belo, et al. (2002). "Transcription elongation factor Spt4 mediates loss of phosphorylated RNA polymerase II transcription in response to DNA damage." Nucleic Acids Res. **30**(16): 3532-3539.

<http://nar.oupjournals.org/cgi/content/abstract/30/16/3532>

Previously, we found that Rad26, the yeast Cockayne syndrome B homolog and the transcription elongation factor Spt4 mediate transcription-coupled repair of UV-induced DNA damage. Here we

studied the effect of DNA damage on transcription by directly analyzing the RNA polymerase II localization at active genes in vivo. A rad26 defect leads to loss of Ser5 phosphorylated RNA polymerase II localization to active genes, while localization is only transiently diminished in wild type cells. In contrast, loss of Ser5-P RNAP II localization is suppressed in spt4 cells. Interestingly, even when DNA damage is persistent the absence of Spt4 leads to a delayed loss of transcription suggesting that Spt4 is directly involved in mediating transcription shutdown. Comparative analysis of phosphorylated and non-phosphorylated RNA polymerase II localization revealed that Ser5-P RNAP II is preferentially lost in the presence of DNA damage. In addition, we found evidence for a transient Rad26 localization to active genes in response to DNA damage. These findings provide insight into the transcriptional response to DNA damage and the factors involved in communicating this response, which has direct implications for our understanding of transcription-repair coupling.

Keith, J. M., D. A. E. Cochran, et al. (2004). "Unlocking hidden genomic sequence." Nucleic Acids Res. **32**(3): e35-.

<http://nar.oupjournals.org/cgi/content/abstract/32/3/e35>

Despite the success of conventional Sanger sequencing, significant regions of many genomes still present major obstacles to sequencing. Here we propose a novel approach with the potential to alleviate a wide range of sequencing difficulties. The technique involves extracting target DNA sequence from variants generated by introduction of random mutations. The introduction of mutations does not destroy original sequence information, but distributes it amongst multiple variants. Some of these variants lack problematic features of the target and are more amenable to conventional sequencing. The technique has been successfully demonstrated with mutation levels up to an average 18% base substitution and has been used to read previously intractable poly(A), AT-rich and GC-rich motifs.

Kermekchiev, M. B., A. Tzekov, et al. (2003). "Cold-sensitive mutants of Taq DNA polymerase provide a hot start for PCR." Nucleic Acids Res. **31**(21): 6139-6147.

<http://nar.oupjournals.org/cgi/content/abstract/31/21/6139>

Although the thermophilic bacterium *Thermus aquaticus* grows optimally at 70{degrees}C and cannot grow at moderate temperatures, its DNA polymerase I has significant activity at 20-37{degrees}C. This activity is a bane to some PCRs, since it catalyzes non-specific priming. We report mutations of KlenTaq (an N-terminal deletion variant) DNA polymerase that have markedly reduced activity at 37{degrees}C yet retain apparently normal activity at 68{degrees}C and resistance at 95{degrees}C. The first four of these mutations are clustered on the outside surface of the enzyme, nowhere near the active site, but at the hinge point of a domain that has been proposed to move at each cycle of nucleotide incorporation. We show that the novel cold-sensitive mutants can provide a hot start for PCR and exhibit slightly improved fidelity.

Kim, J., L. Jia, et al. (2003). "Dynamic methylation of histone H3 at lysine 4 in transcriptional regulation by the androgen receptor." Nucleic Acids Res. **31**(23): 6741-6747.

<http://nar.oupjournals.org/cgi/content/abstract/31/23/6741>

The methylation of histone H3 correlates with either gene expression or silencing depending on

the residues modified. Methylated lysine 4 (H3-K4) is associated with transcription at active gene loci. Furthermore, it was reported that trimethylated but not dimethylated H3-K4 is exclusively associated with active chromatin in *Saccharomyces cerevisiae*. In the present study, we investigated the H3-K4 methylation at the human prostate specific antigen (PSA) locus following gene activation and repression via androgen receptor (AR). We show that ligand-induced, AR-mediated transcription was accompanied by rapid decreases in di- and trimethylated H3-K4 at the PSA enhancer and promoter. Moreover, the observed decreases in H3-K4 methylation were reversed when AR was inhibited by a specific AR antagonist, bicalutamide. In contrast to the decreases in methylation at the 5' transcriptional control regions of the PSA gene, H3-K4 methylation in the coding region steadily increased after a lag period of [~]4 h. The results suggest a novel role of methylated H3-K4 in transcriptional regulation.

Kuiper, R. P., M. Schepens, et al. (2004). "Regulation of the MiTF/TFE bHLH-LZ transcription factors through restricted spatial expression and alternative splicing of functional domains." Nucleic Acids Res. **32**(8): 2315-2322.

<http://nar.oupjournals.org/cgi/content/abstract/32/8/2315>

The MiTF/TFE (MiT) family of basic helix-loop-helix leucine zipper transcription factors is composed of four closely related members, MiTF, TFE3, TFEB and TFEC, which can bind target DNA both as homo- or heterodimers. Using real-time RT-PCR, we have analyzed the relative expression levels of the four members in a broad range of human tissues, and found that their ratio of expression is tissue-dependent. We found that, similar to the MiTF gene, the genes for TFEB and TFEC contain multiple alternative first exons with restricted and differential tissue distributions. Seven alternative 5' exons were identified in the TFEB gene, of which three displayed specific expression in placenta and brain, respectively. A novel TFEC transcript (TFEC-C) encodes an N-terminally truncated TFEC isoform lacking the acidic activation domain (AAD), and is exclusively expressed in kidney and small intestine. Furthermore, we observed that a considerable proportion of the TFEC transcripts splice out protein-coding exons, resulting in transcription factor isoforms lacking one or more functional domains, primarily the basic region and/or the AAD. These isoforms were always co-expressed with the intact transcription factors and may act as negative regulators of MiTF/TFE proteins. Our data reveal that multiple levels of regulation exist for the MiTF/TFE family of transcription factors, which indicates how these transcription factors may participate in various cellular processes in different tissues.

Leh-Louis, V., B. Wirth, et al. (2004). "Differential evolution of the *Saccharomyces cerevisiae* DUP240 paralogs and implication of recombination in phylogeny." Nucleic Acids Res. **32**(7): 2069-2078.

<http://nar.oupjournals.org/cgi/content/abstract/32/7/2069>

Multigene families are observed in all genomes sequenced so far and are the reflection of key evolutionary mechanisms. The DUP240 family, identified in *Saccharomyces cerevisiae* strain S288C, is composed of 10 paralogs: seven are organized as two tandem repeats and three are solo ORFs. To investigate the evolution of the three solo paralogs, YAR023c, YCR007c and YHL044w, we performed a comparative analysis between 15 *S. cerevisiae* strains. These three ORFs are present in all strains and the conservation of synteny indicates that they are not frequently involved in chromosomal reshaping, in contrast to the DUP240 ORFs organized in tandem repeats. Our analysis of nucleotide and amino acid variations indicates that YAR023c and YHL044w fix mutations more easily than YCR007c, although they all belong to the same multigene family. This comparative analysis was also conducted with five arbitrarily chosen Ascomycetes-specific genes and five arbitrarily chosen common genes (genes that have a homolog in at least one non-Ascomycetes organism). Ascomycetes-specific genes appear to be

diverging faster than common genes in the *S.cerevisiae* species, a situation that was previously described between different yeast species. Our results point to the strong contribution, during DNA sequence evolution, of allelic recombination besides nucleotide substitution.

Lindroos, K., S. Sigurdsson, et al. (2002). "Multiplex SNP genotyping in pooled DNA samples by a four-colour microarray system." Nucleic Acids Res. **30**(14): e70-.

<http://nar.oupjournals.org/cgi/content/abstract/30/14/e70>

We selected 125 candidate single nucleotide polymorphisms (SNPs) in genes belonging to the human type 1 interferon (IFN) gene family and the genes coding for proteins in the main type 1 IFN signalling pathway by screening databases and by in silico comparison of DNA sequences. Using quantitative analysis of pooled DNA samples by solid-phase mini-sequencing, we found that only 20% of the candidate SNPs were polymorphic in the Finnish and Swedish populations. To allow more effective validation of candidate SNPs, we developed a four-colour microarray-based mini-sequencing assay for multiplex, quantitative allele frequency determination in pooled DNA samples. We used cyclic mini-sequencing reactions with primers carrying 5'-tag sequences, followed by capture of the products on microarrays by hybridisation to complementary tag oligonucleotides. Standard curves prepared from mixtures of known amounts of SNP alleles demonstrate the applicability of the system to quantitative analysis, and showed that for about half of the tested SNPs the limit of detection for the minority allele was below 5%. The microarray-based genotyping system established here is universally applicable for genotyping and quantification of any SNP, and the validated system for SNPs in type 1 IFN-related genes should find many applications in genetic studies of this important immunoregulatory pathway.

Marras, S. A. E., B. Gold, et al. (2004). "Real-time measurement of in vitro transcription." Nucleic Acids Res. **32**(9): e72-.

<http://nar.oupjournals.org/cgi/content/abstract/32/9/e72>

We have developed a simple method to measure RNA synthesis in real time. In this technique, transcription reactions are performed in the presence of molecular beacons that possess a 2'-O-methylribonucleotide backbone. These probes become fluorescent as they hybridize to nascent RNA during the course of synthesis. We found that molecular beacons synthesized from natural deoxyribonucleotides were not suitable, because they are copied by RNA polymerases, generating complementary product strands that bind to the molecular beacons, causing a conformational change that results in unwanted fluorescence. However, when the molecular beacons are synthesized from 2'-O-methylribonucleotides, they are not copied and fluorescence is strictly dependent upon transcription of the added template. Utilizing these modified molecular beacons, quantitative comparisons were made of the activity of a variety of RNA polymerases and the effect of an inhibitor of transcription was determined.

Matsui, D., M. Sakari, et al. (2002). "Transcriptional regulation of the mouse steroid 5{alpha}-reductase type II gene by progesterone in brain." Nucleic Acids Res. **30**(6): 1387-1393.

<http://nar.oupjournals.org/cgi/content/abstract/30/6/1387>

The steroid 5{alpha}-reductase (5{alpha}-R) plays an important physiological role in the conversion of steroid hormones such as androgen and progesterone to their 5{alpha}-reduced

derivatives. 5 α -R type II (5 α -R2), one of two 5 α -R isoforms, is thought to be a key enzyme in the generation of neuroactive steroids in the brain, particularly allopregnanolone (AP), via the production of its precursor dihydroprogesterone from progesterone. In the present study, we investigated possible regulatory mechanisms of 5 α -R2 gene expression by steroid hormones in the female mouse brain. We first cloned mouse 5 α -R2 (m5 α -R2) cDNA by degenerate PCR, and found that progesterone induced 5 α -R2 gene expression to levels detectable by in situ hybridization in female mouse brains. Functional analysis of the m5 α -R2 gene promoter by a transient expression assay with human progesterone receptor (PR) and androgen receptor (AR) expression vectors identified a progesterone and androgen regulatory element (m5 α -R2 PRE/ARE). Results of an electrophoretic mobility shift assay revealed that both PR and AR homodimers bound directly to m5 α -R2 PRE/ARE sequence. These findings suggest that the gene expression of m5 α -R2 is transcriptionally regulated by progesterone in female brains.

Motz, M., G. Sagner, et al. (2003). "Sequential DEXAS: a method for obtaining DNA sequences from genomic DNA and blood in one reaction." *Nucleic Acids Res.* **31**(20): e121-.

<http://nar.oupjournals.org/cgi/content/abstract/31/20/e121>

Sequential DEXAS (direct exponential amplification and sequencing), a one step amplification and sequencing procedure that allows accurate, inexpensive and rapid DNA sequence determination directly from genomic DNA, is described. This method relies on the simultaneous use of two DNA polymerases that differ both in their ability to incorporate dideoxynucleotides and in the time at which they are activated during the reaction. One enzyme, which incorporates deoxynucleotides and performs amplification of the target DNA sequence, is supplied in an active state whereas the other enzyme, which incorporates dideoxynucleotides and performs the sequencing reaction, is supplied in an inactive state but becomes activated by a temperature step during the thermocycling. Thus, in the initial stage of the reaction, target amplification occurs, while in the second stage the sequencing reaction takes place. We show that Sequential DEXAS yields high quality sequencing results directly from genomic DNA as well as directly from human blood without any prior isolation or purification of DNA.

Napierala, M., D. Michalowski, et al. (2005). "Facile FMR1 mRNA structure regulation by interruptions in CGG repeats." *Nucleic Acids Res.* **33**(2): 451-463.

<http://nar.oupjournals.org/cgi/content/abstract/33/2/451>

RNA metabolism is a major contributor to the pathogenesis of clinical disorders associated with premutation size alleles of the fragile X mental retardation (FMR1) gene. Herein, we determined the structural properties of numerous FMR1 transcripts harboring different numbers of both CGG repeats and AGG interruptions. The stability of hairpins formed by uninterrupted repeat-containing transcripts increased with the lengthening of the repeat tract. Even a single AGG interruption in the repeated sequence dramatically changed the folding of the 5'UTR fragments, typically resulting in branched hairpin structures. Transcripts containing different lengths of CGG repeats, but sharing a common AGG pattern, adopted similar types of secondary structures. We postulate that interruption-dependent structure variants of the FMR1 mRNA contribute to the phenotype diversity, observed in premutation carriers.

Osipovich, A. B., E. K. White-Grindley, et al. (2004). "Activation of cryptic 3' splice sites within introns of

cellular genes following gene entrapment." Nucleic Acids Res. **32**(9): 2912-2924.

<http://nar.oupjournals.org/cgi/content/abstract/32/9/2912>

Gene trap vectors developed for genome-wide mutagenesis can be used to study factors governing the expression of exons inserted throughout the genome. For example, entrapment vectors consisting of a partial 3'-terminal exon [i.e. a neomycin resistance gene (Neo), a poly(A) site, but no 3' splice site] were typically expressed following insertion into introns, from cellular transcripts that spliced to cryptic 3' splice sites present either within the targeting vector or in the adjacent intron. A vector (U3NeoSV1) containing the wild-type Neo sequence preferentially disrupted genes that spliced in-frame to a cryptic 3' splice site in the Neo coding sequence and expressed functional neomycin phosphotransferase fusion proteins. Removal of the cryptic Neo 3' splice site did not reduce the proportion of clones with inserts in introns; rather, the fusion transcripts utilized cryptic 3' splice sites present in the adjacent intron or generated by virus integration. However, gene entrapment with U3NeoSV2 was considerably more random than with U3NeoSV1, consistent with the widespread occurrence of potential 3' splice site sequences in the introns of cellular genes. These results clarify the mechanisms of gene entrapment by U3 gene trap vectors and illustrate features of exon definition required for 3' processing and polyadenylation of cellular transcripts.

Paracchini, S., B. Arredi, et al. (2002). "Hierarchical high-throughput SNP genotyping of the human Y chromosome using MALDI-TOF mass spectrometry." Nucleic Acids Res. **30**(6): e27-.

<http://nar.oupjournals.org/cgi/content/abstract/30/6/e27>

We have established the use of a primer extension/mass spectrometry method (the PinPoint assay) for high-throughput SNP genotyping of the human Y chromosome. 118 markers were used to define 116 haplogroups and typing was organised in a hierarchical fashion. Twenty multiplex PCR/primer extension reactions were set up and each sample could be assigned to a haplogroup with only two to five of these multiplex analyses. A single aliquot of one enzyme was found to be sufficient for both PCR and primer extension. We observed 100% accuracy in blind validation tests. The technique thus provides a reliable, cost-effective and automated method for Y genotyping, and the advantages of using a hierarchical strategy can be applied to any DNA segment lacking recombination.

Pemov, A., H. Modi, et al. (2005). "DNA analysis with multiplex microarray-enhanced PCR." Nucleic Acids Res. **33**(2): e11-.

<http://nar.oupjournals.org/cgi/content/abstract/33/2/e11>

We have developed a highly sensitive method for DNA analysis on 3D gel element microarrays, a technique we call multiplex microarray-enhanced PCR (MME-PCR). Two amplification strategies are carried out simultaneously in the reaction chamber: on or within gel elements, and in bulk solution over the gel element array. MME-PCR is initiated by multiple complex primers containing gene-specific, forward and reverse, sequences appended to the 3' end of a universal amplification primer. The complex primer pair is covalently tethered through its 5' end to the polyacryl- amide backbone. In the bulk solution above the gel element array, a single pair of unattached universal primers simultaneously directs pseudo-monoplex PCR of all targets according to normal solution-phase PCR. The presence of a single universal PCR primer pair in solution accelerates amplification within gel elements and eliminates the problem of primer interference that is common to conventional multiplex PCR. We show 106-fold amplification of

targeted DNA after 50 cycles with average amplification efficiency 1.34 per cycle, and demonstrate specific on-chip amplification of six genes in *Bacillus subtilis*. All six genes were detected at 4.5 pg of bacterial genomic DNA (equivalent to 103 genomes) in 60 independent amplification reactions performed simultaneously in single reaction chamber.

Pincas, H., M. R. Pingle, et al. (2004). "High sensitivity EndoV mutation scanning through real-time ligase proofreading." Nucleic Acids Res. **32**(19): e148-.

<http://nar.oupjournals.org/cgi/content/abstract/32/19/e148>

The ability to associate mutations in cancer genes with the disease and its subtypes is critical for understanding oncogenesis and identifying biomarkers for clinical diagnosis. A two-step mutation scanning method that sequentially used endonuclease V (EndoV) to nick at mismatches and DNA ligase to reseat incorrectly or nonspecifically nicked sites was previously developed in our laboratory. Herein we report an optimized single-step assay that enables ligase to proofread EndoV cleavage in real-time under a compromise between buffer conditions. Real-time proofreading results in a dramatic reduction of background cleavage. A universal PCR strategy that employs both unlabeled gene-specific primers and labeled universal primers, allows for multiplexed gene amplification and precludes amplification of primer dimers. Internally labeled PCR primers eliminate EndoV cleavage at the 5' terminus, enabling high-throughput capillary electrophoresis readout. Furthermore, signal intensity is increased and artifacts are reduced by generating heteroduplexes containing only one of the two possible mismatches (e.g. either A/C or G/T). The single-step assay improves sensitivity to 1:50 and 1:100 (mutant:wild type) for unknown mutations in the p53 and K-ras genes, respectively, opening prospects as an early detection tool.

Ramakrishnan, R., D. Dorris, et al. (2002). "An assessment of Motorola CodeLinkTM microarray performance for gene expression profiling applications." Nucleic Acids Res. **30**(7): e30-.

<http://nar.oupjournals.org/cgi/content/abstract/30/7/e30>

DNA microarrays enable users to obtain information on differences in transcript abundance on a massively parallel scale. Recently, however, data analyses have revealed potential pitfalls related to image acquisition, variability and misclassifications in replicate measurements, cross-hybridization and sensitivity limitations. We have generated a series of analytical tools to address the manufacturing, detection and data analysis components of a microarray experiment. Together, we have used these tools to optimize performance in an expression profiling study. We demonstrate three significant advantages of the Motorola CodeLinkTM platform: sensitivity of one copy per cell, coefficients of variation of 10% in the hybridization signals across slides and across target preparations, and specificity in distinguishing highly homologous sequences. Slides where oligonucleotide probes are spotted in 6-fold redundancy were used to demonstrate the effect of replication on data quality. Lastly, the differential expression ratios obtained with the CodeLinkTM expression platform were validated against those obtained with quantitative reverse transcription-PCR assays for 54 genes.

Rapozzi, V., B. E. A. Burm, et al. (2002). "Antiproliferative effect in chronic myeloid leukaemia cells by antisense peptide nucleic acids." Nucleic Acids Res. **30**(17): 3712-3721.

<http://nar.oupjournals.org/cgi/content/abstract/30/17/3712>

Peptide nucleic acid (PNA) is a synthetic DNA analogue that is resistant to nucleases and proteases and binds with exceptional affinity to RNA. Because of these properties PNA has the potential to become a powerful therapeutic agent to be used in vivo. Until now, however, the use of PNA in vivo has not been much investigated. Here, we have attempted to reduce the expression of the bcr/abl oncogene in chronic myeloid leukaemia KYO-1 cells using a 13mer PNA sequence (asPNA) designed to hybridise to the b2a2 junction of bcr/abl mRNA. To enhance cellular uptake asPNA was covalently linked to the basic peptide VKRKKKP (NLS-asPNA). Moreover, to investigate the cellular uptake by confocal microscopy, both PNAs were linked by their N-terminus to fluorescein (FL). Studies of uptake, carried out at 4 and 37{degrees}C on living KYO-1 cells stained with hexidium iodide, showed that both NLS-asPNA-FL and asPNA-FL were taken up by the cells, through a receptor-independent mechanism. The intracellular amount of NLS-asPNA-FL was about two to three times higher than that of asPNA-FL. Using a semi-quantitative RT-PCR technique we found that 10 {micro}M asPNA and NLS-asPNA reduced the level of b2a2 mRNA in KYO-1 cells to 20 {+/-} 5% and 60 {+/-} 10% of the control, respectively. Western blot analysis showed that asPNA promoted a significant inhibition of p210BCR/ABL protein: residual protein measured in cells exposed for 48 h to asPNA was [~]35% of the control. Additionally, asPNA impaired cell growth to 50 {+/-} 5% of the control and inhibited completion of the cell cycle. In summary, these results demonstrate that a PNA 13mer is taken up by KYO-1 cells and is capable of producing a significant and specific down-regulation of the bcr/abl oncogene involved in leukaemogenesis.

Reyes-Lopez, M. A., A. Mendez-Tenorio, et al. (2003). "Fingerprinting of prokaryotic 16S rRNA genes using oligodeoxyribonucleotide microarrays and virtual hybridization." Nucleic Acids Res. **31**(2): 779-789.

<http://nar.oupjournals.org/cgi/content/abstract/31/2/779>

An oligonucleotide microarray hybridization system to differentiate microbial species was designed and tested. Seven microbial species were studied, including one *Bacillus* and six *Pseudomonas* strains. DNA sequences near the 5' end of 16S rRNA genes were aligned and two contiguous regions of high variability, flanked by highly conserved sequences, were found. The conserved sequences were used to design PCR primers which efficiently amplified these polymorphic regions from all seven species. The amplicon sequences were used to design 88 9mer hybridization probes which were arrayed onto glass slides. Single-stranded, fluorescence-tagged PCR products were hybridized to the microarrays at 15{degrees}C. The experimental results were compared with the $\{\Delta\}G\{\text{degrees}\}$ values for all matched and mismatched duplexes possible between the synthetic probes and the 16S target sequences of the seven test species, calculated using a virtual hybridization' software program. Although the observed hybridization patterns differed significantly from patterns predicted solely on the basis of perfect sequence matches, a unique hybridization fingerprint was obtained for each of the species, including closely related *Pseudomonas* species, and there was a reasonable correlation between the intensity of observed hybridization signals and the calculated $\{\Delta\}G\{\text{degrees}\}$ values. The results suggest that both perfect and mismatched pairings can contribute to microbial identification by hybridization fingerprinting.

Rothenburg, S., T. Schwartz, et al. (2002). "Complex regulation of the human gene for the Z-DNA binding protein DLM-1." Nucleic Acids Res. **30**(4): 993-1000.

<http://nar.oupjournals.org/cgi/content/abstract/30/4/993>

Dlm-1 is a recently described gene which is upregulated in murine stromal cells lining tumors. The function of the 40 kDa DLM-1 protein is poorly understood. DLM-1 contains an N-terminal Z-

DNA binding domain homologous to the Z{alpha} domain in the RNA editing enzyme ADAR1. We report the cloning of human and rat DLM-1. In addition to the Z{alpha} domain, three further conserved regions were identified. One of these is homologous to the second Z-DNA binding domain, Z{beta}, of ADAR1. We find that human DLM-1 is predominantly expressed in lymphatic tissues. The gene spans 17 kb and consists of 10 exons. DNA transcripts are extremely heterogeneous as a result of alternative splicing and the usage of exon variants combined with at least two transcriptional start sites and 3'-terminal exons. The exon coding for the Z{alpha} domain was present in approximately one-third of the analyzed mRNAs. Nearly half of the transcripts contained exon variants that had premature stop codons incorporated. Based on our analysis, over 2000 different mRNAs may be produced due to alternative splicing and usage of different 5' and 3' ends. The cellular function of DLM-1 appears to call for a high degree of adaptation by this complex regulation.

Samuelson, J. C., Z. Zhu, et al. (2004). "The isolation of strand-specific nicking endonucleases from a randomized SapI expression library." *Nucleic Acids Res.* **32**(12): 3661-3671.

<http://nar.oupjournals.org/cgi/content/abstract/32/12/3661>

The Type IIS restriction endonuclease SapI recognizes the DNA sequence 5'-GCTCTTC-3' (top strand by convention) and cleaves downstream (N1/N4) indicating top- and bottom-strand spacing, respectively. The asymmetric nature of DNA recognition presented the possibility that one, if not two, nicking variants might be created from SapI. To explore this possibility, two parallel selection procedures were designed to isolate either top-strand nicking or bottom-strand nicking variants from a randomly mutated SapI expression library. These procedures take advantage of a SapI substrate site designed into the expression plasmid, which allows for in vitro selection of plasmid clones possessing a site-specific and strand-specific nick. A procedure designed to isolate bottom-strand nicking enzymes yielded Nb.SapI-1 containing a critical R420I substitution near the end of the protein. The top-strand procedure yielded several SapI variants with a distinct preference for top-strand cleavage. Mutations present within the selected clones were segregated to confirm a top-strand nicking phenotype for single variants Q240R, E250K, G271R or K273R. The nature of the amino acid substitutions found in the selected variants provides evidence that SapI may possess two active sites per monomer. This work presents a framework for establishing the mechanism of SapI DNA cleavage.

Schwarz, G., S. Baumler, et al. (2004). "Determination of detection and quantification limits for SNP allele frequency estimation in DNA pools using real time PCR." *Nucleic Acids Res.* **32**(3): e24-.

<http://nar.oupjournals.org/cgi/content/abstract/32/3/e24>

The quantification of single nucleotide polymorphism (SNP) allele frequencies in pooled DNA samples using real time PCR is a promising approach for large-scale diagnostics and genotyping. The limits of detection (LOD) and limits of quantification (LOQ) for mutant SNP alleles are of particular importance for determination of the working range, which, in the case of allele-specific real time PCR, can be limited by the variance of calibration data from serially diluted mutant allele samples as well as by the variance of the 100% wild-type allele samples (blank values). In this study, 3{sigma} and 10{sigma} criteria were applied for the calculation of LOD and LOQ values. Alternatively, LOQ was derived from a 20% threshold for the relative standard deviation (%RSD) of measurements by fitting a curve for the relationship between %RSD and copy numbers of the mutant alleles. We found that detection and quantification of mutant alleles were exclusively limited by the variance of calibration data since the estimated LODcalibration (696 in 30 000 000 copies, 0.0023%), LOQ20%RSD (1470, 0.0049%) and LOQcalibration (2319, 0.0077) values were significantly higher than the LODblank (130, 0.0004%) and LOQblank (265, 0.0009%)

values derived from measurements of wild-type allele samples. No significant matrix effects of the genomic background DNA on the estimation of LOD and LOQ were found. Furthermore, the impact of large genome sizes and the general application of the procedure for the estimation of LOD and LOQ in quantitative real time PCR diagnostics are discussed.

Selzer, R. R., S. Nyaga, et al. (2002). "Differential requirement for the ATPase domain of the Cockayne syndrome group B gene in the processing of UV-induced DNA damage and 8-oxoguanine lesions in human cells." Nucleic Acids Res. **30**(3): 782-793.

<http://nar.oupjournals.org/cgi/content/abstract/30/3/782>

Cockayne syndrome (CS) is a rare inherited human genetic disorder characterized by UV sensitivity, developmental abnormalities and premature aging. The cellular and molecular phenotypes of CS include increased sensitivity to oxidative and UV-induced DNA lesions. The CSB protein is thought to play a pivotal role in transcription-coupled repair and CS-B cells are defective in the repair of the transcribed strand of active genes, both after exposure to UV and in the presence of oxidative DNA lesions. A previous study has indicated that a conserved helicase ATPase motif II residue is essential for the function of the CSB protein in responding to UV-induced DNA damage in a hamster cell line. Due to the limitations in studying a complex human disorder in another species, this study introduced the site-directed mutation of the ATPase motif II in the human CSB gene in an isogenic human cell line. The CSB mutant allele was tested for genetic complementation of UV-sensitive phenotypes in the human CS-B cell line CS1AN.S3.G2. In addition, the incision of an 8-oxoguanine lesion by extracts of the CS-B cell lines stably transfected with the wild-type or ATPase mutant CSB gene has been investigated. The ATPase motif II point mutation (E646Q) abolished the function of the CSB protein to complement the UV-sensitive phenotypes of survival, RNA synthesis recovery and apoptosis. Interestingly, whole-cell extract prepared from these mutant cells retained wild-type incision activity on an oligonucleotide containing a single 8-oxoguanine lesion, whereas the absence of the CSB gene altogether resulted in reduced incision activity relative to wild-type. These results suggest damage-specific functional requirements for CSB in the repair of UV-induced and oxidative lesions in human cells. The transfection of the mutant or wild-type CSB gene into the CS1AN.S3.G2 cells did not alter the expression of the subset of genes examined by cDNA array analysis.

Shapero, M. H., J. Zhang, et al. (2004). "MARA: a novel approach for highly multiplexed locus-specific SNP genotyping using high-density DNA oligonucleotide arrays." Nucleic Acids Res. **32**(22): e181-.

<http://nar.oupjournals.org/cgi/content/abstract/32/22/e181>

We have developed a locus-specific DNA target preparation method for highly multiplexed single nucleotide polymorphism (SNP) genotyping called MARA (Multiplexed Anchored Runoff Amplification). The approach uses a single primer per SNP in conjunction with restriction enzyme digested, adapter-ligated human genomic DNA. Each primer is composed of common sequence at the 5' end followed by locus-specific sequence at the 3' end. Following a primary reaction in which locus-specific products are generated, a secondary universal amplification is carried out using a generic primer pair corresponding to the oligonucleotide and genomic DNA adapter sequences. Allele discrimination is achieved by hybridization to high-density DNA oligonucleotide arrays. Initial multiplex reactions containing either 250 primers or 750 primers across nine DNA samples demonstrated an average sample call rate of [~]95% for 250- and 750-plex MARA. We have also evaluated >1000- and 4000-primer plex MARA to genotype SNPs from human chromosome 21. We have identified a subset of SNPs corresponding to a primer conversion rate of [~]75%, which show an average call rate over 95% and concordance >99% across seven DNA

samples. Thus, MARA may potentially improve the throughput of SNP genotyping when coupled with allele discrimination on high-density arrays by allowing levels of multiplexing during target generation that far exceed the capacity of traditional multiplex PCR.

Sironi, M., G. Menozzi, et al. (2004). "Silencer elements as possible inhibitors of pseudoexon splicing." Nucleic Acids Res. **32**(5): 1783-1791.

<http://nar.oupjournals.org/cgi/content/abstract/32/5/1783>

Human pre-mRNAs contain a definite number of exons and several pseudoexons which are located within intronic regions. We applied a computational approach to address the question of how pseudoexons are neglected in favor of exons and to possibly identify sequence elements preventing pseudoexon splicing. A search for possible splicing silencers was carried out on a pseudoexon selection that resembled exons in terms of splice site strength and exon splicing enhancer (ESE) representation; three motifs were retrieved through hexamer composition comparisons. One of these functions as a powerful silencer in transfection-based splicing assays and matches a previously identified silencer sequence with hnRNP H binding ability. The other two motifs are novel and failed to induce skipping of a constitutive exon, indicating that they might act as weak repressors or in synergy with other unidentified elements. All three motifs are enriched in pseudoexons compared with intronic regions and display higher frequencies in intronless gene-coding sequences compared with exons. We consider that a subpopulation of pseudoexons might rely on negative regulators for splicing repression; this hypothesis, if experimentally verified, might improve our understanding of exonic splicing regulatory sequences and provide the identification of a novel mutation target for human genetic diseases.

Svoboda, P., P. Stein, et al. (2004). "Lack of homologous sequence-specific DNA methylation in response to stable dsRNA expression in mouse oocytes." Nucleic Acids Res. **32**(12): 3601-3606.

<http://nar.oupjournals.org/cgi/content/abstract/32/12/3601>

Double-stranded RNA (dsRNA) induces sequence-specific mRNA degradation in most eukaryotic organisms via a conserved pathway known as RNA interference (RNAi). Post-transcriptional gene silencing by RNAi is also connected with transcriptional silencing of cognate sequences. In plants, this transcriptional silencing is associated with sequence-specific DNA methylation. To address whether this mechanism operates in mammalian cells, we used bisulfite sequencing to analyze DNA in mouse oocytes constitutively expressing long dsRNA against the *Mos* gene. Our data show that long dsRNA induces efficient *Mos* mRNA knockdown but not CpG and non-CpG DNA methylation of the endogenous *Mos* sequence in oocytes and early embryos. These data demonstrate that dsRNA does not directly induce DNA methylation in the trans form of this sequence in these mammalian cells.

Takizawa, T., W. Ochiai, et al. (2003). "Enhanced gene activation by Notch and BMP signaling cross-talk." Nucleic Acids Res. **31**(19): 5723-5731.

<http://nar.oupjournals.org/cgi/content/abstract/31/19/5723>

The signaling systems of Notch and bone morphogenetic protein (BMP) are highly conserved from flies to mammals and have been shown to be important in the development of multiple organs. For instance, in the fate determination of mouse neuroepithelial cells, Notch signaling

plays a role in keeping the progenitors from differentiating into neurons. BMP is also known to inhibit neuronal differentiation. In this paper, we show that BMP2 enhances Notch-induced transcriptional activation of Hes-5 and Hesr-1 in mouse neuroepithelial cells. BMP2 stimulation, in addition to the introduction of the intracellular domain of Notch (NIC), resulted in enhanced activation of the Hes-5 gene promoter. RBP-J{ κ } binding to its target sequence is important not only for Notch signaling, but also for BMP2 signaling, to activate the Hes-5 gene promoter. Smad1, a Smad species that is activated by BMP2, barely interacted with NIC, but did form a complex with NIC in the simultaneous presence of the coactivators P/CAF and p300. Recruitment of p300 to the NIC-containing complex was facilitated by activated Smad1, which is suggested to contribute to BMP2-mediated enhancement of Notch-induced Hes-5 expression. These data suggest a novel functional cooperation between Notch signaling and BMP signaling.

Tallet-Lopez, B., L. Aldaz-Carroll, et al. (2003). "Antisense oligonucleotides targeted to the domain IIIId of the hepatitis C virus IRES compete with 40S ribosomal subunit binding and prevent in vitro translation." *Nucleic Acids Res.* **31**(2): 734-742.

<http://nar.oupjournals.org/cgi/content/abstract/31/2/734>

Initiation of protein synthesis on the hepatitis C virus (HCV) mRNA involves a structured element corresponding to the 5' untranslated region and constituting an internal ribosome entry site (IRES). The domain IIIId of the HCV IRES, an imperfect RNA hairpin extending from nucleotides 253 to 279 of the viral mRNA, has been shown to be essential for translation and for the binding of the 40S ribosomal subunit. We investigated the properties of a series of antisense 2'-O-methyloligoribonucleotides targeted to various portions of the domain IIIId. Several oligomers, 14-17 nt in length, selectively inhibited in vitro translation of a bicistronic RNA construct in rabbit reticulocyte lysate with IC₅₀s <10 nM. The effect was restricted to the second cistron (the Renilla luciferase) located downstream of the HCV IRES; no effect was observed on the expression of the first cistron (the firefly luciferase) which was translated in a cap-dependent manner. Moreover, antisense 2'-O-methyloligoribonucleotides specifically competed with the 40S ribosomal subunit for binding to the IRES RNA in a filter-retention assay. The antisense efficiency of the oligonucleotides was nicely correlated to their affinity for the IIIId subdomain and to their ability to displace 40S ribosomal subunit, making this process a likely explanation for in vitro inhibition of HCV-IRES-dependent translation.

Tengs, T., T. LaFramboise, et al. (2004). "Genomic representations using concatenates of Type IIB restriction endonuclease digestion fragments." *Nucleic Acids Res.* **32**(15): e121-.

<http://nar.oupjournals.org/cgi/content/abstract/32/15/e121>

We have developed a method for genomic representation using Type IIB restriction endonucleases. Representation by concatenation of restriction digests, or RECORD, is an approach to sample the fragments generated by cleavage with these enzymes. Here, we show that the RECORD libraries may be used for digital karyotyping and for pathogen identification by computational subtraction.

Thomassin, H., C. Kress, et al. (2004). "MethylQuant: a sensitive method for quantifying methylation of specific cytosines within the genome." *Nucleic Acids Res.* **32**(21): e168-.

<http://nar.oupjournals.org/cgi/content/abstract/32/21/e168>

Here we present MethylQuant, a novel method that allows accurate quantification of the methylation level of a specific cytosine within a complex genome. This method relies on the well-established treatment of genomic DNA with sodium bisulfite, which converts cytosine into uracil without modifying 5-methyl cytosine. The region of interest is then PCR-amplified and quantification of the methylation status of a specific cytosine is performed by methylation-specific real-time PCR with SYBR Green I using one of the primers whose 3' end discriminates between the methylation states of this cytosine. The presence of a locked nucleic acid at the 3' end of the discriminative primer provides the specificity necessary for accurate and sensitive quantification, even when one of the methylation states is present at a level as low as 1% of the overall population. We demonstrate that accurate quantification of the methylation status of specific cytosines can be achieved in biological samples. The method is high-throughput, cost-effective, relatively simple and does not require any specific equipment other than a real-time PCR instrument.

Umeno, D., K. Hiraga, et al. (2003). "Method to protect a targeted amino acid residue during random mutagenesis." Nucleic Acids Res. **31**(16): e91-.

<http://nar.oupjournals.org/cgi/content/abstract/31/16/e91>

To generate a random mutant library that is free from mutation at a particular amino acid residue, we replace the codon of interest with a detachable, short DNA sequence containing a BsaXI recognition site. After PCR mutagenesis, this sequence is removed and intramolecular ligation of the sequences flanking the insert regenerates the gene. The three-base cohesive ends for ligation correspond to the codon for the targeted residue and any sequences with mutations at this site will fail to ligate. As a result, only the variants that are free from mutation at this site are in the proper reading frame. In a random library of C30 carotenoid synthase CrtM, this method was used to exclude readily accessible mutations at position F26, which confer C40 synthase function. This enabled us to identify two additional mutations, W38C and E180G, which confer the same phenotype but are present in the random library at much lower frequencies.

van Eijk, M. J. T., J. L. N. Broekhof, et al. (2004). "SNPWaveTM: a flexible multiplexed SNP genotyping technology." Nucleic Acids Res. **32**(4): e47-.

<http://nar.oupjournals.org/cgi/content/abstract/32/4/e47>

Scalable multiplexed amplification technologies are needed for cost-effective large-scale genotyping of genetic markers such as single nucleotide polymorphisms (SNPs). We present SNPWaveTM, a novel SNP genotyping technology to detect various subsets of sequences in a flexible fashion in a fixed detection format. SNPWave is based on highly multiplexed ligation, followed by amplification of up to 20 ligated probes in a single PCR. Depending on the multiplexing level of the ligation reaction, the latter employs selective amplification using the amplified fragment length polymorphism (AFLP(R)) technology. Detection of SNPWave reaction products is based on size separation on a sequencing instrument with multiple fluorescence labels and short run times. The SNPWave technique is illustrated by a 100-plex genotyping assay for Arabidopsis, a 40-plex assay for tomato and a 10-plex assay for Caenorhabditis elegans, detected on the MegaBACE 1000 capillary sequencer.

Wang, G., C. Brennan, et al. (2004). "Balanced-PCR amplification allows unbiased identification of genomic copy changes in minute cell and tissue samples." Nucleic Acids Res. **32**(9): e76-.

<http://nar.oupjournals.org/cgi/content/abstract/32/9/e76>

Analysis of genomic DNA derived from cells and fresh or fixed tissues often requires whole genome amplification prior to microarray screening. Technical hurdles to this process are the introduction of amplification bias and/or the inhibitory effects of formalin fixation on DNA amplification. Here we demonstrate a balanced-PCR procedure that allows unbiased amplification of genomic DNA from fresh or modestly degraded paraffin-embedded DNA samples. Following digestion and ligation of a target and a control genome with distinct linkers, the two are mixed and amplified in a single PCR, thereby avoiding biases associated with PCR saturation and impurities. We demonstrate genome-wide retention of allelic differences following balanced-PCR amplification of DNA from breast cancer and normal human cells and genomic profiling by array-CGH (cDNA arrays, 100 kb resolution) and by real-time PCR (single gene resolution). Comparison of balanced-PCR with multiple displacement amplification (MDA) demonstrates equivalent performance between the two when intact genomic DNA is used. When DNA from paraffin-embedded samples is used, balanced PCR overcomes problems associated with modest DNA degradation and produces unbiased amplification whereas MDA does not. Balanced-PCR allows amplification and recovery of modestly degraded genomic DNA for subsequent retrospective analysis of human tumors with known outcomes.

Wang, X. and B. Seed (2003). "A PCR primer bank for quantitative gene expression analysis." Nucleic Acids Res. **31**(24): e154-.

<http://nar.oupjournals.org/cgi/content/abstract/31/24/e154>

Although gene expression profiling by microarray analysis is a useful tool for assessing global levels of transcriptional activity, variability associated with the data sets usually requires that observed differences be validated by some other method, such as real-time quantitative polymerase chain reaction (real-time PCR). However, non-specific amplification of non-target genes is frequently observed in the latter, confounding the analysis in [~]40% of real-time PCR attempts when primer-specific labels are not used. Here we present an experimentally validated algorithm for the identification of transcript-specific PCR primers on a genomic scale that can be applied to real-time PCR with sequence-independent detection methods. An online database, PrimerBank, has been created for researchers to retrieve primer information for their genes of interest. PrimerBank currently contains 147 404 primers encompassing most known human and mouse genes. The primer design algorithm has been tested by conventional and real-time PCR for a subset of 112 primer pairs with a success rate of 98.2%.

Wang, Y., D. E. Prosen, et al. (2004). "A novel strategy to engineer DNA polymerases for enhanced processivity and improved performance in vitro." Nucleic Acids Res. **32**(3): 1197-1207.

<http://nar.oupjournals.org/cgi/content/abstract/32/3/1197>

Mechanisms that allow replicative DNA polymerases to attain high processivity are often specific to a given polymerase and cannot be generalized to others. Here we report a protein engineering-based approach to significantly improve the processivity of DNA polymerases by covalently linking the polymerase domain to a sequence non-specific dsDNA binding protein. Using Sso7d from *Sulfolobus solfataricus* as the DNA binding protein, we demonstrate that the processivity of both family A and family B polymerases can be significantly enhanced. By introducing point mutations in Sso7d, we show that the dsDNA binding property of Sso7d is essential for the enhancement. We present evidence supporting two novel conclusions. First, the fusion of a heterologous dsDNA binding protein to a polymerase can increase processivity without

compromising catalytic activity and enzyme stability. Second, polymerase processivity is limiting for the efficiency of PCR, such that the fusion enzymes exhibit profound advantages over unmodified enzymes in PCR applications. This technology has the potential to broadly improve the performance of nucleic acid modifying enzymes.

Wong, K.-K., Y. T. M. Tsang, et al. (2004). "Allelic imbalance analysis by high-density single-nucleotide polymorphic allele (SNP) array with whole genome amplified DNA." Nucleic Acids Res. **32**(9): e69-.

<http://nar.oupjournals.org/cgi/content/abstract/32/9/e69>

Besides their use in mRNA expression profiling, oligonucleotide microarrays have also been applied to single-nucleotide polymorphism (SNP) and loss of heterozygosity (LOH) or allelic imbalance studies. In this report, we evaluate the reliability of using whole genome amplified DNA for analysis with an oligonucleotide microarray containing 11 560 SNPs to detect allelic imbalance and chromosomal copy number abnormalities. Whole genome SNP analyses were performed with DNA extracted from osteosarcoma tissues and patient-matched blood. SNP calls were then generated by Affymetrix(R) GeneChip(R) DNA Analysis Software. In two osteosarcoma cases, using unamplified DNA, we identified 793 and 1070 SNP loci with allelic imbalance, respectively. In a parallel experiment with amplified DNA, 78% and 83% of these SNP loci with allelic imbalance was detected. The average false-positive rate is 13.8%. Furthermore, using the Affymetrix(R) GeneChip(R) Chromosome Copy Number Tool to analyze the SNP array data, we were able to detect identical chromosomal regions with gain or loss in both amplified and unamplified DNA at cytoband resolution.

Wuitschick, J. D., J. A. Gershan, et al. (2002). "A novel family of mobile genetic elements is limited to the germline genome in *Tetrahymena thermophila*." Nucleic Acids Res. **30**(11): 2524-2537.

<http://nar.oupjournals.org/cgi/content/abstract/30/11/2524>

In the ciliated protozoan *Tetrahymena thermophila*, extensive DNA elimination is associated with differentiation of the somatic macronucleus from the germline micronucleus. This study describes the isolation and complete characterization of Tlr elements, a family of approximately 30 micronuclear DNA sequences that are efficiently eliminated from the developing macronucleus. The data indicate that Tlr elements are comprised of an [~]22 kb internal region flanked by complex and variable termini. The Tlr internal region is highly conserved among family members and contains 15 open reading frames, some of which resemble genes encoded by transposons and viruses. The Tlr termini appear to be long inverted repeats consisting of (i) a variable region containing multiple direct repeats which differ in number and sequence from element to element and (ii) a conserved terminal 47 bp sequence. Taken together, these results suggest that Tlr elements comprise a novel family of mobile genetic elements that are confined to the *Tetrahymena* germline genome. Possible mechanisms of developmentally programmed Tlr elimination are discussed.

Xu, H., M. Y. Sha, et al. (2003). "Multiplexed SNP genotyping using the QbeadTM system: a quantum dot-encoded microsphere-based assay." Nucleic Acids Res. **31**(8): e43-.

<http://nar.oupjournals.org/cgi/content/abstract/31/8/e43>

We have developed a new method using the QbeadTM system for high-throughput genotyping of single nucleotide polymorphisms (SNPs). The Qbead system employs fluorescent QdotTM semiconductor nanocrystals, also known as quantum dots, to encode microspheres that subsequently can be used as a platform for multiplexed assays. By combining mixtures of quantum dots with distinct emission wavelengths and intensities, unique spectral barcodes¹ are created that enable the high levels of multiplexing required for complex genetic analyses. Here, we applied the Qbead system to SNP genotyping by encoding microspheres conjugated to allele-specific oligonucleotides. After hybridization of oligonucleotides to amplicons produced by multiplexed PCR of genomic DNA, individual microspheres are analyzed by flow cytometry and each SNP is distinguished by its unique spectral barcode. Using 10 model SNPs, we validated the Qbead system as an accurate and reliable technique for multiplexed SNP genotyping. By modifying the types of probes conjugated to microspheres, the Qbead system can easily be adapted to other assay chemistries for SNP genotyping as well as to other applications such as analysis of gene expression and protein-protein interactions. With its capability for high-throughput automation, the Qbead system has the potential to be a robust and cost-effective platform for a number of applications.

Yoon, Y. G. and M. D. Koob (2003). "Efficient cloning and engineering of entire mitochondrial genomes in *Escherichia coli* and transfer into transcriptionally active mitochondria." Nucleic Acids Res. **31**(5): 1407-1415.

<http://nar.oupjournals.org/cgi/content/abstract/31/5/1407>

We have devised an efficient method for replicating and stably maintaining entire mitochondrial genomes in *Escherichia coli* and have shown that we can engineer these mitochondrial DNA (mtDNA) genome clones using standard molecular biological techniques. In general, we accomplish this by inserting an *E. coli* replication origin and selectable marker into isolated, circular mtDNA at random locations using an in vitro transposition reaction and then transforming the modified genomes into *E. coli*. We tested this approach by cloning the 16.3 kb mouse mitochondrial genome and found that the resulting clones could be engineered and faithfully maintained when we used *E. coli* hosts that replicated them at moderately low copy numbers. When these recombinant mtDNAs were replicated at high copy numbers, however, mtDNA sequences were partially or fully deleted from the original clone. We successfully electroporated recombinant mouse mitochondrial genomes into isolated mouse mitochondria devoid of their own DNA and detected robust in organello RNA synthesis by RT-PCR. This approach for modifying mtDNA and subsequent in organello analysis of the recombinant genomes offers an attractive experimental system for studying many aspects of vertebrate mitochondrial gene expression and is a first step towards true in vivo engineering of mammalian mitochondrial genomes.

Yuryev, A., J. Huang, et al. (2002). "Predicting the success of primer extension genotyping assays using statistical modeling." Nucleic Acids Res. **30**(23): e131-.

<http://nar.oupjournals.org/cgi/content/abstract/30/23/e131>

Using an empirical panel of more than 20 000 single base primer extension (SNP-IT) assays we have developed a set of statistical scores for evaluating and rank ordering various parameters of the SNP-IT reaction to facilitate high-throughput assay primer design with improved likelihood of success. Each score predicts either signal magnitude from primer extension or signal noise caused by mispriming of primers and structure of the PCR product. All scores have been shown to correlate with the success/ failure rate of the SNP-IT reaction, based on analysis of assay results. A logistic regression analysis was applied to combine all scored parameters into one measure predicting the overall success/failure rate of a given SNP marker. Three training sets for

different types of SNP-IT reaction, each containing about 22 000 SNP markers, were used to assign weights to each score and optimize the prediction of the combined measure. c-Statistics of 0.69, 0.77 and 0.72 were achieved for three training sets. This new statistical prediction can be used to improve primer design for the SNP-IT reaction and evaluate the probability of genotyping success for a given SNP based on analysis of the surrounding genomic sequence.

Zeschnigk, M., S. Bohringer, et al. (2004). "A novel real-time PCR assay for quantitative analysis of methylated alleles (QAMA): analysis of the retinoblastoma locus." Nucleic Acids Res. **32**(16): e125-.

<http://nar.oupjournals.org/cgi/content/abstract/32/16/e125>

Altered methylation patterns have been found to play a role in developmental disorders, cancer and aging. Increasingly, changes in DNA methylation are used as molecular markers of disease. Therefore, there is a need for reliable and easy to use techniques to detect and measure DNA methylation in research and routine diagnostics. We have established a novel quantitative analysis of methylated alleles (QAMA) which is essentially a major improvement over a previous method based on real-time PCR (MethyLight). This method is based on real-time PCR on bisulfite-treated DNA. A significant advantage over conventional MethyLight is gained by the use of TaqMan probes based on minor groove binder (MGB) technology. Their improved sequence specificity facilitates relative quantification of methylated and unmethylated alleles that are simultaneously amplified in single tube. This improvement allows precise measurement of the ratio of methylated versus unmethylated alleles and cuts down potential sources of inter-assay variation. Therefore, fewer control assays are required. We have used this novel technical approach to identify hypermethylation of the CpG island located in the promoter region of the retinoblastoma (RB1) gene and found that QAMA facilitates reliable and fast measurement of the relative quantity of methylated alleles and improves handling of diagnostic methylation analysis. Moreover, the simplified reaction setup and robustness inherent to the single tube assay facilitates high-throughput methylation analysis. Because the high sequence specificity inherent to the MGB technology is widely used to discriminate single nucleotide polymorphisms, QAMA potentially can be used to discriminate the methylation status of single CpG dinucleotides.

Nutrition Research (1)

Nakamura, M., M. Tanaka, et al. (2000). "Association between beta 3-adrenergic receptor polymorphism and a lower reduction in the ratio of visceral fat to subcutaneous fat area during weight loss in Japanese obese women." Nutrition Research **20**(1): 25.

<http://www.sciencedirect.com/science/article/B6TB1-3YS2C5R-5/2/a5c77d05b78037df7da63b16db25d7f0>

We investigated whether amino acid substitution of tryptophan by arginine at the residue 64 (64 Arg) of beta 3-adrenergic receptor affects on the degree of reduction in the abdominal fat distribution during a 3-month weight reduction program in either pre- or postmenopausal Japanese women. Beta 3-adrenergic receptor gene polymorphism was examined in 90 Japanese obese women by restriction-enzyme cleavage conformation. The visceral and subcutaneous fat area was measured by magnetic resonance imaging. The baseline body mass index, body

weight, fat mass and abdominal subcutaneous and total fat area in 15 obese postmenopausal women with a beta 3-adrenergic receptor (64 Arg) were significantly higher than those in 25 postmenopausal obese women with a beta 3-adrenergic receptor (64 Trp/64 Trp). In contrast, no such differences were found in the 50 premenopausal obese women. After a 3-month weight reduction period, the ratio of visceral to subcutaneous fat areas tended to be lower in both pre- and post-menopausal women with a beta 3-adrenergic receptor (64 Arg), but statistically significant in only the premenopausal obese women ($p < 0.05$). The absolute changes in visceral fat areas in 5 homozygotes with a beta 3-adrenergic receptor (64 Arg/64 Arg) was significantly smaller than those in 50 obese women with a beta 3-adrenergic receptor (64 Trp/64 Trp). These results thus suggest that an amino acid substitution at residue 64 of beta 3-adrenergic receptor may play an important role in the regulation of fat distribution in Japanese obese women.

Obes. Res. (8)

Baghaei, F., R. Rosmond, et al. (2003). "The CYP19 Gene and Associations with Androgens and Abdominal Obesity in Premenopausal Women." *Obes. Res.* **11**(4): 578-585.

<http://www.obesityresearch.org/cgi/content/abstract/11/4/578>

Objective: Elevated androgens in women are associated with type 2 diabetes and are dependent on the conversion to estrogens by aromatase cytochrome P450. Polymorphisms of a tetranucleotide repeat [TTTA] $_n$ in the fourth intron of the CYP19 gene are associated with endocrine-dependent diseases and were examined in relation to hormone levels and disease risk factors in premenopausal women. **Research Methods and Procedures:** A population sample of women born in 1956 ($n = 270$) were genotyped for this polymorphism and the results set in relation to steroid hormones, including saliva cortisol, anthropometric variables, estimates of insulin, glucose and lipid metabolism, and blood pressure. **Results:** Seven tetranucleotide repeat [TTTA] $_n$ alleles were detected with allelic sizes of 168 to 195 bp, with a TCT deletion/insertion (168/171 bp) upstream of this microsatellite. Smoking was associated with elevated androgens ($p = 0.005$ to 0.019). Using the median (average stretch, 177.5 bp) as a dividing line, nonsmoking women with the shorter microsatellite had higher free testosterone ($p = 0.018$) and lower sex hormone binding globulin ($p = 0.033$). These differences were pronounced with the 168-bp allele. Such women were also characterized by a less-substantial decrease of morning cortisols ("unwinding"; $p = 0.035$) and central obesity (abdominal sagittal diameter, $p = 0.049$) and had waist/hip circumference ratios of borderline significance ($p = 0.064$). **Discussion:** The results indicate that, in premenopausal women, a short microsatellite in the fourth intron of the CYP19 gene, caused by a TCT deletion upstream the [TTTA] $_n$ tract, is associated with elevated androgens, perturbed regulation of the hypothalamic-pituitary-adrenal axis, and abdominal obesity.

Bosse, Y., J.-P. Despres, et al. (2003). "The Peroxisome Proliferator-Activated Receptor {alpha} L162V Mutation Is Associated with Reduced Adiposity." *Obes. Res.* **11**(7): 809-816.

<http://www.obesityresearch.org/cgi/content/abstract/11/7/809>

Objective: To determine the contribution of the peroxisome proliferator-activated receptor {alpha} (PPAR{alpha}) L162V mutation to the variation of several indexes of body fatness obtained from

healthy adults who participated in the Quebec Family Study. Research Methods and Procedures: The PPAR{alpha} L162V mutation was determined by a mismatch polymerase chain reaction method. Adiposity phenotypes were obtained by standardized anthropometric measurements, underwater weighing technique, and computed tomography. Results: For all adiposity phenotypes, subjects carrying the V162 allele had lower values compared with L162 homozygotes (HMZs) [BMI (kg/m²): 27.8 {+/-} 7.6 vs. 26.0 {+/-} 5.6, $p < 0.05$; percentage body fat: 28.5 {+/-} 10.7 vs. 25.7 {+/-} 10.1, $p < 0.05$; waist circumference (cm): 89.0 {+/-} 18.1 vs. 85.7 {+/-} 15.8, $p = 0.07$; total computed tomography abdominal fat areas (cm²): 406 {+/-} 221 vs. 359 {+/-} 192, $p = 0.15$; means {+/-} SD for L162 HMZs vs. V162 carriers, respectively]. Differences in cross-sectional abdominal adipose tissue areas and waist circumference were abolished after adjustment for total body fat mass. Similar trends were observed when results were analyzed by gender, although associations seemed stronger in women. The odds ratio of having a BMI above 30 kg/m² reached 1.77 (1.02; 3.07, 95% confidence intervals) for L162 HMZs. This risk could be considered marginal on an individual basis, but because 85% of the subjects are affected by this small risk, the impact on the population is important. Discussion: The PPAR{alpha} V162 allele is associated with reduced adiposity and has a substantial population-attributable risk.

Di Rocco, P., M. Manco, et al. (2004). "Lowered Tumor Necrosis Factor Receptors, but Not Increased Insulin Sensitivity, with Infliximab." *Obes. Res.* **12**(4): 734-739.

<http://www.obesityresearch.org/cgi/content/abstract/12/4/734>

Objectives: To verify whether Infliximab could modify insulin sensitivity and TNF- α and GLUT4 mRNA expression in muscle and adipose tissue of morbidly obese subjects. Soluble TNF receptors I and II (TNFR-I and TNFR-II) were also assayed. Research Methods and Procedures: Six obese subjects were investigated before and 2 weeks after a single intravenous administration of 5 mg/kg Infliximab; insulin sensitivity was evaluated by euglycemic hyperinsulinemic clamp, and TNF- α and GLUT4 mRNA expression were assessed by reverse-transcriptase polymerase chain reaction on muscle and adipose tissue. TNF- α , TNFR-I, and TNFR-II were determined using the ELISA technique. Results: Infliximab infusion did not affect fasting plasma insulin or fasting plasma glucose levels; whole body glucose uptake did not change significantly. TNF- α and GLUT4 mRNA did not show any significant change in muscle or adipose tissue. Serum TNF- α was undetectable before and after treatment, whereas TNFR-I and TNFR-II concentrations significantly decreased ($p < 0.01$). Discussion: An explanation for the absence of effect of Infliximab on insulin resistance in morbidly obese subjects may be the paracrine way of action of this cytokine. Because Infliximab is predominantly distributed within the vascular compartment, its effectiveness in penetrating muscle and adipose tissue is potentially low. The significant decrease of TNFR-I and TNFR-II might be ascribed to a targeted effect of Infliximab on the immune system.

Ellsworth, D. L., S. A. Coady, et al. (2005). "Interactive Effects Between Polymorphisms in the {beta}-Adrenergic Receptors and Longitudinal Changes in Obesity." *Obes. Res.* **13**(3): 519-526.

<http://www.obesityresearch.org/cgi/content/abstract/13/3/519>

We assessed interactions between polymorphisms in the {beta}-adrenergic receptor genes and longitudinal changes in obesity from childhood to adulthood using longitudinal data collected over a 24-year period from 1973 to 1996. Sex- and age-stratified analyses using random coefficients models were used to examine gene-gene interaction effects on obesity measures in 1179 African-American and white men and women (71% white, 57% women). Suggestive evidence for an interaction ($p = 0.022$) between the {beta}1- and {beta}2-adrenergic receptors was observed in men for longitudinal change in BMI. Men with Gly/Gly genotypes for both the {beta}1 and {beta}2

receptors showed significant increases ($\sim 0.6\%/yr$) in BMI from childhood to adulthood. Women showed suggestive evidence for an interaction ($p = 0.035$) between the β_1 - and β_3 -adrenergic receptors for change over time in BMI. Women with Gly/Gly genotypes at the β_1 -receptor and carrying at least one β_3 -Arg allele showed notable increases in BMI. The regulation of lipolysis and development of obesity differ markedly between men and women and may be influenced by genetic polymorphisms, which contribute to the efficiency of the β -adrenergic receptors, and hormonal effects on adrenergic receptor activity.

Epstein, L. H., J. L. Jaroni, et al. (2002). "Dopamine Transporter Genotype as a Risk Factor for Obesity in African-American Smokers." *Obes. Res.* **10**(12): 1232-1240.

<http://www.obesityresearch.org/cgi/content/abstract/10/12/1232>

Objective: To assess the association between a polymorphism related to dopamine function, dopamine transport (SLC6A3), and obesity in smokers. Research Methods and Procedures: Logistic regression was used to assess the relationship between this genetic polymorphism and obesity (body mass index ≥ 30 kg/m²) from a sample of 510 smokers who smoked at least 10 cigarettes per day and who were participating in a study designed to examine genetic and nongenetic predictors of response to a pharmacological treatment. Results: The likelihood of obesity in African Americans ($N = 90$) with the 10/10 SLC6A3 genotype was 5.16 times that of African Americans with 9/9 or 9/10 SLC6A3 genotypes (odds ratio = 5.16, confidence interval = 1.60 to 16.65). There was no association of the SLC6A3 genotype with obesity for non-Hispanic whites ($N = 420$). Discussion: These results suggest that variants of the dopamine transporter gene may be related to obesity in African-American smokers. Possible mechanisms responsible for the association between dopamine transport and obesity in African-American smokers are discussed.

Heilbronn, L. K., A. E. Civitarese, et al. (2005). "Glucose Tolerance and Skeletal Muscle Gene Expression in Response to Alternate Day Fasting." *Obes. Res.* **13**(3): 574-581.

<http://www.obesityresearch.org/cgi/content/abstract/13/3/574>

Objective: Alternate day fasting may extend lifespan in rodents and is feasible for short periods in nonobese humans. The aim of this study was to examine the effects of 3 weeks of alternate day fasting on glucose tolerance and skeletal muscle expression of genes involved in fatty acid transport/oxidation, mitochondrial biogenesis, and stress response. Research Methods and Procedures: Glucose and insulin responses to a standard meal were tested in nonobese subjects (eight men and eight women; BMI, 20 to 30 kg/m²) at baseline and after 22 days of alternate day fasting (36 hour fast). Muscle biopsies were obtained from a subset of subjects ($n = 11$) at baseline and on day 21 (12-hour fast). Results: Glucose response to a meal was slightly impaired in women after 3 weeks of treatment ($p < 0.01$), but insulin response was unchanged. However, men had no change in glucose response and a significant reduction in insulin response ($p < 0.03$). There were no significant changes in the expression of genes involved in mitochondrial biogenesis or fatty acid transport/oxidation, although a trend toward increased CPT1 expression was observed ($p < 0.08$). SIRT1 mRNA expression was increased after alternate day fasting ($p = 0.01$). Discussion: Alternate day fasting may adversely affect glucose tolerance in nonobese women but not in nonobese men. The gene expression results indicate that fatty acid oxidation and mitochondrial biogenesis are unaffected by alternate day fasting. However, the increased expression in SIRT1 suggests that alternate day fasting may improve stress resistance, a commonly observed feature of calorie-restricted rodents.

Pico, C., J. Sanchez, et al. (2002). "Leptin Production by the Stomach Is Up-Regulated in Obese (fa/fa) Zucker Rats." Obes. Res. **10**(9): 932-938.

<http://www.obesityresearch.org/cgi/content/abstract/10/9/932>

Objective: Genetically obese (fa/fa) Zucker rats display markedly elevated circulating leptin levels compared with their lean counterparts; this is expected because of the lack of a LepR-mediated feedback inhibition. The aim of this study was to determine the effect of the leptin receptor mutation in the Zucker rat on gastric leptin production and on the response to 14 hours of starvation. The response to a short-term period of food intake (20 minutes) on gastric leptin release was also analyzed. **Research Methods and Procedures:** Leptin mRNA expression in the gastric mucosa and in adipose tissue depots (epididymal, retroperitoneal, mesenteric, and inguinal) was assessed by reverse transcriptase-polymerase chain reaction and serum and stomach leptin content by enzyme-linked immunosorbent assay. **Results:** Obese Zucker rats overexpressed leptin in the stomach. They overexpress leptin in the inguinal adipose tissue but not in visceral adipose tissue depots, indicating tissue-specific obesity-dependent differences. Gastric leptin expression is regulated by feeding conditions in lean but not in obese (fa/fa) rats. In lean animals, leptin mRNA levels decrease in fasting conditions and increase rapidly with a short period of food intake. Obese Zucker rats also overdisplay stomach leptin levels. Feeding acutely stimulates leptin secretion by the stomach in lean, and to a lesser extent, in obese rats. **Discussion:** These results indicate impaired regulation of leptin expression in the stomach of obese (fa/fa) Zucker rats. However, there is still an effect of the nutritional status on gastric leptin levels despite the lack of a functional leptin receptor.

Rosa, G., P. Di Rocco, et al. (2003). "Reduced PDK4 Expression Associates with Increased Insulin Sensitivity in Postobese Patients." Obes. Res. **11**(2): 176-182.

<http://www.obesityresearch.org/cgi/content/abstract/11/2/176>

Objective: The aim of this study was to verify whether changes in PDK4 mRNA expression in skeletal muscle in formerly obese subjects who underwent malabsorptive bariatric surgery [bilio-pancreatic diversion (BPD)] might be related to insulin sensitivity improvement, and if these possible modifications might correlate with a reduction of the intramyocytic lipid level. **Research Methods and Procedures:** Six obese women (body mass index 46.6 ± 8.2 kg/m²) were enrolled in the study. Body composition, euglycemic-hyperinsulinemic clamp and muscle biopsies for skeletal muscle lipid analysis, and semiquantitative reverse transcriptase-polymerase chain reaction were performed before and 3 years after BPD. **Results:** The average weight loss observed after surgery was $\sim 42\%$. Increased glucose uptake was accompanied by a significant decrease of PDK4 mRNA ($R^2 = 0.71$, $p < 0.001$). The amounts of intramyocytic triglycerides correlate directly with PDK4 mRNA ($R^2 = 0.87$, $p = 0.005$) and inversely with glucose uptake values ($R^2 = 0.75$, $p < 0.001$). **Discussion:** Our results support the concept that a reduced tissue availability of fatty acids consequent to a massive lipid malabsorption influences glucose metabolism acting through the regulation of PDH complex. In fact, as shown in animals, a higher level of FFA availability is likely to induce overexpression of PDK4 also in humans.

Kahn, J. A., G. B. Slap, et al. (2004). "Comparison of Adolescent and Young Adult Self-Collected and Clinician-Collected Samples for Human Papillomavirus." Obstet. Gynecol. **103**(5): 952-959.

<http://www.greenjournal.org/cgi/content/abstract/103/5/952>

OBJECTIVE: To examine the concordance between self-collected and clinician-collected samples for human papillomavirus (HPV) DNA. **METHODS:** Sexually active adolescent and young adult women aged 14-21 years (N = 101) were enrolled in a prospective cohort study of HPV testing. Participants self-collected vaginal samples for HPV DNA, and clinicians collected cervicovaginal samples for HPV DNA and a cervical cytology specimen. We determined concordance between the results of self- and clinician-collected specimens using a {kappa} statistic and McNemar's test. **RESULTS:** Of the 51% of participants who were HPV positive, 53% had 1 type, 25% had 2 types, and 22% had 3 types or more; 25 different HPV types were identified. Self-collected samples detected more participants with HPV than clinician-collected samples (45% versus 42%, P = .65). When results were categorized into presence or absence of high-risk HPV types, agreement between self- and clinician-collected specimens was high ({kappa} 0.72) and the difference between test results was not significant (McNemar's P = .41). However, when all HPV types detected were considered, agreement was perfect in only 51% of those with 1 or more types of high-risk HPV type. There was no association between agreement and age or HPV type. **CONCLUSION:** Self testing for HPV DNA may be sufficiently sensitive for the detection of high-risk HPV DNA among adolescent and young adult women in clinical settings. **LEVEL OF EVIDENCE:** II-3

Obstetrics & Gynecology (6)

Eppel, W., C. Worda, et al. (2000). "Human papillomavirus in the cervix and placenta." Obstetrics & Gynecology **96**(3): 337.

<http://www.sciencedirect.com/science/article/B6TB2-411PH91-4/2/11e48e51fc07b9e107922e9dec214c6>

Objective: To determine the prevalence and association of human papillomavirus (HPV) infection in the cervixes and placentas of pregnant women. **Methods:** Cervical samples were taken from 179 of 226 women who had placental biopsies because of abnormal ultrasound findings or were older than 35 years, to detect HPV infections with hybrid capture II tests. Polymerase chain reaction (PCR) was done on placental tissue of 147 of the 226 women to detect HPV DNA. **Results:** We found 44 of 179 women (24.6%, 95% confidence interval 18.3, 31.0) to test positive for HPV in their cervixes. Logistic regression analyses showed decreased prevalence of HPV infection with increased maternal age (P = .039). The HPV DNA E6 PCR from the villus tissue was negative in the 147 cases examined. However, a significant contingency coefficient between low-risk HPV infection and elevated risk of chromosome aberration was found ([phi] = V = 0.15, P = .050). **Conclusion:** The infection rate of 24.6% in women without clinical symptoms of HPV infection was high, but there seemed to be no virus transmission to the placenta in women with subclinical infections. Low-risk cervical HPV infection might be associated with a slightly higher risk of abnormal fetal karyotype.

Garcia, F., B. Barker, et al. (2003). "Cross-sectional study of patient- and physician-collected cervical cytology and human papillomavirus." Obstetrics & Gynecology **102**(2): 266.

<http://www.sciencedirect.com/science/article/B6TB2-496F1R7-D/2/7eddeabcaf772d718487ffa51dbb6b>

ObjectiveTo compare the performance of patient- and physician-obtained cytology and human papillomavirus (HPV) testing for the detection of high-grade cervical intraepithelial neoplasia.**Methods**A cross-sectional study was performed involving 334 women seen at three colposcopy clinics (Tucson, Arizona; Hermosillo, Mexico; and Lima, Peru). All women used a cytobrush to self-collect specimens for cervical cytology and HPV testing. Subjects subsequently underwent physician collection for cytology and HPV, followed by a complete colposcopic evaluation with directed biopsy. Cytology was processed using thin-layer technology, and HPV was determined using the polymerase chain reaction technique. Test performance characteristics were determined using the histopathologic diagnosis as the reference standard and designating high-grade cervical intraepithelial neoplasia as clinically significant disease for the purpose of the analysis.**Results**The sensitivity of patient-collected cytology was significantly lower (55.0%) and specificity was significantly better (84.1%) than those of physician-directed sampling (85.2% and 73.4%, respectively). Patient-collected HPV had significantly lower sensitivity (49.0%) than physician sampling (82.2%), although specificity did not significantly differ.**Conclusion**Patient collection is a feasible although inferior alternative to physician-collected cervical cytology and HPV testing.

Hsu, K.-F., S.-C. Huang, et al. (2003). "Clinical significance of serum human papillomavirus DNA in cervical carcinoma." Obstetrics & Gynecology **102**(6): 1344.

<http://www.sciencedirect.com/science/article/B6TB2-4B4P70J-V/2/3bc6092821c4002928b97e54462d7c72>

ObjectiveTo study the association between serum human papillomavirus (HPV) deoxyribonucleic acid (DNA) and clinicopathologic prognostic factors and the clinical usefulness of serum HPV DNA in early-stage cervical cancer.**Methods**Deoxyribonucleic acids extracted from cervical tissues and sera of patients with stage IB or IIA cervical cancer and 40 controls including patients with cervical carcinoma in situ or benign disease were examined for HPV DNA with L1 consensus and types 16- and 18-specific E7 primers. Multivariable logistic regression was used to determine significant correlates of positive serum HPV DNA, and the receiver operating characteristic curve was applied in risk-factor assessment.**Results**Human papillomavirus DNA was not detected in sera from patients with carcinoma in situ or benign disease. Among the 112 patients with cervical cancer, we detected 27 positive samples (24.1%) in serum. Positive HPV DNA in serum was significantly associated with lymphovascular invasion and deep stromal invasion with or without parametrial extension (P = .001), large tumor size, and elevated levels of serum squamous cell carcinoma antigen (P ConclusionThe presence of serum HPV DNA in patients with early-stage cervical cancer was correlated with poor prognosis factors that warrant adjuvant therapy.

Laivuori, H., R. Kaaja, et al. (2000). "677 C->T polymorphism of the methylenetetrahydrofolate reductase gene and preeclampsia." Obstetrics & Gynecology **96**(2): 277.

<http://www.sciencedirect.com/science/article/B6TB2-40SFFDM-T/2/4be1f52155ddca619cd66623ab40aa24>

Objective: To evaluate C to T substitution at nucleotide 677 of N5,N10-methylenetetrahydrofolate

reductase gene in women with prior preeclamptic or normotensive pregnancies. Methods: Methylenetetrahydrofolate reductase genotypes were determined in 113 Finnish women with preeclamptic first pregnancies and 103 controls with one or more normotensive pregnancies, using polymerase chain reaction and restriction enzyme analysis. Preeclampsia was defined as severe in 100 women who fulfilled one or more of the subsequent criteria: systolic blood pressure (BP) at least 160 mmHg, diastolic BP at least 110 mmHg, or proteinuria at least 2 g per 24-hour urine collection. Results: There were no significant differences in prevalences of the methylenetetrahydrofolate reductase genotypes (CC, CT, and TT) between groups (57%, 40%, and 3% in the preeclamptic group and 54%, 39%, and 7%, respectively, in controls). The frequency of the T677 allele was 0.23 in the preeclamptic group and 0.26 in the control group (difference 0.03; 95% confidence interval -0.08, 0.14; $P = .51$). Our sample had 60% power to detect a difference of the allele frequencies similar to that (0.12) reported previously. The result was similar when analysis was restricted to patients with severe preeclampsia (T677 allele frequency 0.22). Conclusion: A carrier status for the T677 allele of the methylenetetrahydrofolate reductase gene does not predispose to preeclampsia, at least in the Finnish population.

Neuman, R. J., P. C. Huettner, et al. (2000). "Association between DQB1 and cervical cancer in patients with human papillomavirus and family controls." *Obstetrics & Gynecology* **95**(1): 134.

<http://www.sciencedirect.com/science/article/B6TB2-3Y6GT5-W/2/bda8415a152ed169e7ca64c01499ce0d>

Objective: The role of human leukocyte antigen (HLA) DQB1 alleles and human papillomavirus (HPV) as contributing factors to invasive cervical cancer was investigated. To overcome problems of misleading causal inferences common in traditional case-control studies, a family-based test, the transmission/disequilibrium test, was used. Methods: Ninety-six patients with pathologically confirmed invasive cervical cancer were ascertained. Human papillomavirus types were determined in 80 patients, of whom 81.25% were HPV-positive, and 18.75% were HPV-negative. Deoxyribonucleic acid was extracted from samples, taken from patients and their parents, and sequenced to determine DQB1 genotypes. Nuclear family data were used to test whether the DQB1 locus is associated with invasive cervical cancer while controlling for high-risk HPV-positive patients. The transmission/disequilibrium test evaluates whether the frequency of transmission of parental marker alleles to their affected offspring deviates from the expected Mendelian frequency of 50%. Results: The HLA DQB1 locus showed evidence for allelic association with invasive cervical cancer in high-risk HPV-positive patients ($P = .006$). The transmission/disequilibrium test showed that the DQB1*0303 allele was transmitted to high-risk HPV patients more often than expected by chance, $[\chi^2]_{21} = 8.0$, $P = .005$ ($P = .035$ when correcting for multiple tests). Tests of association were negative when applied to all 96 patients, irrespective of HPV status. No significant differences were found in the distribution of the DQB1 alleles among HPV-positive patients compared with those who were HPV-negative, indicating that HLA alleles are not associated with susceptibility to HPV infection. Conclusion: These results suggest that the DQB1*0303 allele increases the risk for invasive cervical cancer in women who are HPV-positive.

Zhong, X. Y., M. R. Burk, et al. (2000). "Fluctuation of maternal and fetal free extracellular circulatory DNA in maternal plasma." *Obstetrics & Gynecology* **96**(6): 991.

<http://www.sciencedirect.com/science/article/B6TB2-41NK7G0-T/2/d29f2afbdad5fb08a44cd8238d949d20>

Objective: To examine whether concentrations of free extracellular fetal circulatory DNA in maternal plasma are stable or fluctuate. Methods: Consecutive blood samples were drawn from

13 healthy nonpregnant volunteers and from 16 healthy pregnant women over 3 days. DNA was isolated from the plasma fraction and quantified by real-time polymerase chain reaction (PCR). Results: In nonpregnant controls the total amount of cell free DNA fluctuated by an average of 13.5-fold. In samples obtained from pregnant women the amount of maternal cell free DNA varied by an average of 21.5-fold. Because ten of those women were pregnant with male fetuses, the concentration of free fetal DNA in these cases was determined by a real-time PCR assay for the Y chromosome. The mean variation in free fetal DNA levels in male fetuses was 2.2-fold. Conclusion: The degree of variation in free fetal DNA concentrations observed in this study was similar to published values, so these results imply that care should be exercised when considering quantitation of this fetal material for potential diagnostic or screening purposes.

Ophthalmology (5)

Akpek, E. K., C.-C. Chan, et al. (2004). "Lack of herpes virus DNA in choroidal tissues of a patient with serpiginous choroiditis." *Ophthalmology* **111**(11): 2071.

<http://www.sciencedirect.com/science/article/B6VT2-4DJ46SK-8/2/adae0c146c0c8d58c39e4a71ae1a39ef>

ObjectiveTo investigate the role of herpes viruses in the etiology of serpiginous choroiditis.
DesignInterventional case report.
ParticipantA 59-year-old male patient with long-term history of serpiginous choroiditis.
InterventionThe patient's affected eye was obtained during autopsy. Polymerase chain reaction was performed in the microdissected choroidal tissues.
ResultsHistopathologic examination demonstrated active inflammation with lymphocytic infiltration of the choroid. No viral DNA was amplified using pairs of herpes simplex virus (HSV) P1/P2 (for HSV-1, HSV-2, Epstein-Barr virus [EBV], cytomegalovirus [CMV] and human herpes virus [HHV]-8), and varicella-zoster virus [VZV] P1/P2 (for VZV, HHV-6, HHV-7) in the infiltrating lymphocytes or choroidal tissues.
ConclusionsThe current observation suggests a lack of a role for herpetic viral etiology in the etiopathogenesis of serpiginous choroiditis.

Bowyer, J. D., P. D. Gormley, et al. (1999). "Choroidal tuberculosis diagnosed by polymerase chain reaction: A clinicopathologic case report: Historical image." *Ophthalmology* **106**(2): 290.

<http://www.sciencedirect.com/science/article/B6VT2-49CM8C4-64/2/539e7d4ade6b2d6b7861fef40456a4b4>

PurposeTo show the use of the polymerase chain reaction (PCR) in a granulomatous choroidal lesion to support a diagnosis of tuberculosis.
DesignObservational case report.
TestingNucleic acid target amplification of a choroidal specimen using PCR for detection of *Mycobacterium tuberculosis* was tested.
Main outcome measuresPositive nucleic acid target amplification for *M. tuberculosis* in the ocular sample was measured.
ResultsPCR was positive for *M. tuberculosis* with appropriate negative controls.
ConclusionsPCR was thought to be a useful supportive technique in the diagnosis of choroidal tuberculosis.

Cockerham, G. C., K. Bijwaard, et al. (2000). "Primary graft failure: A clinicopathologic and molecular

analysis." *Ophthalmology* **107**(11): 2083.

<http://www.sciencedirect.com/science/article/B6VT2-41HMC5-1D/2/5ec0f5b61f1bb75606d8107bcb15495b>

Objective Primary graft failure (PGF) corneal tissues were analyzed for herpes simplex virus (HSV) and varicella-zoster virus (VZV). **Design** Retrospective, noncomparative case series. **Materials** Formalin-fixed, paraffin-embedded tissue of 21 donor corneas and 14 recipient corneas of PGF cases, as well as 10 control corneas. **Methods** Clinical, histologic, immunohistochemical, polymerase chain reaction (PCR), and, in selected cases, transmission electron microscopic characteristics were studied. **Main outcome measures** Evidence of HSV or VZV in donor tissues. **Results** Median patient age was 65 years, and median donor age was 48 years. Donor cornea parameters, including endothelial cell counts, death-to-preservation time, and time in storage, were generally within accepted standards. Stromal edema was found in all 21 donor corneas with PGF. Eighteen donor corneas demonstrated severely reduced or absent endothelium and mild to moderate lymphocytic infiltration without necrosis. Three donor corneas (14%) had necrotizing stromal keratitis (NSK) with keratic precipitates. Positive immunohistochemical staining of keratocytes for HSV was present in two of two donor corneas with NSK and was negative in 18 other donor corneas. Polymerase chain reaction analysis revealed the DNA of HSV type 1 (HSV1) in all donor corneas with NSK and in four donor corneas without NSK (33%). Recipient corneal tissue was negative for HSV1 DNA in three patients with NSK and positive in two of the four other PCR-positive patients. Transmission electron microscopy analysis showed viral particles in two donor corneas with NSK. Polymerase chain reaction analysis revealed no evidence of HSV type 2 or VZV in any cornea. All control corneas were negative for viral DNA. Sixteen corneas remained clear and two had failed after regraft for PGF, with a median follow-up of 3.6 years. **Conclusions** Herpes simplex virus type 1 DNA was present in 33% of patients of PGF. Herpetic stromal keratitis was found in some failed corneas; the lack of HSV in the paired recipient suggests importation within the donor cornea. The overall prognosis for regrafting after PGF is good.

Coupland, S. E., L. Krause, et al. (1998). "Lymphoproliferative lesions of the ocular adnexa: Analysis of 112 cases." *Ophthalmology* **105**(8): 1430.

<http://www.sciencedirect.com/science/article/B6VT2-49CMCY2-RD/2/602ccaf9b43c1d701978c94ce4dd734f>

Objective Lymphoproliferative lesions of the ocular adnexa were analyzed to examine (1) the suitability of the Revised European-American Lymphoma (REAL) classification for the subtyping of the lymphomas in these sites; (2) the predictive value of the REAL classification for the evolution of these tumors; and (3) the frequency and prognostic impact of tumor type, location, proliferation rate (Ki-67 index), p53, and CD5 positivity and the presence of monoclonality within these tumors. **Design** Retrospective review. **Methods** The clinical, histomorphologic, immunohistochemical, and molecular biologic (polymerase chain reaction [PCR]) features of lymphoid proliferations of the ocular adnexa were studied. **Study materials** The ocular adnexal lymphoproliferative lesions were located as follows: orbit in 52 patients (46%), conjunctiva in 32 patients (29%), eyelid in 23 patients (21%), and caruncle in 5 patients (4%). **Results** Reactive lymphoid hyperplasia was diagnosed in 12 cases and lymphoma in 99 cases; 1 case remained indeterminate. The five main subtypes of lymphoma according to the REAL classification were extranodal marginal-zone B-cell lymphoma (64%), follicle center lymphoma (10%), diffuse large cell B-cell lymphoma (9%), plasmacytoma (6%), and lymphoplasmacytic lymphoma (5%). Age, gender, and anatomic localization of the lymphomas did not have prognostic significance during a follow-up period of 6 months to 16.5 years (mean, 3.3 years). Extent of disease at time of presentation was the most important clinical prognostic factor: advanced disease correlated with increased risk ratios of having persistent disease at the final follow-up and with lymphoma-related

death (P P P P P ConclusionThe REAL classification is suitable for the subdivision of the ocular adnexal lymphomas. The MIB-1 proliferation rate and p53 positivity may aid the prediction of disease stage and disease progression, whereas PCR can support the diagnosis and reduce the number of histologically indeterminate lesions.

Gonzalez-Fernandez, F., S. A. Kaltreider, et al. (1998). "Sebaceous carcinoma: Tumor progression through mutational inactivation of p53." *Ophthalmology* **105**(3): 497.

<http://www.sciencedirect.com/science/article/B6VT2-49CR821-17/2/6c52a0477b7d2a1c4d41405f5cbba0b6>

BackgroundSebaceous carcinoma may masquerade for years as an inflammatory condition. In many cases, this may be because of the presence of longstanding intraepithelial disease (e.g., dysplasia or carcinoma in situ), which eventually progresses to invasive carcinoma recognized through tumefaction and a worsening clinical presentation. The mechanism for this tumor progression is unknown. In the Far East, human papilloma virus (HPV) has been suggested to play a role in the development of sebaceous carcinoma by inactivating tumor suppressor gene p53. Here, the authors explore the molecular basis of the progression of ocular sebaceous carcinoma.**Methods**Cases of sebaceous carcinoma seen at the University of Virginia, Department of Ophthalmology, during the period from 1989 to 1996 were analyzed for HPV infection by in situ hybridization and polymerase chain reaction. The expression of p53, p21WAF-1, Bcl-2, and epithelial membrane antigen was examined by immunohistochemistry. In one of the cases, frozen tumor was available, allowing exons 5 through 9 of the p53 gene to be sequenced.**Results**Seven cases were identified, all of which were from women. All were negative for HPV. In cases in which disease was restricted to dysplasia (carcinoma in situ), p53 but not p21WAF-1 was negative. In contrast, cases that contained a component of invasive or metastatic carcinoma showed striking hyperexpression of nuclear p53 in all of the malignant cells. In one of these cases, a G:C -> T:A transversion was found in the p53 gene. This mutation, characteristic of bulky carcinogens, substituted phenylalanine for cysteine 277, a residue that participates in hydrogen bonding to the p53 DNA binding consensus sequence.**Conclusions**Mutational inactivation of p53 may be involved in the progression of sebaceous carcinoma.

Oral Oncology (14)

Chen, Q., L. P. Samaranayake, et al. (2000). "Homozygous deletion of the PTEN tumor-suppressor gene is not a feature in oral squamous cell carcinoma." *Oral Oncology* **36**(1): 95.

<http://www.sciencedirect.com/science/article/B6TB6-3Y51HW4-K/2/e14d7e80469b4efc80a6d68ad652a6ed>

A recently identified putative tumor-suppressor gene, PTEN, at 10q23 has been described as mutated or homozygously deleted in many different human tumors. To determine the role of the homozygous deletion of this PTEN gene in oral squamous cell carcinoma (OSCC), we screened two cell lines derived from the latter tissue and 28 tumor samples from patients with OSCC, using a differential display polymerase chain reaction (PCR) system and, direct DNA sequencing methods. All of the nine exons of the PTEN could be successfully amplified using DNA from tumor tissues and the cell lines using this system. DNA sequencing confirmed the accuracy of the

PCR procedures. However, none of the samples, either from the cancer tissues or from the cell lines, showed homozygous deletion of PTEN. These data suggest that homozygous deletion of the PTEN gene is unlikely to be a feature of OSCCs.

Cruz, I. B. F., P. J. F. Snijders, et al. (1996). "Age-dependence of human papillomavirus DNA presence in oral squamous cell carcinomas." *Oral Oncology* **32**(1): 55.

<http://www.sciencedirect.com/science/article/B6TB6-3Y2MYY0-1C/2/44fcd149be38a4d856d765a9c97a17d8>

The aetiology of oral cancer is thought to be multifactorial. Apart from the two known major risk factors (tobacco and alcohol), a viral aetiology has been proposed, with special reference to human papillomavirus (HPV). 35 cases of oral squamous cell carcinoma (OSCC), seen at the Departments of Oral & Maxillofacial Surgery and Oral Pathology and Otolaryngology of the Free University of Amsterdam, were analysed as well as 12 biopsies of clinically and histologically normal gingival mucosa collected from healthy individuals after tooth extractions, using the polymerase chain reaction (PCR) and two different sets of primers that are able to detect a broad spectrum of HPV types. An overall HPV positivity of 54.3% in OSCC was found, the majority of positive cases (78.9%) harbouring HPV type 16. In contrast, no positivity for HPV was detected in the clinically normal oral mucosal samples analysed. Furthermore, a significant association between HPV presence and age was found: patients older than 60 years showed a lower prevalence of the virus (29.4%) compared with patients below this age (77.8%) ($P < 0.05$). The results from the present study suggest an association between HPV and OSCC, particularly in patients under the seventh decade.

D'Costa, J., D. Saranath, et al. (1998). "Detection of HPV-16 genome in human oral cancers and potentially malignant lesions from India." *Oral Oncology* **34**(5): 413.

<http://www.sciencedirect.com/science/article/B6TB6-3VYXY78-K/2/d3c9364fe47cb02207fff69e3b9a2f01>

The presence of high risk human papilloma virus (HPV) 16 and 18 was examined in 100 oral cancer patients of Indian descent, 80 patients with potentially malignant oral lesions and corresponding clinically normal mucosa from 48 of these patients. Additionally, presence of HPV-33, -6 and -11 was also studied in 86 oral cancers, 50 potentially malignant oral lesions and 30 corresponding normal oral mucosa. All the patients with oral cancer and oral lesions, were long term tobacco-chewers, and a majority of the patients were in Advanced Stages III and IV. The DNA samples were amplified by polymerase chain reaction (PCR) using HPV L1 consensus primers. Typing of HPV was performed by Southern hybridization analysis of the PCR products using HPV-16, -18, -33, -6 and -11 type specific oligonucleotide probes. HPV-16 was detected in 15 out of 100 (15%) oral tumours, 27 out of 80 (34%) potentially malignant lesions and 15 out of 48 (31%) of the corresponding normal mucosa in the patients with oral lesions. HPV-18 was not detected in any of the oral cancers, oral lesions and normal mucosa. HPV-33 and the low-risk HPV-6 and -11 were also not detected in the oral cancers, oral lesions and corresponding normal mucosa. A significantly higher prevalence of HPV-16 was observed in oral lesions (27 out of 80, 34%) as compared to oral cancers (15 out of 100, 15%). The observed difference of 19% (95% confidence interval [CI]: 6%, 31%), between these two proportions was statistically significant at the 5% level of significance. Our data indicates that HPV-16 may play a direct role in a certain proportion of oral cancers; whereas in a subpopulation of oral cancers HPV-16 infection may be vital in the early events associated with development of potentially malignant oral lesions, and the presence of the virus not essential in the progression of the oral lesion to frank malignancy.

Helmke, B. M., C. Reisser, et al. (2004). "Expression of SGLT-1 in preneoplastic and neoplastic lesions of the head and neck." Oral Oncology **40**(1): 28.

<http://www.sciencedirect.com/science/article/B6TB6-49BY4C9-9/2/141879f1015466e1b8a62fe26f7b0911>

Tumors show an increased glucose uptake that is mediated by glucose transport proteins. We have analyzed the expression of the sodium-dependent glucose co-transporters SGLT-1 and-2 in short-term cultures of squamous cell carcinomas of the head and neck (HNSCC) by RT-PCR. Distribution of the SGLT-1 protein in HNSCC tissues was investigated by immunohistochemistry. While we observed in 17/36 HNSCC short-term cultures the SGLT-1 mRNA, we found no SGLT-2 expression. SGLT-1 mRNA expression occurred preferentially in cultures originating from moderately and well differentiated HNSCC. In tumor tissues a heterogeneous SGLT-1 staining restricted to differentiated tumor cells was seen in 27/30 HNSCC analyzed. In normal mucosa SGLT-1 staining was also confined to differentiated compartments and lacked in dysplastic areas. Our data indicate a differentiation-dependent expression of SGLT-1 in HNSCC. This is important knowledge for the planning of glucose-targeting therapies and suggest SGLT-1 as a differentiation marker in head and neck tissues.

Ibrahim, S. O., N. Aarsaether, et al. (2003). "Gene expression profile in oral squamous cell carcinomas and matching normal oral mucosal tissues from black Africans and white Caucasians: the case of the Sudan vs. Norway." Oral Oncology **39**(1): 37.

<http://www.sciencedirect.com/science/article/B6TB6-460WNHM-1/2/d0534d71c2f4ffcc2ab6bb20906bf004>

Expression profile of 588 known genes relating to tumour biology, was examined between oral squamous cell carcinomas (OSCCs) and matching normal oral mucosal tissues (NOMTs) obtained from Sudanese (n=11) and Norwegian (n=11) patients. cDNA probes were synthesised from total RNA and hybridised with the Atlas human cancer cDNA expression array membranes. RT-PCR and immunohistochemistry were applied to confirm the expression pattern of a subset of the 588 genes. Differences in expression of the genes examined were found between the OSCCs and the NOMTs on the Atlas membranes. Several of these genes were either up- or down-regulated 1.6-fold or higher in the OSCCs compared to the NOMTs in the cases from the two populations. We found that 181 (31%) and 195 (33%) genes were either up-regulated or down-regulated in the OSCCs from the Sudan and Norway, respectively. From the total number of genes (n=376) found expressed in the OSCCs investigated from the two countries, 53 genes (14%) showed common expression profile [35 (66%) were up-regulated and 18 (34%) were down-regulated] and 70 genes (19%) showed opposite regulation status. Results of the RT-PCR and immunohistochemistry confirmed the hybridisation data. These findings may provide an OSCCs-specific gene expression profile in patients from the two countries, suggesting that alterations of 123 genes are common in these OSCCs regardless of ethnic differences or other socio-cultural risk factors between the patients from the two countries. The findings might further suggest that specific genes are frequently involved in these OSCCs, which may provide novel clues as diagnostic, prognostic biomarkers and/or targets for therapy. The Atlas human cancer cDNA expression array technique can be useful to examine and describe the expression profile of known genes frequently involved in OSCCs from different populations.

Ibrahim, S. O., J. R. Lillehaug, et al. (1999). "Expression of biomarkers (p53, transforming growth factor

alpha, epidermal growth factor receptor, c-erbB-2/neu and the proliferative cell nuclear antigen) in oropharyngeal squamous cell carcinomas." *Oral Oncology* **35**(3): 302.

<http://www.sciencedirect.com/science/article/B6TB6-3W19WVP-D/2/62eca3ee658bdecea90437db6beb0015>

Using immunohistochemistry, expression of p53, transforming growth factor-alpha (TGF-[alpha]), epidermal growth factor receptor (EGFR), c-erbB-2/neu and proliferating cell nuclear antigen (PCNA) was examined in 26 fresh frozen tissue specimens of oropharyngeal squamous cell carcinomas (SCCs). p53 gene mutations were examined by polymerase chain reaction (PCR)/DNA sequencing methods in 22 carcinomas. The findings were examined for correlations with patients' clinicopathological parameters. Expressions of p53 and PCNA were also examined in 21 formalin-fixed corresponding tissues. Of the fresh frozen tissue specimens, 77% (20/26) showed expression and 68% (15/22) showed mutations (substitutions) of the p53, with significant clustering of the mutations in exons 5 (8/22; 36%), 7 (4/22; 18%) and 8 (5/22; 23%). No mutations were found in exon 6. There was a discordance between expression of p53 protein and mutations of the gene. Parallel to expression and mutations of the p53 found in most of the specimens, expression of TGF-[alpha], EGFR, c-erbB-2/neu and PCNA was found in 88% (22/25), 92% (23/25), 58% (14/24) and 91% (21/23) of the specimens, respectively. For the formalin-fixed tissue specimens, 62% (13/21) and 90% (19/21) expressed p53 and PCNA, respectively. Examining for correlations with patients' clinicopathological parameters, expression of p53, TGF-[alpha], EGFR and c-erbB-2/neu seemed to negatively correlate with the increase of the tumour grade. The present work suggests that: (1) lack of negative growth regulation due to inactivation of the p53 gene together with activation of other proto-oncogenes are necessary genetic events in the carcinogenesis of oropharyngeal SCCs; (2) in oropharyngeal SCCs, p53 gene mutations were clustered in exons 5 (codons 130-186), 7 (codons 230-248) and 8 (codons 271-282) which perhaps suggests that tobacco carcinogens probably affect the mutational hot spots of the p53 gene at codons 157, 175, 186, 248, 273 and 282; and (3) fresh frozen and formalin-fixed tissue specimens give similar results when an immunohistochemical method is applied. The importance of p53, TGF-[alpha], EGFR, c-erbB-2/neu and PCNA as biomarkers in oropharyngeal SCCs deserves particular attention because it might offer further understanding of the development of these carcinomas.

Lopes, M. A., N. G. Nikitakis, et al. (2001). "Amplification and protein expression of chromosome 12q13-15 genes in osteosarcomas of the jaws." *Oral Oncology* **37**(7): 566.

<http://www.sciencedirect.com/science/article/B6TB6-43YR0B8-5/2/3ef78ec1804098fb87eb59e301008c09>

Overexpression and amplification of several genes (MDM2, CDK4 and SAS) located on chromosome 12q13-15 have been noted to occur in various human sarcomas. As a result, two major growth regulation pathways may be inhibited. MDM2 may down regulate the p53-mediated growth control and CDK4 may affect pRB-mediated events. To determine the frequency of alterations in these genes and their correlation with clinicopathologic features, we analyzed the MDM2 and CDK4 protein levels by immunohistochemistry and assessed MDM2, CDK4 and SAS amplification by real-time PCR in nine osteosarcomas of the jaws. Positive staining for CDK4 and MDM2 was observed in eight cases (88.8%) and five cases (55.5%), respectively. Intense CDK4 staining was noted in four cases (two high grade, one intermediate grade and one low grade). Intense MDM2 staining was observed in the same four previous cases, as well as, one additional high-grade tumor. Individual DNA amplification for CDK4, MDM2 and SAS was observed in six cases for each gene. Co-amplification was observed in five cases that showed CDK4 and MDM2 concomitant amplification and four cases that displayed amplification for all of the genes. In addition, among the five cases that presented CDK4 and MDM2 amplification, strong overexpression of CDK4 and MDM2 was observed in three and in four cases, respectively (three

high grade and one intermediate grade). These results suggest that 12q13-15 genes are involved in neoplastic disease and concurrent amplification and overexpression of these genes might help to define high-grade tumors.

Macabeo-Ong, M., C. H. Shiboski, et al. (2003). "Quantitative analysis of cathepsin L mRNA and protein expression during oral cancer progression." *Oral Oncology* **39**(7): 638.

<http://www.sciencedirect.com/science/article/B6TB6-491J58Y-1/2/9beac13a4e883ded79551e9688b38a1e>

Although an important risk factor for oral cancer is the presence of epithelial dysplasia, most of these lesions will not progress to malignancy. Presently, for the individual patient with dysplasia, there are few reliable markers that may indicate the likelihood of progression to oral cancer. Cathepsin L is a lysosomal protease that degrades extracellular matrix material. Because cathepsin L is frequently overexpressed in oral squamous cell carcinoma (SCC) we hypothesized that it is also overexpressed in oral premalignancy and that premalignant lesions that progressed to oral cancer expressed higher levels of cathepsin L than those premalignant lesions that did not. In this retrospective pilot study we examined changes in cathepsin L expression at the mRNA level using quantitative TaqMan RT-PCR and at the protein level by immunohistochemistry in 33 routinely processed oral dysplastic lesions and 14 SCCs obtained from 33 patients. Sixteen of the dysplastic lesions progressed to oral SCC and 17 did not after several years of follow-up. Cathepsin L mRNA was overexpressed in 16/33 (48%) dysplastic lesions and in 9/14 (64%) oral SCC. Cathepsin L protein was also overexpressed in a large proportion of dysplasias and cancers. Overexpression was independent of dysplasia grade and identified in both those patients who progressed to oral SCC and in those who did not. Levels of cathepsin L mRNA and protein did not differ significantly in the progressing versus non-progressing dysplasias ($P=0.27$). However, cathepsin L mRNA and protein were significantly lower in the non-progressing dysplasias when compared to the oral cancers ($P=0.03$) but not in the progressing dysplasias suggesting a trend for dysplasias with overexpressed cathepsin L to be more likely to progress to oral cancer.

Nikitakis, N. G., M. A. Lopes, et al. (2002). "Oral leiomyosarcoma: review of the literature and report of two cases with assessment of the prognostic and diagnostic significance of immunohistochemical and molecular markers." *Oral Oncology* **38**(2): 201.

<http://www.sciencedirect.com/science/article/B6TB6-454F7D0-D/2/3b86e597155d24daca5ad4b0cfe9e335>

Leiomyosarcoma of the oral cavity is a very rare tumor that is associated with aggressive clinical behavior and low survival. In this paper, we report two new cases of leiomyosarcoma affecting the mandibular gingiva and mandible of a 35-year-old male and the mandible of a 51-year-old female. Given the difficulty in the histopathologic discrimination between benign and malignant smooth muscle tumors and the absence of reliable histologic parameters for prognostication of leiomyosarcomas, we evaluated the diagnostic and prognostic value of various immunohistochemical and molecular markers. By means of immunohistochemistry and quantitative real-time PCR analysis, we detected protein expression of PCNA, bcl-2, CDK4, p53 and MDM2 in both our cases and MDM2 amplification in our second case. The literature, pertinent to oral leiomyosarcoma and to molecular analysis of smooth muscle tumors, is reviewed.

Nishimoto, I. N., N. A. Pinheiro, et al. (2004). "Cyclin D1 gene polymorphism as a risk factor for squamous cell carcinoma of the upper aerodigestive system in non-alcoholics." Oral Oncology **40**(6): 604.

<http://www.sciencedirect.com/science/article/B6TB6-4BSW5SW-B/2/0a509c1f60da3965c58b8180928fbf57>

Squamous cell carcinoma of the upper aerodigestive tract (UADT) is associated with environmental factors, especially tobacco and alcohol consumption. Genetic factors, including cyclin D1 (CCND1) polymorphism have been suggested to play an important role in tumorigenesis and progression of UADT cancer. To investigate the relationship between CCND1 polymorphism on susceptibility for UADT cancers, 147 cancer and 135 non-cancer subjects were included in this study. CCND1 genotype at codon 242(G870A) in exon 4 was undertaken using denaturing high performance liquid chromatography (DHPLC) and DNA sequencing. Significant odds ratio (OR) of the AA + GA genotypes [OR=7.5 (95% CI: 1.4-39.7)] was observed in non-drinkers but for non-smokers a non-significant [OR=5.4 (95% CI: 0.9-31.4)] was found in the adjusted model. These results suggest that allele A may be a risk factor for UADT cancer, especially in non-alcoholics. However, further epidemiological studies are needed to establish the exact role of CCND1 polymorphism and the development of UADT cancers.

Patrikidou, A., J. Bennett, et al. (2002). "A novel, de novo germline TP53 mutation in a rare presentation of the Li-Fraumeni syndrome in the maxilla." Oral Oncology **38**(4): 383.

<http://www.sciencedirect.com/science/article/B6TB6-462BN8J-C/2/80d6f5e8870dfd9d0c848b424885302c>

We undertook the genetic analysis of a classic Li-Fraumeni syndrome (LFS) family with clustering of primary tumours including two maxillary sarcomas, a rare LFS site of tumour occurrence. Our aim was to investigate the presence of a specific type of TP53 mutation that could be associated with this unusual predilection of site for cancer occurrence. Mutational screening of the coding region of TP53 revealed an A>T transversion in codon 144 of exon 5 (CAG>CTG, Gln>Leu) in the germline of one of the three affected members, with loss of heterozygosity (LOH) in the tumour tissue. All other affected members were negative for germline or somatic TP53 mutations. TP53 immunohistochemistry was uninformative. The mutation we report is a de novo constitutional TP53 mutation that has not been previously described in the literature. It could explain the more burdened phenotype of the affected patient (died at 21 months). Alternative mechanisms to explain the overall family phenotype are discussed.

Saranath, D., A. T. Tandle, et al. (1999). "p53 inactivation in chewing tobacco-induced oral cancers and leukoplakias from India." Oral Oncology **35**(3): 242.

<http://www.sciencedirect.com/science/article/B6TB6-3W19WVP-3/2/db3b44673e394cab93d0d60d952c06d0>

The inactivation of p53 tumour suppressor gene vis-a-vis point mutation, overexpression and degradation due to Human Papilloma virus (HPV) 16/18 infection, was examined in chewing tobacco-associated oral cancers and oral leukoplakias from India. The analysis of mutations was assessed by polymerase chain reaction (PCR) with single strand conformation polymorphism (PCR-SSCP) of exons 5-9 on DNA from 83 oral cancer cases, and the mutations confirmed by direct nucleotide sequencing of the PCR products. p53 protein expression was evaluated by immunohistochemical analysis on paraffin-embedded sections of 62 representative oral cancer biopsies and 22 leukoplakias, using p53-specific monoclonal antibody DO-7. The presence of

HPV16/18 was detected in the 83 oral cancer cases by PCR analysis using HPV L1 consensus sequences, followed by Southern hybridization with type-specific oligonucleotide probes. Forty-six per cent (38/83) of oral cancer tumours showed p53 alterations, with 17% (14/83) showing point mutations, 37% (23/62) with overexpression and 25% (21/83) with presence of HPV16 wherein the E6 HPV16 protein degrades p53. HPV18 was not detected in any of the samples. Ninety-two per cent concordance was observed between missense point mutations and overexpression of p53 protein. A significant correlation was not observed between p53 alterations in oral cancer and clinico-pathological profile of the patients. Twenty-seven per cent (6/22) of oral leukoplakias showed p53 overexpression. The overall p53 alterations in oral cancer tissues and oral lesions are comparable to data from the oral cancers reported in the Western countries with smoking and alcohol-associated oral cancers, and suggest a critical role for p53 gene in a significant proportion of oral cancers from India. The overexpression of p53 protein in leukoplakias may serve as a valuable biomarker for identifying individuals at high risk of transformation to malignant phenotype.

Tjebbes, G. W. A., P. A. Kreijveld, et al. (2002). "P53 tumor suppressor gene mutations in laryngeal cancer and in recurrent disease following radiation therapy." *Oral Oncology* **38**(3): 296.

<http://www.sciencedirect.com/science/article/B6TB6-45MVW76-C/2/521ba2037c848de7953c4ca4b200826b>

In this study we performed p53 sequencing based mutation analysis in laryngeal cancers and matched recurrent disease following irradiation. The question is if irradiation affects the DNA and introduces or deletes mutations so that p53 cannot be used as a clonal marker anymore. P53 mutations were identified in fresh-frozen laryngectomy specimens with either primary laryngeal cancers, treated by surgery and irradiation post-operative with local failure during follow-up, or with recurrent laryngeal cancers following primary irradiation. In 21 tumors the p53 status was analyzed by direct sequencing full-length mRNA through RT-PCR. DNA sequencing analysis of exons 2 through 11 was performed when RNA isolation could not be performed. The marker mutation identified in this way was detected by DNA sequencing of the corresponding exon in formalin-fixed deparaffinized tumor biopsy samples in respectively matched recurrent disease following surgery and irradiation or primary tumor before irradiation. DNA sequencing analysis of the corresponding exon of peripheral blood leukocytes excluded the presence of germline mutations or polymorphisms. In 16 out of 21 tumors (71%), a mutation was identified. Fifteen of these marker mutations were detected in the matched tumor biopsy sample (94%). The only case lacking the marker mutation probably was a second primary tumor. We conclude that we find no direct evidence for induction or loss of p53 mutations following irradiation. Consequently, p53 may be used as a diagnostic tool when histological examination fails, for example in discriminating between the presence of a second primary tumor in the same area versus recurrent disease.

Vera-Sempere, F. J., J. S. Burgos, et al. (1996). "Immunohistochemical expression of Epstein-Barr virus-encoded latent membrane protein (LMP-1) in paraffin sections of EBV-associated nasopharyngeal carcinoma in Spanish patients." *Oral Oncology* **32**(3): 163.

<http://www.sciencedirect.com/science/article/B6TB6-3Y2MYWM-5/2/32a02e0d794fc82aa94a6190a6a501e5>

Nasopharyngeal carcinoma (NPC) paraffin samples, from Spanish patients, of distinct histological types, including squamous cell carcinoma (10 cases), nonkeratinising carcinoma (12 cases) and undifferentiated carcinoma (29 cases) were analysed for Epstein-Barr virus (EBV) detection and EBV-encoded latent membrane protein (LMP-1) expression using a sensitive nested-polymerase

chain reaction with four oligonucleotide primers specific for EBV genome (EB-1, 2, 3, 4) and immunohistochemistry by means of CS1-4 pool monoclonal antibody. EBV genome was detected regardless of histological type in 100% of samples with sufficient DNA quality to permit viral diagnosis (50 out of 51 cases), supporting the previous view that all types of NPC are variants of an EBV-associated malignancy. However LMP-1, an EBV-encoded oncogenic protein, was detected in 40 out of 51 samples (78.4%) and LMP-1 immunohistochemical expression was not apparently influenced by histological type, primary or metastatic site, clinical stage, age or sex. This high percentage of detection of LMP-1 in our cases supports a role for EBV in the pathogenesis of different types of NPC, but the lack of constant expression of LMP-1 in NPC remains unclear and various reasons are postulated to explain the absence of this oncogenic protein in some EBV-associated NPCs.

Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology & Endodontics (1)

Nikitakis, N. G., M. A. Lopes, et al. (2001). "MDM2+/CDK4+/p53+ oral liposarcoma: Case report and review of the literature." Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology & Endodontics **92**(2): 194.

<http://www.sciencedirect.com/science/article/B6WP1-45SRBVR-66/2/861e20686f40259dde489192c869c8ed>

Although liposarcoma is one of the most common soft tissue sarcomas, its location in the oral cavity is very rare. To our knowledge, only 43 cases of liposarcoma originating in the oral tissues have been reported in the English-language literature. In this article, we report a case of well-differentiated liposarcoma affecting the cheek of a 28-year-old man and review the oral liposarcoma literature. Immunohistochemical analysis of the tumor revealed an MDM2+/CDK4+/p53+ immunophenotype that is consistent with the immunohistochemical profile of well-differentiated liposarcoma originating in other areas of the body. Quantitative polymerase chain reaction analysis of the DNA levels of the MDM2 (human homologue of the murine double-minute type 2), CDK4 (cyclin-dependent kinase 4), and SAS (sarcoma amplified sequence), genes was performed, revealing only SAS gene amplification. The possibility of misdiagnosis of oral liposarcoma because of its sometimes inconspicuous clinical and microscopic features is emphasized. Careful pathologic examination of liposarcoma is essential for discrimination from benign adipose tissue neoplasms and for precise histologic classification, both of major prognostic significance. Possible implications of molecular and cytogenetic analysis for unraveling the pathogenesis and determining the prognosis of liposarcoma are discussed. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;92:194-201)

Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology (2)

Kurose, K., M. Terai, et al. (2004). "Low prevalence of HPV infection and its natural history in normal oral mucosa among volunteers on Miyako Island, Japan." Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology **98**(1): 91.

<http://www.sciencedirect.com/science/article/B6WP1-4CSCVP9-X/2/c40780dcb9615370ee14150317190ed6>

ObjectiveTo investigate the prevalence of human papillomavirus (HPV) infection in normal oral mucosa, and to observe the natural history in the oral cavity in oral swab samples collected from healthy volunteers on Miyako Island, Okinawa, Japan.**Study design**The prevalence of HPV infection in oral buccal mucosa cell scrapes collected between 2000 and 2002 from a cohort of 668 healthy volunteers was determined. HPV DNA was detected by consensus polymerase chain reaction (PCR) using MY09/MY11 primers followed by direct cycle sequencing. Just over 2 years later the HPV-positive participants were reevaluated.**Results**Of the 668 subjects, 662 samples were analyzed for HPV. HPV DNA was detected in 4 (0.6%) specimens. HPV type 16 (HPV16), HPV53, and HPV71, mucosal types, and HPV12, a cutaneous type, were all identified by direct sequencing. In the follow-up survey, the HPV71- and HPV12-positive participants again tested positive, while HPV DNA was not detected in the HPV16- and HPV53-positive participants.**Conclusion**The results of this study among healthy individuals from Miyako Island suggest that oral HPV infection is uncommon. In this cohort, HPV71 and HPV12 were persistent, while HPV16 and HPV53 were transient in normal oral mucosa.

Takezawa, K., C. Jackson, et al. (1998). "Molecular characterization of Warthin tumor." Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology **85**(5): 569.

<http://www.sciencedirect.com/science/article/B6WP1-4CRH670-3M/2/209dee1cb420efd8432505d1e57b8f3e>

Objective. Warthin tumor of the salivary gland is composed of oncocytic epithelium with a prominent follicular lymphoid infiltrate. The purpose of this study was to characterize the clonality of this lymphoid component by means of polymerase chain reaction technology.**Study design.** DNA was isolated from paraffin-embedded tissue from 20 cases of typical Warthin tumor of the salivary gland and amplified by polymerase chain reaction to assess B- and T-cell clonality.**Results.** No dominant clonal populations were identified in any tumor. However, minor clonal expansions of both B and T cells were detected in up to 50% of tumors (immunoglobulin H, 50%; T-cell antigen receptor [beta], 10%; T-cell antigen receptor [gamma], 5%). No tumors showed evidence of bcl-2 proto-oncogene translocation, whereas 95% contained detectable Epstein-Barr virus DNA.**Conclusion.** The B- and T-cell components of Warthin tumor are polyclonal with oligoclonal expansion of both T and B cells in some lesions.

Osteoarthritis and Cartilage (7)

Arai, M., D. Anderson, et al. (2004). "Effect of adenovirus-mediated overexpression of bovine ADAMTS-4 and human ADAMTS-5 in primary bovine articular chondrocyte pellet culture system." Osteoarthritis and Cartilage **12**(8): 599.

<http://www.sciencedirect.com/science/article/B6WP3-4CP6999-1/2/a9620198a5f1afcb9191a74146efa0ac>

IntroductionArticular cartilage matrix synthesis and degradation are dynamic processes that must be balanced for proper maintenance of the tissue. In osteoarthritis (OA), this balance is skewed

toward degradation and ultimate loss of matrix. The transcriptional and/or activity levels of hundreds of genes are dysregulated in chondrocytes from osteoarthritic cartilage, and a subset of these genes may represent pivotal factors that could be modulated if their specific role in the disease process could be identified. **Objective** To investigate the role of ADAMTS-4 and ADAMTS-5 in cartilage matrix degradation by developing a chondrocyte pellet culture assay in combination with adenoviral gene expression, and to demonstrate the utility of this assay by assessing the specific functional contribution of these genes to cartilage matrix metabolism. **Methods** A full-length cDNA for bovine ADAMTS-4 (bADAMTS-4) was isolated, and used to evaluate the expression, regulation, and activity of this gene in bovine cartilage. Adenoviruses expressing bADAMTS-4, human ADAMTS-5 (hADAMTS-5) or human bone morphogenetic protein 2 (BMP-2) were used to infect primary chondrocytes, and their effect on extracellular matrix metabolism was assessed by monitoring the accumulation and release of glycosaminoglycans (GAG) in three-dimensional chondrocyte pellet cultures. **Results** Analysis of bADAMTS-4 transcriptional regulation in chondrocytes revealed that interleukin-1[alpha] (IL-1[alpha]) was the most potent inducer of bADAMTS-4 mRNA and subsequent aggrecan degradation in cartilage explant cultures of those cytokines tested. bADAMTS-4 mRNA induction by IL-1[alpha] was greater in nasal cartilage than in articular cartilage. Chondrocytes infected with adenovirus expressing either bADAMTS-4 or hADAMTS-5 genes showed increased aggrecan degradation in newly synthesized matrix by pellet cultures while chondrocytes overexpressing BMP-2 showed increased aggrecan synthesis. **Conclusion** Adenoviral delivery of genes to primary bovine chondrocytes, followed by culture in three-dimensional pellet format and evaluation of extracellular matrix protein metabolism, is a useful functional assay for assessing the role of genes on cartilage matrix synthesis and degradation.

Barbero, A., S. Grogan, et al. (2004). "Age related changes in human articular chondrocyte yield, proliferation and post-expansion chondrogenic capacity." *Osteoarthritis and Cartilage* **12**(6): 476.

<http://www.sciencedirect.com/science/article/B6WP3-4C47MGY-1/2/1be851e6dfb9704524a1e1cfe0603408>

Objective: We investigated how aging effects human chondrocyte yield, proliferation, post-expansion chondrogenic capacity, and response to specific growth factors supplemented during expansion. **Methods:** Fifty-two samples of human articular cartilage were harvested from cadavers 20 to 91 years old and grouped into age decades. Cell yields were normalised to tissue wet weight. Cell proliferation rates were calculated during expansion in medium without (CTR) or with TGF[beta]1, FGF-2 and PDGF-BB (TFP). Chondrogenic capacity of CTR- and TFP-expanded cells was assessed by cultivation as 3D pellets in a defined serum-free medium, followed by histological, immunohistochemical, biochemical and real-time RT-PCR analyses. **Results:** Cell yields were similar in donors up to 40 years of age and significantly lower (1.8-fold) in older donors. Cell proliferation rates in CTR medium significantly decreased after 30 years of age and remained similar in older donors. In the presence of TFP, proliferation rates were higher (up to 3.7-fold) in all age groups and decreased only slightly with age. The glycosaminoglycan (GAG) content of pellets obtained from CTR-expanded cells was not correlated with age. Pellets from TFP-expanded cells reproducibly contained more GAG (up to 3.2-fold) than those from CTR-expanded cells only if donors were younger than 40. Safranin O staining intensity and collagen type II expression and accumulation were consistent with GAG contents. **Conclusion:** Medium supplementation with the growth factor combination TFP during chondrocyte expansion supports higher proliferation rates at any age and higher post-expansion chondrogenic capacity in donors up to 40 years. These findings may be relevant for chondrocyte-based cartilage repair procedures.

Daouti, S., B. Latario, et al. "Development of comprehensive functional genomic screens to identify novel

mediators of osteoarthritis." Osteoarthritis and Cartilage In Press, Corrected Proof
<http://www.sciencedirect.com/science/article/B6WP3-4FSCMF8-5/2/96006cf3f4dfb125b603a82f22c05681>

SummaryObjectiveThe aim of this study was to develop high-throughput assays for the analysis of major chondrocyte functions that are important in osteoarthritis (OA) pathogenesis and methods for high-level gene expression and analysis in primary human chondrocytes.**Methods**In the first approach, complementary DNA (cDNA) libraries were constructed from OA cartilage RNA and full-length clones were selected. These cDNAs were transferred into a retroviral vector using Gateway Technology. Full-length clones were over-expressed in human articular chondrocytes (HAC) by retroviral-mediated gene transfer. The induction of OA-associated markers, including aggrecanase-1 (Agg-1), matrix metalloproteinase-13 (MMP-13), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), collagen IIA and collagen X was measured by quantitative real-time polymerase chain reaction (QPCR). Induction of a marker gene was verified by independent isolation of 2-3 clones per gene, re-transfection followed by QPCR as well as nucleotide sequencing. In the second approach, whole cDNA libraries were transduced into chondrocytes and screened for chondrocyte cluster formation in three-dimensional agarose cultures.**Results**Using green fluorescent protein (eGFP) as a marker gene, it was shown that the retroviral method has a transduction efficiency of >90%. A total of 40 verified hits were identified in the QPCR screen. The first set of 19 hits coordinately induced iNOS, COX-2, Agg-1 and MMP-13. The most potent of these genes were the tyrosine kinases Axl and Tyro-3, receptor interacting kinase-2 (RIPK2), tumor necrosis factor receptor 1A (TNFR1A), fibroblast growth factor (FGF) and its receptor FGFR, MUS81 endonuclease and Sentrin/SUMO-specific protease 3. The second set of seven hits induced both Agg-1 and MMP-13 but none of the other markers. Five of these seven genes regulate the phosphoinositide-3-kinase pathway. The most potently induced OA marker was iNOS. This marker was induced 20-500 fold by seven genes. Collagen IIA was also induced by seven genes, the most potent being transforming growth factor [beta] (TGF[beta])-stimulated protein TSC22, vascular endothelial growth factor (VEGF) and splicing factor 3a. This screening assay did not identify inducers of collagen X. The second chondrocyte cluster formation screen identified 14 verified hits. Most of the genes inducing cluster formation were kinases. Additional genes had not been previously known to regulate chondrocyte cluster formation or any other chondrocyte function.**Conclusions**The methods developed in this study can be applied to screen for genes capable of inducing an OA-like phenotype in chondrocytes on a genome-wide scale and identify novel mediators of OA pathogenesis. Thus, coordinated functional genomic approaches can be used to delineate key genes and pathways activated in complex human diseases such as OA.

Nishida, Y., C. B. Knudson, et al. (2004). "Osteogenic Protein-1 inhibits matrix depletion in a hyaluronan hexasaccharide-induced model of osteoarthritis." Osteoarthritis and Cartilage **12**(5): 374.

<http://www.sciencedirect.com/science/article/B6WP3-4BT1RSB-1/2/0c8ce162339a5a98d0b7a46f8bbdee40>

Objective: To examine the capacity of recombinant osteogenic protein-1 (OP-1) to inhibit the cartilage extracellular matrix damage that follows treatment with hyaluronan hexasaccharides (HA6).**Design:** The effects of OP-1 were examined on isolated human chondrocytes grown in alginate beads as well as articular cartilage slices treated with hyaluronan hexasaccharides. Changes in the relative expression of messenger RNA for hyaluronan synthase- 2, aggrecan and CD44 were determined by competitive quantitative reverse transcriptase-polymerase chain reaction. Cartilage proteoglycan biosynthesis was examined by a ³⁵S-sulfate incorporation assay. Cell-associated matrix of human chondrocytes was visualized by the use of particle exclusion assay, and alcian blue staining. Cartilage slices were examined for accumulation of proteoglycan by Safranin-O, and hyaluronan by a specific biotinylated probe.**Results:** Combined OP-1 and HA6 treatment resulted in enhanced expression of mRNA for aggrecan and HAS-2,

compared to the treatment with HA6 only. This increased expression of aggrecan mRNA was paralleled by an increased synthesis of cartilage proteoglycan especially retained in the cell-associated matrix. Co-treatment with OP-1 inhibited the HA6-induced depletion of cell-associated matrices as well as HA6-induced depletion of hyaluronan and proteoglycan within cartilage tissue slices. **Conclusions:** These results demonstrate that OP-1 can abrogate the catabolic events associated with a HA6-induced matrix depletion model of osteoarthritis. The mRNA levels of two major cartilage extracellular matrix components, aggrecan and hyaluronan synthase-2 are enhanced above values obtained by either OP-1 or HA6 treatments alone.

Patwari, P., G. Gao, et al. (2005). "Analysis of ADAMTS4 and MT4-MMP indicates that both are involved in aggrecanolysis in interleukin-1-treated bovine cartilage." Osteoarthritis and Cartilage **13**(4): 269.

<http://www.sciencedirect.com/science/article/B6WP3-4FK3P8P-1/2/dc3b4fc82a3cfb636a995394748281cd>

SummaryObjectiveTo investigate the mechanism of aggrecanolysis in interleukin-1 (IL-1)-treated cartilage tissue by examining the time course of aggrecan cleavages and the tissue and medium content of membrane type 4-matrix metalloproteinases (MT4-MMP) and a disintegrin and metalloproteinase with thrombospondin type I motifs (ADAMTS)4.**Methods**Articular cartilage explants were harvested from newborn bovine femoropatellar groove. The effects of IL-1 treatment with or without aggrecanase blockade were investigated by Western analysis of aggrecan fragment generation, ADAMTS4 species (p68 and p53), and MT4-MMP, as well as by realtime PCR (polymerase chain reaction) for ADAMTS4 and 5. Aggrecanase was blocked with mannosamine (ManN), an inhibitor of glycosylphosphatidylinositol anchor synthesis, and esculetin (EST), an inhibitor of MMP-1, MMP-3, and MMP-13 gene expression.**Results**IL-1 treatment caused a major increase in MT4-MMP abundance in the tissue and medium. ADAMTS4 (p68) was abundant in fresh cartilage and this was retained in the tissue in untreated cartilage. IL-1 treatment for 6 days caused a marked loss of p68 from the cartilage and the appearance of p53 in the medium. Addition of either 1.35 mM ManN or 31-500 [μ M] EST blocked IL-1-mediated aggrecanolysis and this was accompanied by nearly complete inhibition of the MT4-MMP increase, the p68 loss and the formation of p53. IL-1 treatment increased mRNA abundance for ADAMTS4 ([not, vert, similar]3-fold) and ADAMTS5 ([not, vert, similar]10-fold) but this was not accompanied by a marked change in enzyme protein abundance.**Conclusion**These studies support a central role for MT4-MMP in IL-1-induced cartilage aggrecanolysis and are consistent with the identification of p68 as the aggrecanase that cleaves within the CS2 domain, and of p53 as the aggrecanase that generates G1-NITEGE. Since the induction by IL-1 was not accompanied by marked changes in total ADAMTS4 protein, but rather in partial conversion of p68 to p53 and release of both from the tissue, we conclude that aggrecanolysis in this model system results from MT4-MMP-mediated processing of a resident pool of ADAMTS4 and release of the p68 and p53 from their normal association with the cell surface.

Stern, A. G., M. R. C. de Carvalho, et al. (2003). "Association of erosive hand osteoarthritis with a single nucleotide polymorphism on the gene encoding interleukin-1 beta." Osteoarthritis and Cartilage **11**(6): 394.

<http://www.sciencedirect.com/science/article/B6WP3-48M7SCC-1/2/24f565be3c6fc166263e482daaa327d4>

Objective: Certain forms of primary osteoarthritis (OA), particularly those affecting hand joints, have a genetic component. Recent studies have shown suggestive evidence that hand and knee OA are linked with the interleukin-1 (IL-1) region on human chromosome 2q. This study was

undertaken to assess the association of primary OA of the hand (hand OA) with IL-1 region markers. Methods: Sixty-eight US Caucasoid cases and 51 US Caucasoid controls aged 60 years or older were recruited from the Mid-Atlantic region of the United States. Hand OA was classified by American College of Rheumatology (ACR) Clinical Criteria, and cases were subjected to radiographic examination for subgrouping. Genotyping was done for seven previously described single nucleotide polymorphisms (SNPs) of genes for IL-1[alpha] (encoded by IL1A), IL-1[beta] (IL1B), and the IL-1 receptor antagonist (IL1RN), as well as an IL1RN variable number of tandem repeat (VNTR) marker. Six microsatellite markers on other chromosomes (null loci) were also typed. Results: The IL1B 5810 G>A SNP genotypes marker were not in Hardy-Weinberg equilibrium ($p=0.007$). This IL1B 5810 AA genotype association was also significant between erosive and non-erosive hand OA subjects (relative risk 4.01, $p=0.008$). As expected, significant linkage disequilibrium was present between IL1B 5810 SNP and IL1A (-889 SNP, other IL1B SNPs, and the nearest IL1RN SNP examined. The IL1B 5810A allele occurs most frequently on haplotypes with the SNP alleles IL1B 1423C, IL1B 1903T, IL1B 5887C, and IL1A (-889C. Genotypes at null loci failed to show evidence suggesting population stratification that might account for spurious association. Conclusion: Statistical evidence shows association between erosive hand OA and a genomic region containing the IL1B 5810 SNP in a US Caucasoid population. This supports a potential role for IL-1 in the pathogenesis of a severe phenotype of hand OA.

Takahashi, N., K. Rieneck, et al. "Elucidation of IL-1/TGF-[beta] interactions in mouse chondrocyte cell line by genome-wide gene expression." *Osteoarthritis and Cartilage In Press, Corrected Proof*
<http://www.sciencedirect.com/science/article/B6WP3-4FRJKX7-3/2/b234f77a406c40d5c85354dd29e4fbdc>

Summary Objective To elucidate the antagonism between interleukin-1 (IL-1) and transforming growth factor-[beta] (TGF-[beta]) at the gene expression level, as IL-1 and TGF-[beta] are postulated to be critical mediators of cartilage degeneration/protection in rheumatic diseases. **Methods** The H4 chondrocyte cell line was validated by comparing metalloproteinase expression profile with intact murine cartilage by reverse transcription polymerase chain reaction. Genome-wide gene expression in the H4 cells in response to IL-1 and TGF-[beta], alone and in combination, was analyzed by using oligonucleotide arrays negotiating approximately 12,000 genes. **Results** The response of cartilage and the H4 cell line to IL-1 and TGF-[beta] was comparable. Oligonucleotide array analysis demonstrated a mutual but asymmetrical antagonism as the dominant mode of interaction between IL-1 and TGF-[beta]. Cluster analysis revealed a remarkable selectivity in the mode of action exerted by TGF-[beta] on IL-1 regulated genes: antagonistic on pro-inflammatory genes whereas additive on growth regulators such as vascular endothelial growth factor (VEGF) and connective tissue growth factor (CTGF). While the former cluster underlined the protective effect of TGF-[beta], the latter underscored the adverse effect of TGF-[beta]. We further identified potentially novel classes of target genes under control of TGF-[beta] such as ras family, histones, proteasome components, and ubiquitin family, highlighting the importance of such genes in TGF signaling besides the well-characterized SMAD pathway. **Conclusions** We identified a cluster of genes as potential targets mediating the adverse effect of TGF-[beta] such as fibrosis. Transcriptional regulation of ras GTPase and ubiquitin/proteasome pathways is likely to be a novel mechanism mediating the effect of TGF-[beta] and its interaction with IL-1. These down-stream genes and pathways can be targets in future therapy.

Dolan, S., J. G. Kelly, et al. (2004). "Differential expression of central metabotropic glutamate receptor (mGluR) subtypes in a clinical model of post-surgical pain." *Pain* **110**(1-2): 369.

<http://www.sciencedirect.com/science/article/B6T0K-4CGN1WH-1/2/b18622b3de2c34f1f4044f239abba3ce>

Tissue damage during surgery can induce 'central sensitization' and the development of pain and hyperalgesia post-operatively. Metabotropic glutamate receptors (mGluRs) contribute to nociception, inflammatory pain and hyperalgesia. This study characterized the temporal expression of group I (mGluR1, mGluR5) and II (mGluR2, mGluR3) mGluRs in spinal cord following abdominal surgery. Lumbar spinal cord was recovered from adult sheep euthanased 5 h, 1, 2, 3 and 6 days after undergoing a midline laparotomy, and processed for mGluR mRNA (real-time PCR, in situ hybridization) and protein (Western blotting). mGluR5 mRNA was up-regulated 5 h and 1 day post-surgery in laminae I-II of the spinal cord dorsal horn. mGluR5 protein was increased 1 day post-surgery. A delayed induction of mGluR2 and mGluR3 mRNAs and mGluR2/3 protein occurred in spinal cord 3 days after surgery. By 6 days, mGluR2 mRNA levels had returned to normal, however, mGluR3 mRNA and mGluR2/3 protein remained elevated. No change was detected in mGluR1. These results demonstrate that mGluRs are differentially regulated following surgery and support a link between mGluR-mediated activity and post-surgical pain.

Dolan, S., J. G. Kelly, et al. (2003). "Up-regulation of metabotropic glutamate receptor subtypes 3 and 5 in spinal cord in a clinical model of persistent inflammation and hyperalgesia." *Pain* **106**(3): 501.

<http://www.sciencedirect.com/science/article/B6T0K-4B0WSF5-4/2/79fb396b25dfbe47079752c1f36f05d4>

Evidence from experimental pain research has revealed that metabotropic glutamate receptors (mGluRs) play a pivotal role in nociceptive processing, inflammatory pain and hyperalgesia. The aim of this study was to characterise expression of group I and II mGluRs in spinal cord in a model of naturally occurring persistent inflammation (sheep with unilateral lameness due to inflammation of the digital tissues of the feet, estimated to have been affected by the condition for >2 weeks) and an experimental model of acute inflammation (injection of intradermal carrageenan into lower forelimb in sheep). Animals with unilateral clinical inflammation displayed significant mechanical hyperalgesia on the affected limb. Carrageenan treatment produced significant bilateral limb mechanical hyperalgesia 3 h post-injection. Up-regulation of mGluR3 and mGluR5 mRNA was observed in ipsilateral spinal cord recovered from clinically lame animals, restricted to laminae II-V and I-II, respectively. Western blot analyses of protein extracts revealed a bilateral increase in mGluR2/3 and mGluR5. No change was detected in spinal cord mGluR1 or mGluR2 mRNA. There was no change in mGluR1,2,3,5 subtype mRNA or proteins in spinal cord recovered from animals 3 h post-carrageenan. These results demonstrate for the first time that mGluR subtypes are differentially expressed in spinal cord dorsal horn in response to persistent inflammation, and suggest that mGluR activity may be involved in mediating altered behaviours associated with clinical inflammatory pain.

Fehrenbacher, J. C., T. H. Burkey, et al. (2005). "Tumor necrosis factor [alpha] and interleukin-1[beta] stimulate the expression of cyclooxygenase II but do not alter prostaglandin E2 receptor mRNA levels in cultured dorsal root ganglia cells." *Pain* **113**(1-2): 113.

<http://www.sciencedirect.com/science/article/B6T0K-4DS6YD9-4/2/b147436a7560e4ad567d914244148afb>

Tumor necrosis factor [alpha] (TNF[alpha]) and interleukin 1[beta] (IL-1[beta]) are pro-inflammatory cytokines capable of altering the sensitivity of sensory neurons. Because sensitization elicited by IL-1[beta] and TNF[alpha] is blocked by inhibition of the inducible enzyme, cyclooxygenase-II (COX-2), we examined whether these cytokines could increase COX-2 expression in dorsal root ganglion (DRG) cultures. Treatment of cell cultures with either IL-1[beta] or TNF[alpha] increases immunoreactive COX-2, as measured by immunoblotting, in a time- and concentration-dependent manner. A 24-h pretreatment with 10 ng/ml IL-1[beta] or 50 ng/ml TNF[alpha] augmented COX-2 expression 50- and 8-fold over basal levels, respectively. Immunohistochemistry established the presence of COX-2-like immunoreactivity in both neuronal and non-neuronal cells in culture. The addition of IL-1 receptor antagonist blocked the induction of COX-2 expression by IL-1[beta], but did not alter TNF[alpha]-stimulated increases in COX-2, indicating that the mechanism of TNF[alpha] is not limited to increasing the expression of IL-1[beta]. The basal and TNF[alpha]-induced expression of COX-2 was not dependent on the presence of NGF in the growth media. IL-1[beta] and TNF[alpha] treatment for 24 h enhanced prostaglandin E2 (PGE2) production 2-4-fold, which was blocked by pretreatment with the COX-2 inhibitor, NS-398. Exposing cultures to PGE2, IL-1[beta], or TNF[alpha] for 24 h did not alter PGE2 receptor (EP) mRNA levels. These results indicate that TNF[alpha] and IL-1[beta] induce the functional expression of COX-2 but not EP receptors in DRG cells in culture and suggest that cytokine-induced sensitization of sensory neurons is secondary to prostaglandin production and not alterations in EP receptors.

Gold, M. S., S. Dastmalchi, et al. (1997). "[alpha]2-Adrenergic receptor subtypes in rat dorsal root and superior cervical ganglion neurons." *Pain* **69**(1-2): 179.

<http://www.sciencedirect.com/science/article/B6T0K-3PF5D07-T/2/06826a75f76b16857f34ccd7835d5e32>

To determine which [alpha]2-adrenergic receptor subtypes are present in primary afferent and sympathetic postganglionic neurons we have performed in situ hybridization and immunohistochemistry experiments on rat dorsal root and superior cervical ganglia. Reverse transcriptase polymerase chain reaction was used as a preliminary screen for the presence of mRNA encoding [alpha]2-adrenergic subtypes in dorsal root and superior cervical ganglia; polymerase chain reaction primers amplified distinct regions of the rat [alpha]2A- (RG20), [alpha]2B- (RNG) and [alpha]2C- (RG10) adrenergic receptor subtypes in mRNA extracted from lumbar dorsal root and superior cervical ganglia. To localize receptors to cell types in the ganglia, in situ hybridization was performed on cryosections of dorsal root and superior cervical ganglia with oligonucleotide probes designed to distinguish between mRNA encoding for [alpha]2-adrenergic receptor subtypes. Immunohistochemistry was performed with a polyclonal antibody against the [alpha]2A-adrenergic receptor subtype. Our results with reverse transcriptase polymerase chain reaction indicate that all three [alpha]2-adrenergic receptor subtypes are expressed in dorsal root and superior cervical ganglia. Data from the in situ hybridization experiments indicated that the mRNA detected with the reverse transcriptase polymerase chain reaction was present in neuronal cell bodies, except for the mRNA encoding the [alpha]2A-adrenergic receptor which was not detectable in dorsal root ganglia. The distribution of mRNA encoding [alpha]2B- and [alpha]2C-adrenergic receptor subtypes among dorsal root ganglion neurons and [alpha]2A-, [alpha]2B- and [alpha]2C-adrenergic receptor subtypes among superior cervical ganglion neurons suggests that multiple adrenergic receptor subtypes are present in a single neuron. Neuronal cell bodies in both the dorsal root and superior cervical ganglion consistently demonstrated [alpha]2A-adrenergic receptor-like immunoreactivity. The apparent co-expression of multiple [alpha]2-adrenergic receptor subtypes in dorsal root and superior cervical ganglion neurons enables a single transmitter to produce a number of effects in the same neuron;

which receptors are functionally active may vary with the presence of nerve injury, inflammation or other physiological and pathophysiological conditions.

Molliver, D. C., J. Lindsay, et al. (2005). "Overexpression of NGF or GDNF alters transcriptional plasticity evoked by inflammation." *Pain* **113**(3): 277.

<http://www.sciencedirect.com/science/article/B6T0K-4F8SV30-3/2/9704c08ac77a2068b8f837d02249f812>

Transcriptional changes evoked in nociceptive sensory neurons by inflammatory injury play a substantial role in the generation of and recovery from painful hypersensitivity. Transgenic mice overexpressing nerve growth factor (NGF) or glial cell line-derived neurotrophic factor (GDNF) in the skin possess a greatly increased number of nociceptors. Surprisingly, NGF-overexpressers display reduced hypersensitivity and recovered more rapidly in response to inflammation, suggesting a compensatory suppression of nociceptive transmission in these mice. To determine whether these transgenic mice show changes in inflammation-evoked transcriptional plasticity, we examined the expression of a panel of genes implicated in nociceptive signaling in response to injection of complete Freund's adjuvant into the hindpaw. Relative mRNA levels were quantified 1, 4 and 15 days after injection using real-time PCR. In wild type mice CFA injection elicited a reproducible pattern of altered gene expression that returned to baseline over a 2-week period. In mice overexpressing NGF or GDNF the expression patterns for several genes were substantially altered; these changes in injury-evoked patterns of gene expression suggest the existence of endogenous regulatory mechanisms that can compensate for increased nociceptive input by modulating the expression of a limited subset of genes.

Obata, K., H. Tsujino, et al. (2002). "Expression of neurotrophic factors in the dorsal root ganglion in a rat model of lumbar disc herniation." *Pain* **99**(1-2): 121.

<http://www.sciencedirect.com/science/article/B6T0K-45G037C-2/2/836218c927ff22eaf93dbc1d967e27a4>

A variety of molecules released by inflammatory reactions in the dorsal root and dorsal root ganglion (DRG) may play important roles in the pathology of neuronal abnormalities in lumbar disc herniation. In order to elucidate the pathophysiological mechanisms of painful radiculopathy, secondary to lumbar disc herniation, we evaluated pain-related behavior and the change of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) expression in the DRG and dorsal root using a rat model of lumbar disc herniation. In the nucleus pulposus (NP) group, the left L4/5 nerve roots were exposed after hemilaminectomies and autologous intervertebral discs, which were obtained from coccygeal intervertebral discs, were implanted on each of the exposed nerve roots without mechanical compression. Rats in the NP group, but not the sham-operated rats, developed mechanical allodynia on the ipsilateral hind paw for 1 day after surgery and showed a significant increase in the number of NGF-immunoreactive (IR) cells in the nerve root and DRG. NGF-IR cells in the nerve root and DRG included macrophages and Schwann cells, because these cells were labeled for NGF and ED-1 or glial fibrillary acid protein by dual immunostaining. A significant increase in the percentage of BDNF-IR neurons in the DRG was observed in the NP group at 3 days after surgery and the increase in BDNF mRNA expression was confirmed using in situ hybridization histochemistry and reverse transcription-polymerase chain reaction. We also injected NGF into the endoneurial space of the normal rat spinal nerve root and found that the NGF injection produced dose-dependent mechanical allodynia on the ipsilateral hind paw at 1 day after surgery and an increase in the percentage of BDNF-IR neurons in the DRG at 3 days after surgery compared to the group receiving saline injection. These findings suggest that in the lumbar disc herniation model, i.e. neuritis of the nerve root, increased

NGF produced by the inflammatory responses in the dorsal root and DRG tissues may affect the production of BDNF in the DRG and may play important roles in the modulation of the dorsal horn neurons. These changes in neurotrophic factors in the primary afferents may be involved in the pathophysiological mechanisms of neuropathic pain produced by lumbar disc herniation.

Okamoto, K., H. Imbe, et al. (2002). "5-HT_{2A} receptor subtype in the peripheral branch of sensory fibers is involved in the potentiation of inflammatory pain in rats." *Pain* **99**(1-2): 133.

<http://www.sciencedirect.com/science/article/B6T0K-45GKR5N-2/2/8ca5641301895ec620c0bdbcd8f66f56>

One of the major serotonin (5-HT) receptor subtypes expressed in the rat dorsal root ganglion (DRG) neurons is the 5-HT_{2A} receptor. We have previously shown that 5-HT_{2A} receptors in the peripheral sensory terminals are responsible for 5-HT-induced pain and hyperalgesia. In the present study, we characterized neurons expressing 5-HT_{2A} receptors in the rat DRG neurons by means of in situ hybridization, immunohistochemistry, reverse transcription-polymerase chain reaction (RT-PCR) and behavioral tests. In situ hybridization on consecutive sections revealed that 5-HT_{2A} receptor mRNA is colocalized with calcitonin-gene related peptide (CGRP) mRNA (100/104; 96.2%) but not with c-Ret mRNA (1/115; 0.9%). Signals for 5-HT_{2A} receptor mRNA were found in 9.4±2.2% of normal DRG (L5) neurons, most of which were small to medium in size. Four days of complete Freund's adjuvant-induced inflammation of the hindpaw doubled the incidence of 5-HT_{2A} receptor mRNA-expressing neurons to 19.3±2.8%. The level of 5-HT_{2A} receptor mRNA in DRGs of normal and various pathological conditions was then determined by RT-PCR. The level was up-regulated by peripheral inflammation, but not by axotomy or chronic constriction of the peripheral nerve. Systemic administration of 5-HT_{2A} receptor antagonist (Sarpogrelate HCl) produced analgesic effects on thermal hyperalgesia caused by peripheral inflammation, but failed to attenuate thermal hyperalgesia in chronic constriction injury model. These findings suggest that 5-HT_{2A} receptors are mainly expressed in CGRP-synthesizing small DRG neurons and may be involved in the potentiation of inflammatory pain in the periphery.

Sakurada, S., H. Watanabe, et al. (2004). "Involvement of the histaminergic system in the nociceptin-induced pain-related behaviors in the mouse spinal cord." *Pain* **112**(1-2): 171.

<http://www.sciencedirect.com/science/article/B6T0K-4DDXVX0-4/2/30fd6192ecd44e60abaf3b5fc56d0fbb>

Intrathecal (i.t.) injection of nociceptin elicited a behavioral response mainly consisting of biting and licking, which were eliminated by the i.t. co-administration of opioid receptor-like-1 (ORL-1) receptor antagonists. The behavioral response induced by nociceptin was characteristically similar to that by i.t.-administered histamine, and was attenuated by i.t. co-administration of the H₁ receptor antagonists, but not by the H₂ receptor antagonists, whereas the H₃ receptor antagonist promoted the nociceptin-induced behavior. H₁ receptor knockout (H₁R-KO) mice did not show the nociceptin-induced nociceptive behavior, which was observed in wild-type mice. Pretreatment with a histamine antiserum or a histidine decarboxylase inhibitor resulted in a significant reduction of the response to nociceptin. The previous studies showed that NK₁ receptor antagonists and a novel substance P (SP)-specific antagonist given i.t. could reduce the behavioral response to nociceptin and histamine. On the other hand, the nociceptive response induced by nociceptin, but not histamine, was completely attenuated by the i.t. co-administration of agonists for GABA_A and GABA_B receptors. In contrast, the antagonists for GABA_A and GABA_B receptors injected i.t. showed same nociceptive response with nociceptin and histamine, and their nociceptive responses were significantly blocked by the i.t. co-administration of the H₁ receptor antagonists, but not H₂ receptor antagonists or ORL-1 receptor antagonists. The present

results suggest that the activation of the ORL-1 receptor by nociceptin may induce the disinhibition of histaminergic neuron and enhance the release of histamine, which subsequently acts on the H1 receptor located on the SP-containing neurons to produce the spinal cord-mediated nociceptive response.

Shrikhande, S. V., H. Friess, et al. (2001). "NK-1 receptor gene expression is related to pain in chronic pancreatitis." *Pain* **91**(3): 209.

<http://www.sciencedirect.com/science/article/B6T0K-42MFFR7-3/2/1ac95477d2c6e973712291731b4cb57d>

Recent theories of pathogenesis of pain in chronic pancreatitis (CP) are neuroimmune interactions of intrapancreatic nerves and inflammatory cells and increase in levels of pain neurotransmitters such as substance P (SP). This study analyzed the expression and localization of neurokinin 1 receptor (NK-1R), which binds SP, and its association with pain and inflammation in CP. Pancreatic tissues from 31 patients (22 males, nine females; mean age 45.9+/-9.4 years) with CP were evaluated. Nine normal pancreases (five males, four females; mean age 42.9+/-9.5 years) served as controls. Quantitative PCR was used to determine the NK-1R mRNA expression levels and in situ hybridization and immunohistochemistry were used to localize expression sites of NK-1R mRNA and protein, respectively. We also analyzed whether an association exists between NK-1R mRNA expression and pain and inflammation. In CP samples, in situ hybridization and immunohistochemistry localized NK-1R mRNA expression and protein mainly in the nerves, ganglia, blood vessels, inflammatory cells and occasionally in fibroblasts. In patients with mild to moderate and strong intensity of pain, NK-1R mRNA levels were increased 14- and 30-fold over controls, respectively. There was a significant relationship between NK-1R mRNA levels and intensity of pain ($r=0.46$, $P=0.03$), NK-1R mRNA and the frequency of pain ($r=0.51$, $P=0.04$), and NK-1 mRNA and duration of pain ($r=0.46$, $P=0.01$) in CP patients, but not with the degree of tissue inflammation. NK-1R signaling may be involved in the pain syndrome of CP. The expression of NK-1R in inflammatory cells and blood vessels also points to an interaction of immunoreactive substance P nerves, inflammatory cells and blood vessels, and further supports the existence of a neuroimmune interaction that probably influences the pain syndrome and chronic inflammatory changes so characteristic of CP.

Solovieva, S., P. Leino-Arjas, et al. (2004). "Possible association of interleukin 1 gene locus polymorphisms with low back pain." *Pain* **109**(1-2): 8.

<http://www.sciencedirect.com/science/article/B6T0K-4BM8KGG-1/2/a846d3f760d79f30e36ada5500a0c651>

Based on a hypothesis that interleukin 1 (IL-1) activity is associated with low back pain (LBP), we investigated relationships between previously described functional IL-1 gene polymorphisms and LBP. The subjects were a subgroup of a Finnish study cohort. The IL-1[alpha](C889-T), IL-1[beta](C3954-T) and IL-1 receptor antagonist (IL-1RN)(G1812-A, G1887-C and T11100-C) polymorphisms were genotyped in 131 middle-aged men from three occupational groups (machine drivers, carpenters and office workers). A questionnaire inquired about individual and lifestyle characteristics and the occurrence of LBP, the number of days with pain and days with limitation of daily activities because of pain, and pain intensity, during the past 12 months. Lumbar disc degeneration was determined with magnetic resonance imaging. Carriers of the IL-1RNA1812 allele had an increased risk of LBP (OR 2.5, 95% CI 1.0-6.0) and carriers of this allele in combination with the IL-1[alpha]T889 or IL-1[beta]T3954 allele had a higher risk of and more days with LBP than non-carriers. Pain intensity was associated with the simultaneous carriage of the IL-1[alpha]T889 and IL-1RNA1812 alleles (OR 3.7, 95% CI 1.2-11.9). Multiple regression

analyses allowing for occupation and disc degeneration showed that carriage of the IL-1RNA1812 allele was associated with the occurrence of pain, the number of days with pain and days with limitations of daily activities. Carriage of the IL-1[beta]T3954 allele was associated with the number of days with pain. The results suggest a possible contribution of the IL-1 gene locus polymorphisms to the pathogenesis of LBP. The possibility of chance findings cannot be excluded due to the small sample size.

van de Beek, W.-J. T., B. O. Roep, et al. (2003). "Susceptibility loci for complex regional pain syndrome." Pain **103**(1-2): 93.

<http://www.sciencedirect.com/science/article/B6T0K-487F009-3/2/a4156b6570f2e60eaa9e05f44de4ebb7>

An association between HLA-DR13 and patients with complex regional pain syndrome (CRPS) who progressed towards multifocal or generalized tonic dystonia was recently reported. We now report on a new locus, centromeric in HLA-class I, which was significantly associated with a spontaneous development of CRPS, suggesting an interaction between trauma severity and genetic factors conferring CRPS susceptibility. Additionally, an association with the D6S1014 locus was found, supporting the previous finding of an association with HLA-DR13.

Zwick, M., D. C. Molliver, et al. (2003). "Transgenic mice possessing increased numbers of nociceptors do not exhibit increased behavioral sensitivity in models of inflammatory and neuropathic pain." Pain **106**(3): 491.

<http://www.sciencedirect.com/science/article/B6T0K-4B0WSF5-3/2/88fead1d7052e2f17ede7d8a43e508ef>

At least two classes of nociceptors can be distinguished based on their growth factor requirements: glial cell-line derived neurotrophic factor (GDNF)- and nerve growth factor (NGF)-dependent primary afferent neurons. Based on numerous anatomical and biochemical differences, GDNF- and NGF-dependent neurons have been proposed to be involved in the development of different types of persistent pain. To examine this hypothesis we used two lines of transgenic mice that contained a supernormal number of either NGF- or GDNF-dependent neurons (referred to as NGF-OE and GDNF-OE mice, respectively). These mice were tested in a model of inflammatory pain (induced by injection of complete Freund's adjuvant) and neuropathic pain (using a spinal nerve ligation protocol). Contrary to expectations, neither line of transgenic mice became more hyperalgesic following induction of persistent pain. In fact, NGF-OE mice recovered more rapidly and became hypoalgesic despite extensive paw swelling in the inflammatory pain model. In the neuropathic pain model, only wildtype mice became hyperalgesic. Real-time PCR analysis showed that the NGF-OE and GDNF-OE mice exhibited changes in neuronal-specific mRNAs in the dorsal root ganglia but not the spinal cord dorsal horn. These results indicate that increasing the number of nociceptors results in potent compensatory mechanisms that may begin with changes in the sensory neurons themselves.

Hatabu, T., Y. Matsumoto, et al. (2002). "The expression system of biologically active canine interleukin-8 in *Leishmania* promastigotes." Parasitology International **51**(1): 63.

<http://www.sciencedirect.com/science/article/B6TB7-44V20RR-1/2/c4240505ef2cd6894961cc16d41d6bca>

It has been reported that *Leishmania* promastigotes have ability to express foreign genes on drug selectable plasmids. To investigate further abilities of the recently described expression vector, P6.5, in the transfection of *Leishmania* organisms (Chen D-Q, Kolli BK, Yadava N et al. Episomal expression of specific sense and antisense mRNAs in *Leishmania amazonensis*: modulation of gp63 levels in promastigotes and their infection of macrophages in vitro. *Infect Immun* 2000;68:80-86), the constructed expression vector, which contains canine interleukin-8 (cIL-8) coding cDNA, was introduced by electroporation to promastigotes of four species of the genus *Leishmania*: *Leishmania amazonensis*, *L. equatorensis*, *L. donovani* and *L. infantum*. Extrachromosomal DNAs and total RNAs from the transfected promastigotes were subjected to polymerase chain reaction (PCR) and reverse transcriptase-PCR, respectively, using cIL-8 gene specific primers, and a predicted product of 330 bp was detected. Western blot analysis using a mouse monoclonal antibody raised against cIL-8 demonstrated the successful expression of cIL-8 in the transfectants and culture supernatants. Culture supernatants of the transfected *L. amazonensis* and *L. equatorensis* promastigotes showed a high chemotactic activity to both dog and mouse polymorphonuclear leukocytes. These results indicate that *Leishmania* promastigotes transfected with the expression vector P6.5 containing cIL-8 cDNA are capable of producing biologically active cIL-8. The *Leishmania* expression system using the P6.5 vector might be a useful alternative for the production of biologically active recombinant cytokines.

Robinson, B. S., P. T. Monis, et al. (2004). "Detection and significance of the potentially pathogenic amoeboflagellate *Naegleria italica* in Australia." Parasitology International **53**(1): 23.

<http://www.sciencedirect.com/science/article/B6TB7-4BRJH86-3/2/d1a8c12abb30a5a026376dcc12dfd80d>

Thermophilic amoeboflagellates in the genus *Naegleria* include both virulent and benign species. One of the less studied species, *N. italica*, has not been detected in the environment since the first reports from Italy in the 1980s; its virulence is known only from infection of laboratory mice. Two recent strains from recreational water in Western Australia (AWQC NG960, NG961) were tentatively identified as *N. italica* from the characteristic mobilities of seven isozymes. Sequences of the 5.8S rRNA gene and its flanking ITS aligned with a 380+bp length of the published sequence for *N. italica* with 98% identity. Differences from the type strain were confined to ITS2. Shorter alignments (*Naegleria* species, corresponding to conserved regions of the 5.8S gene and ITS. Unlike the European type strain of *N. italica*, the Australian isolates failed to infect laboratory mice intranasally, confirming that infectivity of this species is variable and often lower than in *N. fowleri*.

Safitri, I., A. Jalloh, et al. (2003). "Sequence diversity in the amino-terminal region of the malaria-vaccine candidate serine repeat antigen in natural *Plasmodium falciparum* populations." Parasitology International **52**(2): 117.

<http://www.sciencedirect.com/science/article/B6TB7-47T8SF7-1/2/cf103bd509b085de7321772035787d14>

The amino-terminal region of the serine repeat antigen (SERA) of *Plasmodium falciparum* is a

major malaria-vaccine candidate. Variation in this molecule is essentially dimorphic and alleles may be grouped into the types FCR3, K1 and Honduras1. The Honduras1-type is thought to be the product of homologous recombination between FCR3 and K1 alleles. Here we have examined patterns of sequence diversity in exon II of SERA gene, which encodes most of the amino-terminal region of the antigen, in wild *P. falciparum* isolates from Indonesia (n=60), Myanmar (n=10) and Thailand (n=14). Among the Indonesian isolates the FCR-3 type predominated (56/60), twenty of which we characterized as novel alleles. A new K1-type allele was also found. In Myanmar, however, all isolates displayed K1-type SERA sequences, which included one new allele. The Honduras1-type was not detected in both countries. In contrast, the 14 isolates from Thailand displayed all three allelic types, with one new Honduras1-type and three new K1-type alleles. On examining the global distribution of SERA alleles by combining previously published sequence data with our results, the FCR3-type alleles predominated in Indonesia, Brazil, and Solomon Islands, but were not found in wild isolates from Myanmar and Africa. Brazil was the only area where K1-type alleles were not found. The distribution of Honduras1-type alleles seems to be mostly restricted to parasite populations from Vietnam, Thailand and Africa. In the allelic families FCR3 and K1, most diversity resulted from variation in sequence and number of octamer repeat units and of allotypes encoding the stretch of serine residues. Sequence analysis indicated that both insertions and deletions of repetitive motifs (creating variation within dimorphic allelic families) and homologous recombination between alleles belonging to different allelic families (creating Honduras1-type alleles) play a role in generating new SERA alleles. Since repeat motifs in the amino-terminal region of SERA contain epitopes recognized by parasite-inhibitory antibodies, sequence variation in exon II may represent one of the parasite's immune-evasion strategies.

Sakihama, N., T. Matsuo, et al. (2004). "Relative frequencies of polymorphisms of variation in Block 2 repeats and 5' recombinant types of *Plasmodium falciparum* msp1 alleles." Parasitology International **53**(1): 59.

<http://www.sciencedirect.com/science/article/B6TB7-4BFVSG0-2/2/0f823b2de04a4a987069bb9efc318a66>

The mechanisms producing the genetic polymorphism at *Plasmodium falciparum* merozoite surface antigen-1 locus (pfmsp1) include the insertion and deletion of the different type of dimorphic Block 2 9-nucleotide repeat units as well as the intragenic recombination. To study relative occurrence frequencies of these two distinct mechanisms, we have developed a sensitive PCR strategy to identify both 5' recombinant types and the number of Block 2 repeats from the same sample. This method can specifically detect the target 5' recombinant type (Blocks 2-6) at the sensitivity of 1-4 copies of the pfmsp1. Applying the new method to field isolates from the Solomon Islands enabled us to identify six different 5' recombinant types and variation in Block 2 repeat number in three of them, thus distinguishing 10 different alleles. Distribution of these alleles in local three villages in the study area suggests that frequencies of variation in the number of Block 2 9-bp repeats and recombination events within Blocks 2-6 are mutually independent and the frequency of repeat variation is relatively high as compared to that of recombination events at the pfmsp1 locus in *P. falciparum* populations from the Solomon Islands.

Stunzenas, V., J. R. Cryan, et al. (2004). "Comparison of rDNA sequences from colchicine treated and untreated sporocysts of *Phyllodistomum folium* and *Bucephalus polymorphus* (Digenea)." Parasitology International **53**(3): 223.

<http://www.sciencedirect.com/science/article/B6TB7-4CCCP61-3/2/5765c3cfe9f0562838df3c77f36b5883>

The most frequently used antimitotic agent in cytogenetic studies is colchicine. We investigated whether the initial treatment of trematodes for karyological analysis with colchicine would have mutagenic or degradational effect on rDNA sequences. *Dreissena polymorpha* is the intermediate host of *Phyllodistomum folium* and *Bucephalus polymorphus*, and the sporocyst stage of these trematode species develop, respectively, in the gills and gonads of this mussel. Sporocysts of *P. folium* and *B. polymorphus* were obtained from *D. polymorpha* collected from waterbodies in Belarus and in Lithuania. 5.8S and 28S rDNA genes, ITS1 and ITS2 of *P. folium* and *B. polymorphus* were sequenced and compared, and no nucleotide sequence differences between colchicine treated and untreated trematodes were found. Based on these results, we conclude that colchicine treatment for 3-5 h has no mutagenic or degradational effect on rDNA sequences. During the course of this investigation, two genetically different *P. folium* samples were noted in Belarus.

Parkinsonism & Related Disorders (2)

Kobayashi, T., M. Wang, et al. (2000). "Exonic deletion mutations of the Parkin gene among sporadic patients with Parkinson's disease." Parkinsonism & Related Disorders 6(3): 129.

<http://www.sciencedirect.com/science/article/B6TB9-408C8T4-1/2/ae49d0d843ad1da21da203d87fb14284>

Exonic deletions of the Parkin gene are common in the autosomal recessive form of juvenile parkinsonism. Here we report Parkin gene mutations among apparently sporadic Parkinson's disease (PD) patients. We screened 200 patients with PD (103 women and 97 men). The age of onset was 54.2 $\pm$ 10.3 years (mean $\pm$ S.D.). Four out of the 200 patients had homozygous exonic deletions in the Parkin gene. The clinical features of these four patients were essentially the same as those of idiopathic PD. The age of onset was consistently younger (33, 38, 47 and 48 years, respectively). On medication, all of them were at Hoehn and Yahr stage II or III even after 12-16 years from the onset of the disease. Thus 2% of apparently sporadic PD patients in Japan have homozygous Parkin gene mutations. This positive rate was 6.3% among the patients with the age of onset below 50. Our study suggests that the prevalence of the carrier state of Parkin gene may be more than that we expected. Our study warrants further studies on Parkin gene mutations in apparently sporadic PD patients.

Shyu, W.-C., S.-Z. Lin, et al. "Early-onset Parkinson's disease in a Chinese population: 99mTc-TRODAT-1 SPECT, Parkin gene analysis and clinical study." Parkinsonism & Related Disorders **In Press, Corrected Proof** <http://www.sciencedirect.com/science/article/B6TB9-4FSX6FW-1/2/2e28a71b88dc6429543c33ffecd0d226>

Early Onset Parkinson's Disease (EOPD) is characterized by selective degeneration of nigrostriatal dopaminergic neurons and a marked response to levodopa. However, at present, few methods are available as diagnostic tools for EOPD except for 18F-DOPA PET. In addition, little is known about the correlation between clinical severity, neuroimaging grading and genetic susceptibility. In the present study, 99mTc-TRODAT-1 SPECT and brain MRI were used to identify 30 cases of non-familial EOPD from a Chinese cohort of 230. All 30 PD patients had an age of onset of less than 55 years (mean age at onset, 41.5[plus-or-minus sign]9.3 years). Each of the 30 EOPD cases was sub-classified into one of five stages based on the 99mTc-TRODAT-1

SPECT findings. In the early stages of PD (stages 1 and 2), a lower uptake of 99mTc-TRODAT-1 in the putamen was found, while uptake in the caudate nucleus was normal. In the latter stages (stages 3, 4, 5), 24 patients revealed a diffuse and uniform loss of 99mTc-TRODAT-1 uptake in the putamen and the caudate nucleus. Further, in conventional genetic studies of the 30 patients, six novel mutations were found in the Parkin gene, and these included five heterozygous point mutations (C441R, Q311H, V258M, C212G, and S193I) and one homozygous deletion (exon 10-12). Known polymorphisms (Ser167Asn, Val380Leu) were also found in a number of patients. However, gene dosage analysis did not reveal any compound heterozygous mutations in these 30 patients using quantitative duplex PCR. This is the first study to examine EOPD patients of Chinese ethnic background (not exhibiting a definite familial trait), to offer a complete genetic analysis of the Parkin gene, and to correlate clinical stages of the disease with dopamine re-uptake.

Pathology - Research and Practice (3)

Haas, C. J., T. Wagner, et al. (2005). "Combined application of RT-PCR and immunohistochemistry on paraffin embedded sentinel lymph nodes of prostate cancer patients." Pathology - Research and Practice **200**(11-12): 763.

<http://www.sciencedirect.com/science/article/B7GW5-4F2V54W-3/2/cc806a08fbf5ad77d52d2c803fb01967>

The detection of tumor cells in the sentinel lymph node (SLN) is of great importance for the prognosis of cancer patients. At present, immunohistochemistry and RT-PCR for tumor marker expression are the most sensitive techniques available for this analysis. However, so far, most RT-PCR-based analyses of SLNs have been performed on fresh material, excluding a direct comparison with the (immuno)histologic results. In our view, this does not entirely aid routine diagnosis. We established an efficient method for RNA extraction and RT-PCR from paraffin sections of SLNs from prostate cancer patients and compared the results with the (immuno)histologic data of adjacent sections. Amplifiable RNA was obtained from 133 SLNs of 68 prostate cancer patients. Correlation of PSA-specific RT-PCR with (immuno)histologic findings showed a positive and negative predictive value of 83% and 100%, respectively, for the prostate cancer patients investigated. Four of 12 patients with biochemical relapse, but without (immuno)histologically detectable tumor cells were RT-PCR-positive for PSA. We found that single sections of paraffin-embedded SLNs are suitable for routinely performed RT-PCR. Combined with (immuno)histology, PSA-specific RT-PCR is a revealing supplementary technique for the detection of tumor cells in SLNs of prostate cancer patients.

Kim, G. Y., J.-H. Park, et al. (2004). "Absence of amplification of HER-2/neu (c-erbB-2) gene in Ewing's Sarcoma: a real-time polymerase chain reaction method." Pathology - Research and Practice **200**(10): 663.

<http://www.sciencedirect.com/science/article/B7GW5-4DGMRP0-2/2/c1046fbf44359515ce78dcdf3bd4e59d>

Amplification and overexpression of the HER-2/neu (c-erbB-2) oncogene have been observed in many cancers and are associated with a poor prognosis particularly in breast cancer. The human

epidermal growth factor (HER)-2 receptor has recently been implicated in Ewing's sarcoma tumor cell line growth and chemosensitivity. The present study evaluates the amplification of HER-2/neu gene in paraffin sections from 42 cases of Ewing's sarcoma by a real-time quantitative polymerase reaction method using LightCycler system (Roche diagnostics, GmbH Mannheim, Germany). The relative copy number of HER-2/neu versus [beta]-globin was calculated at the crossing point. The mean calculated copy number in these cases of Ewing's sarcoma and normal controls was 26.43 and 26.93, respectively. The p value was 0.215 (p). Our results demonstrated an absence of HER-2/neu oncogene amplification in Ewing's sarcomas, and we suggest that HER-2/neu is not a biologically or therapeutically important pathway in Ewing's sarcoma.

Park, H.-R., W. Won Jung, et al. (2004). "Molecular analysis of p53, MDM2 and H-ras genes in low-grade central osteosarcoma." Pathology - Research and Practice **200**(6): 439.

<http://www.sciencedirect.com/science/article/B7GW5-4CPBG0F-3/2/579e43a776880fe6b368219970962244>

Low-grade central osteosarcoma is an uncommon form that is characterized by a long premorbid history, and is compatible with prolonged survival after treatment. However, molecular abnormalities are rare in low-grade central osteosarcomas, whereas p53 mutations occur in approximately 20% of conventional high-grade osteosarcomas. In this study, 21 cases of low-grade central osteosarcoma were analyzed for mutations of the p53 gene, amplification of the MDM2 gene, and mutations of the H-ras gene using formalin-fixed, paraffin-embedded materials. We also examined the expression of p53, MDM2, and p21WAF1 protein immunohistochemically and assessed the proliferation activities using the monoclonal antibody MIB-1. One case (4.7%) showed strong p53 immunoreactivity, whereas p53 gene mutations were not detected at all. Seven cases (33.3%) showed immunoreactivity for MDM2 protein. As for gene alterations, MDM2 amplification was found in four cases (19.0%). p21WAF1 expression was detected in 12 cases (57.1%). MIB-1-LI showed very low levels in all the cases and no significant correlation with p53 or MDM2 immuno-reactivity. None of the tumors showed H-ras mutations. In conclusion, the number of p53 gene alterations in low-grade central osteosarcomas is lower than that in conventional high-grade osteosarcomas. MDM2 alterations and p21WAF1 expression might be involved in the tumorigenesis of low-grade central osteosarcomas.

Pediatr. Res. (18)

Adachi, S., S. Zelenin, et al. (2004). "Cellular Response to Renal Hypoxia Is Different in Adolescent and Infant Rats." Pediatr. Res. **55**(3): 485-491.

<http://www.pedresearch.org/cgi/content/abstract/55/3/485>

Immature renal tubules are more tolerant to ischemia than mature renal tubules. Here we compared the developmental pattern for some cellular responses evoked by hypoxia and reoxygenation in renal proximal tubules from 10- and 40-day-old rats. Redistribution of Na⁺-K⁺-ATPase from the plasma membrane was studied by confocal microscopy techniques in primary cultured renal proximal tubular cells. The developmental expression of Na⁺-K⁺-ATPase, {micro}-calpain and heme oxygenase-1 was measured by RT-PCR techniques in rat renal cortex. In response to hypoxia Na⁺-K⁺-ATPase redistribution from the plasma membrane was almost 2-fold

increased in cells isolated from mature kidneys compared with cells isolated from immature kidneys. Reoxygenation resulted in a complete reestablishment of Na⁺-K⁺-ATPase in the plasma membrane in the immature but not in the mature cells. The dissociation of Na⁺-K⁺-ATPase from the plasma membrane was associated with a reduced activity and a reduced expression of Na⁺-K⁺-ATPase in the mature but not in the immature tubular cells. The expression of {micro}-calpain, a factor shown to induce ischemic injury to proximal tubular cells, was significantly lower in the immature compared with the mature kidney, whereas the expression of heme oxygenase-1, a factor shown to protect from renal ischemic injury, was significantly higher in the immature kidney. The results help to explain the increased tolerance of the immature kidney to injury caused by ischemia and reperfusion.

Agren, J., S. Zelenin, et al. (2003). "Transepidermal Water Loss in Developing Rats: Role of Aquaporins in the Immature Skin." *Pediatr. Res.* **53**(4): 558-565.

<http://www.pedresearch.org/cgi/content/abstract/53/4/558>

In the extremely preterm infant, high transepidermal water loss (TEWL) can result in severe dehydration. TEWL has been attributed to the structural properties of the epidermis but might also be influenced by mechanisms that facilitate water transport. To investigate whether aquaporins (AQP) may be involved in the extreme losses of water through immature skin, we examined the presence and cellular distributions of AQP-1 and AQP-3 in embryonic and adult rat skin by immunohistochemistry. The expression of AQP mRNA in skin was analyzed with the use of semiquantitative reverse transcription-PCR. In rat pups of different embryonic (E) and postnatal (P) ages (days), TEWL and skin hydration were measured. AQP-1 was detected in dermal capillaries, and AQP-3 was abundant in basal epidermal layers. Both AQP displayed several times higher expression in embryonic than in adult skin. TEWL was highest at embryonic day 18 (E18) (133 {+/-} 18 g/m²h) and lower at E20 (25 {+/-} 1 g/m²h) and P4 (9 {+/-} 2 g/m²h). Skin hydration measured as skin electrical capacitance paralleled TEWL, being highest in fetal skin (794 {+/-} 15 pF at E18) and decreasing to 109 {+/-} 11 pF at E20 and to 0 {+/-} 0 pF at P4. We conclude that, as in infants, water loss through the skin of rats decreases markedly with maturation during the perinatal period. The expression and cellular localization of the AQP are such that they might influence skin hydration and water transport and contribute to the high losses of water through the immature skin.

Berner, R., M. Ruess, et al. (2002). "Polymorphisms in the Cell Wall-Spanning Domain of the C Protein {beta}-Antigen in Clinical Streptococcus agalactiae Isolates Are Caused by Genetic Instability of Repeating DNA Sequences." *Pediatr. Res.* **51**(1): 106-111.

<http://www.pedresearch.org/cgi/content/abstract/51/1/106>

The C protein {alpha}- and {beta}-antigens are immunodominant components of the surface of Streptococcus agalactiae, the most frequent cause of neonatal sepsis. Both proteins are thought to contribute significantly to virulence of S. agalactiae. They are mainly expressed by serotypes Ia, Ib, and II. The C protein {beta}-antigen (C{beta}-protein) binds to the Fc portion of human IgA and seems to be of importance in bacterial resistance to mucosal immune defense mechanisms. In this study, PCR analysis of S. agalactiae isolates obtained from 189 neonates and 112 pregnant women revealed the presence of the C{beta}-protein gene in 19% and 22% of the isolates, respectively. Size polymorphisms of the PCR products within the gene region encoding the cell wall-spanning domain indicated a high degree of genetic variability. Thirteen different variants of the amplified region were differentiated among the 60 C{beta}-protein-positive isolates by sequence analysis. In all variants, the polymorphisms were caused by insertions and deletions of repetitive DNA elements that did not alter the open reading frame. Comparison of the C{beta}-

protein gene polymorphisms showed a significantly higher rate of isolates carrying deletions >50 bp in serotype Ib than in serotype II isolates ($p = 0.001$); this was also true for neonatal isolates analyzed separately ($p = 0.01$). Neonatal isolates carried a higher rate of large deletions when compared with maternal isolates; this difference, however, did not reach statistical significance ($p = 0.08$). We hypothesize that polymorphisms in the cell wall-spanning domain of the C β -protein are of functional relevance with regard to maternofetal transmission of the pathogen.

Carter, B. W. J., L. G. Chicoine, et al. (2004). "L-Lysine Decreases Nitric Oxide Production and Increases Vascular Resistance in Lungs Isolated from Lipopolysaccharide-Treated Neonatal Pigs." Pediatr. Res. **55**(6): 979-987.

<http://www.pedresearch.org/cgi/content/abstract/55/6/979>

Nitric oxide (NO) production may depend on the uptake of L-arginine (L-arg), the substrate for NO synthase in inflammatory lung diseases. The cellular transport of L-arg occurs via the cationic amino acid transporters (CAT), and L-lysine (L-lys) competitively inhibits CAT. Neonatal pigs were treated with lipopolysaccharide (LPS) or vehicle for 4 h. LPS increased exhaled NO (exNO; 0.026 ± 0.003 to 0.046 ± 0.003 nmol $\cdot$ kg $^{-1}$ $\cdot$ min $^{-1}$; $p < 0.005$) and decreased mean systemic arterial blood pressure (89 ± 4 to 67 ± 4 mm Hg; $p < 0.05$), whereas vehicle did not affect exNO or mean systemic arterial blood pressure. The lungs were then isolated and perfused; exNO was greater in lungs from LPS-treated animals (0.08 ± 0.01 nmol/kg/min) than in lungs from vehicle-treated animals (0.05 ± 0.01 nmol $\cdot$ kg $^{-1}$ $\cdot$ min $^{-1}$; $p < 0.05$). The addition of L-arg (0.3 mM) significantly ($p < 0.05$) increased exNO production in both groups of lungs (mean increase 0.04 ± 0.01 nmol $\cdot$ kg $^{-1}$ $\cdot$ min $^{-1}$ LPS-treated lungs, $p < 0.05$; mean increase 0.02 ± 0.01 nmol $\cdot$ kg $^{-1}$ $\cdot$ min $^{-1}$ vehicle-treated lungs); however, L-arg decreased pulmonary vascular resistance (PVR) only in LPS-treated lungs (mean decrease 0.03 ± 0.01 mm Hg $\cdot$ ml $^{-1}$ $\cdot$ kg $^{-1}$ $\cdot$ min $^{-1}$, $p < 0.05$). L-lys caused a dose-dependent decrease in exNO production and a dose-dependent increase in PVR in LPS-treated lungs. L-lys decreased exNO only at 30 mM and had no effect on PVR in vehicle-treated lungs. In four lungs each from vehicle- and LPS-treated animals, reverse transcriptase-PCR demonstrated CAT-2 mRNA only in LPS-treated animals. These results suggest that the increased NO production in the lungs from LPS-treated animals depends on the uptake of vascular L-arg.

Castanet, M., L. Leenhardt, et al. (2005). "Thyroid Hemiagenesis Is a Rare Variant of Thyroid Dysgenesis with a Familial Component but without Pax8 Mutations in a Cohort of 22 Cases." Pediatr. Res.: 01.PDR.0000161409.04177.36.

<http://www.pedresearch.org/cgi/content/abstract/01.PDR.0000161409.04177.36v1>

Thyroid hemiagenesis is a rare form of thyroid dysgenesis of which some familial cases have been reported, including one associated with a heterozygous mutation in the Pax8 gene. However, the physiopathology remains not well known. The objectives of this study were 1) to describe the clinical features, 2) to look for familial clustering, and 3) to search for Pax8 mutations in a relatively large cohort of affected patients. A family history of thyroid dysgenesis was found in nine patients (40%), whose affected relatives had ectopic thyroid ($n = 4$), athyreosis ($n = 1$), thyroid hemiagenesis ($n = 2$), or thyroglossal duct cysts ($n = 2$). Screening for Pax8 mutations identified abnormal migration profiles by SSCP analysis in 3 patients, but direct sequencing did not show coding region mutations in any of the 22 patients. In conclusion, this study provides the first evidence that thyroid hemiagenesis can occur as a familial disorder associated with any form of thyroid dysgenesis. This finding supports both a common underlying mechanism to the various abnormalities in thyroid development and a role for genetic factors; however, our results from

Pax8 analysis suggest that this gene may not be a key factor.

Chou, I. C., C.-T. Peng, et al. (2003). "Association Analysis of γ 2 Subunit of γ -Aminobutyric Acid Type A Receptor Polymorphisms with Febrile Seizures." *Pediatr. Res.* **54**(1): 26-29.

<http://www.pedresearch.org/cgi/content/abstract/54/1/26>

An alternation of γ -aminobutyric acid (GABA)-ergic neurotransmission has been implicated as an etiologic factor in epileptogenesis. Missense mutations in the GABRG2 gene, which encodes the γ 2 subunit of central nervous GABAA receptors, have recently been described in one family with childhood absence epilepsy and febrile seizures (FSs). FSs represent the majority of childhood seizures and have a genetic predisposition. It is not known, however, whether polymorphisms in those genes involved in familial epilepsies also contribute to the pathogenesis of FSs. By performing an association study, we used single-nucleotide polymorphisms to investigate the distribution of genotypes of GABRG2 in patients with FSs. A total of 104 children with FSs and 83 normal control subjects were included in the study. PCR was used to identify the C/T and A/G polymorphisms of the GABRG2 gene on chromosome 5q33. Genotypes and allelic frequencies for the GABRG2 gene polymorphisms in both groups were compared. The GABRG2 (nucleotide position 3145 in intron G[>A]) gene in both groups was not significantly different. In contrast, the number of individuals with the GABRG2 (SNP211037)-C/C genotype in patients with FSs was significantly greater compared with that in healthy control subjects ($p = 0.017$), and the GABRG2 (SNP211037)-C allele frequency in patients with FSs was significantly higher than that in healthy control subjects ($p = 0.009$). The odds ratio for developing FSs in individuals with the GABRG2 (SNP211037)-C/C genotype was 2.56 compared with individuals with the GABRG2 (SNP211037)-T/T genotype. These data suggest that the GABRG2 gene might be one of the susceptibility factors for FSs.

Claud, E. C., D. A. N. Li, et al. (2002). "Platelet-Activating Factor Regulates Chloride Transport in Colonic Epithelial Cell Monolayers." *Pediatr. Res.* **52**(2): 155-162.

<http://www.pedresearch.org/cgi/content/abstract/52/2/155>

Platelet-activating factor (PAF) has been implicated in the pathogenesis of gastrointestinal diseases such as necrotizing enterocolitis, Crohn's disease, and ulcerative colitis. However, neither the physiologic role of PAF in the intestine, nor the mechanisms by which PAF participates in the pathogenesis of disease are well understood. The aim of the present study was to determine the direct effect of PAF on intestinal epithelial cell ion transport, and to delineate the mechanisms of regulation. Ion transport was evaluated by measuring short circuit current (Isc) in HT29-CL19A cell monolayers using Ussing chambers. PAF receptor polarity was assessed using domain-selective biotinylation followed by immunoprecipitation and streptavidin blotting of intact epithelial monolayers. PAF (1-200 μ M) stimulated Isc that followed the direction of a Cl⁻ gradient and was specifically inhibited by the Cl⁻ channel blockers glybenclamide, 2,2' iminodibenzoic acid and 4,4' diisothiocyano stilbene-2, 2' disulfonic acid, but was unaffected by the inhibition of prostaglandin synthesis with indomethacin. Stimulated Isc was only detected after apical addition of PAF, correlating with the results of biotinylation experiments indicating an exclusive apical polarity of the PAF receptor. PAF receptor antagonists CV6209 and octylonium bromide abolished PAF-stimulated Isc. Thus, mucosal acting PAF directly and specifically stimulates ion transport via activation of an apical Cl⁻ channel in intestinal epithelial cell monolayers independent of prostaglandin biosynthesis.

Item, C., B. P. Hagerty, et al. (2002). "Mutations at the Galactose-1-P-Uridyltransferase Gene in Infants with a Positive Galactosemia Newborn Screening Test." Pediatr. Res. **51**(4): 511-516.

<http://www.pedresearch.org/cgi/content/abstract/51/4/511>

Newborn screening for galactosemia yields a high number of false-positive results. Confirmatory DNA testing for unknown galactosemia mutations on the initial positive sample using novel techniques of mutation detection tends to reduce the recall rate. The potential benefits of confirmatory DNA testing, however, could be offset by the detection of a high percentage of galactosemia carriers, Duarte/galactosemia compound heterozygotes, and infants with benign sequence changes in the galactose-1-phosphate uridyltransferase (GALT) gene among infants with a positive biochemical screening test. Our aim was to determine the frequency and allelic distribution of all sequence changes in the GALT gene in 110 newborns with a positive total galactose screening test among 43,688 Austrian newborns screened consecutively. We found that only 20 of the 110 probands carried at least one known or novel candidate galactosemia mutation (one galactosemia homozygote, 7 Duarte/galactosemia compounds, 12 carriers) as judged by denaturing gradient gel electrophoresis and cleavage fragment length polymorphism analysis. Four novel galactosemia candidate mutations (Q9H, A46fsdelCAGCT, M129T, L342I) were identified. Sixty-seven probands had no detectable sequence changes and 23 carried only the benign Duarte or Los Angeles variant alleles or silent mutations. We conclude that a rapid and automatable confirmation test for unknown GALT mutations, e.g. on a high-density oligonucleotide array basis, has the potential to lower the recall rate of galactosemia screening in our population by about five-fold from 0.25 to 0.046%. Further research, however, will be required before the development of such a test can be advocated.

Jimenez, D. F. and A. F. Tarantal (2003). "Quantitative Analysis of Male Fetal DNA in Maternal Serum of Gravid Rhesus Monkeys (*Macaca mulatta*). " Pediatr. Res. **53**(1): 18-23.

<http://www.pedresearch.org/cgi/content/abstract/53/1/18>

The isolation of human fetal DNA from the maternal circulation has provided a source of fetal material for prenatal diagnosis. The objective of this study was to investigate whether a similar pattern could be observed in the maternal circulation of male-bearing gravid rhesus monkeys. A real-time PCR TaqMan system for the rhesus Y-chromosome sex determining region was used to determine fetal sex and to quantify fetal DNA concentrations. Results in 14 healthy pregnancies indicated that fetal male DNA could be routinely detected in maternal serum by 50 d of gestation (late first trimester; term 165 {+/-} 10 d). Fetal DNA concentrations increased with advancing gestation, reaching a mean of 341 genome equivalents/mL of serum (range 11-1570 copies/mL) in the last trimester of gestation, similar to findings in humans. The fetal DNA concentration corresponded to 2.7% of the total maternal serum DNA in the third trimester. Similar to findings in humans, male fetal DNA sequences were not detected postpartum (through 4 wk postpartum) or in animals with a previous history of delivering male offspring. These data indicate that fetal male DNA is present in the maternal circulation of gravid rhesus monkeys comparable to findings in humans and further support the use of this nonhuman primate species as a model to investigate fetomaternal cell trafficking and microchimerism.

Molloy, E. J., et al. (2005). "Granulocyte Colony-Stimulating Factor and Granulocyte-Macrophage Colony-Stimulating Factor Have Differential Effects on Neonatal and Adult Neutrophil Survival and Function." Pediatr. Res.: 01.PDR.0000156500.13600.B5.

<http://www.pedresearch.org/cgi/content/abstract/01.PDR.0000156500.13600.B5v1>

Neutropenia is a common sequela of neonatal sepsis. Recent clinical trials have shown the beneficial effects of colony-stimulating factors (CSFs) on outcome in this group, but the exact mechanism remains unknown. Neonates and mothers who were at high-risk for infection were recruited for cord blood sampling in a university tertiary referral maternity hospital. Neonatal and adult neutrophils were evaluated for their ability to combat bacterial infection by examining their functional activity (CD11b and reactive oxygen intermediates) and their persistence at inflammatory sites (apoptosis). The mechanism for altered apoptotic responses was assessed by caspase activation assays, X chromosome-linked inhibitor of apoptosis protein expression, and cytosolic cytochrome c release. Although granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) significantly delayed neutrophil apoptosis in normal adults, only G-CSF had a similar effect in normal neonates. Neutrophils from neonates who are at high risk for infection are unresponsive to the antiapoptotic effects of G-CSF or GM-CSF, unlike maternal neutrophils, which have delayed apoptosis in response to GM-CSF. However, CD11b expression and reactive oxygen intermediate production were significantly increased in normal neonatal neutrophils that were incubated with GM-CSF versus controls but not G-CSF or lipopolysaccharide. Decreased cytosolic cytochrome c release and caspases 3 and 9 activity are associated with the CSF-mediated delay in apoptosis in adults but not in newborns. The antiapoptotic X chromosome-linked inhibitor of apoptosis protein is up-regulated in neonates compared with adults and may mediate their differential spontaneous apoptosis. These results have important implications for the use of CSFs in neonatal sepsis, as responses differ from those seen in adults. Further delineation of neonatal neutrophil responses to CSFs may improve their therapeutic potential.

Ni, Y.-H., M.-H. Chang, et al. (2004). "Longitudinal Study on Mutation Profiles of Core Promoter and Precore Regions of the Hepatitis B Virus Genome in Children." Pediatr. Res. **56**(3): 396-399.

<http://www.pedresearch.org/cgi/content/abstract/56/3/396>

Precore nucleotide 1896 and core promoter mutations may account for hepatitis B e antigen (HBeAg) seroconversion in chronic hepatitis B virus (HBV) infection, yet the mutational profiles of the core promoter are largely unknown in children. An age-matched, case-control study enrolled 110 chronic HBV-infected children, including 55 HBeAg seroconverters and 55 nonseroconverters. Precore and core promoter genes of HBV were sequenced and the serum viral genomes were genotyped from three serial serum samples of the seroconverters and from one serum sample of the nonseroconverters. Higher frequency of A1775G and G1799C mutation rates and lower frequency of A1752G mutation rate were found in the seroconverters. Precore 1896 mutation appeared more in seroconverters than in nonseroconverters (45.5% versus 10.9%; $p < 0.001$). 1762 + 1764 mutation rates were not different between the seroconverters (9.1%) and the nonseroconverters (5.5%). Genotype B was the major type. Genotype C was associated with core promoter 1762 + 1764 mutations in the seroconverter group ($p = 0.023$). The conclusions of this study include the following: 1) mutations of core promoter at nucleotide position 1752, 1775, and 1799 have significant correlations with HBeAg seroconversion; 2) core promoter 1762 + 1764 mutations play a minimal role in HBeAg seroconversion; 3) precore 1896 mutant accounted for half of childhood HBeAg seroconversion; 4) genotype C is associated with 1762 + 1764 mutations during the process of HBeAg seroconversion.

Piedimonte, G., R. G. Hegele, et al. (2004). "Persistent Airway Inflammation after Resolution of Respiratory Syncytial Virus Infection in Rats." Pediatr. Res. **55**(4): 657-665.

<http://www.pedresearch.org/cgi/content/abstract/55/4/657>

Neurogenic inflammation is markedly potentiated in airways that are infected with respiratory syncytial virus (RSV). Aims of this study were to determine whether this potentiation persists after the virus is cleared, investigate the mechanism of postviral potentiation, and define whether prophylaxis with a MAb against the RSV fusion protein (palivizumab) prevents this effect. Thirty days after inoculation, no evidence of active RSV infection was found in the airway epithelium by plaque assay or immunostaining and no viral nucleic sequences were detected by PCR, yet capsaicin-induced plasma extravasation in the airways that were infected 30 d earlier with RSV was still significantly larger compared with pathogen-free controls. Substance P content in lung tissues and capsaicin-induced release of this peptide from sensory nerves were significantly increased at 30 d. The administration of palivizumab 24 h before virus inoculation prevented the development of abnormal neurogenic inflammatory responses. Our data suggest that the airways remain abnormally susceptible to the proinflammatory effects of sensory nerves after RSV infection is cleared, as a result of changes in sensory innervation, and that this abnormality can be prevented by passive prophylaxis against RSV.

Sato, U., S. Kitanaka, et al. (2005). "Functional Characterization of LMX1B Mutations Associated with Nail-Patella Syndrome." Pediatr. Res.: 01.PDR.0000157674.63621.2C.

<http://www.pedresearch.org/cgi/content/abstract/01.PDR.0000157674.63621.2Cv1>

Nail-patella syndrome (NPS) is an autosomal dominant disease characterized by dysplastic nails, absent or hypoplastic patellae, elbow dysplasia, and nephropathy. Recently, it was shown that NPS is the result of heterozygous mutations in the LIM-homeodomain gene, LMX1B. Subsequently, many mutations of the LMX1B gene have been reported in NPS patients. However, functional analyses of the mutant proteins have been performed in only a few mutations. Furthermore, the mechanisms of dominant inheritance in humans have not been established. In the present study, we analyzed the LMX1B gene in three Japanese patients with NPS and identified two novel mutations, 6 nucleotide deletion ({Delta}246N 247Q) and V242L. These two mutations are located in the homeodomain of LMX1B. Functional analyses of the LMX1B mutants revealed that these mutants had diminished transcriptional activity and had lost DNA binding ability. Furthermore, we demonstrated that each mutant did not manifest a dominant-negative effect on the transcriptional activity of wild-type LMX1B. These results suggested that NPS is caused by loss-of-function mutations of LMX1B, and haploinsufficiency of LMX1B should be the predominant pathogenesis of NPS in humans.

Van Woerkom, R., K. D. A. Beharry, et al. (2004). "Influence of Morphine and Naloxone on Endothelin and its Receptors in Newborn Piglet Brain Vascular Endothelial Cells: Clinical Implications in Neonatal Care." Pediatr. Res. **55**(1): 147-151.

<http://www.pedresearch.org/cgi/content/abstract/55/1/147>

The present study examines the hypothesis that morphine exposure alters newborn brain vascular endothelial cell production of endothelin (ET)-1, as well as the mRNA expression of its receptors. Newborn piglet vascular endothelial cells were treated with morphine (100 ng/mL media), naloxone (100 ng/mL media), or drug-free media (control) for 6, 24, 48, and 96 h. Media was analyzed for ET-1 and big ET-1 levels and the cells were assessed for ETA and ETB receptor mRNA expression. Morphine exposure progressively increased ET-1 production from 6 to 96 h with concurrent reductions in big ET-1 levels starting at 24 h to almost undetectable levels by 96 h. Whereas ETA receptor mRNA expression increased 2-fold at 6 h and 4-fold at 96 h, ETB receptor mRNA expression remained unchanged. Naloxone exposure caused significant decreases in ET-1 levels, whereas an opposite effect was noted in big ET-1 levels, which increased from 6 through 96 h. Naloxone caused a progressive decrease in ETA receptor mRNA

expression at 6 h through 96 h and a 2-fold increase in ETB receptor mRNA expression at 48 and 96 h. Increased ET-1 and its receptors in response to morphine may suggest altered cerebrovascular perfusion and brain metabolism in the immature piglet brain.

Williams, S. J., I. C. McMillen, et al. (2002). "Placental Restriction Increases the Expression of Prostaglandin Endoperoxide G/H Synthase-2 and EP2 mRNA in the Fetal Sheep Kidney during Late Gestation." Pediatr. Res. **52**(6): 879-885.

<http://www.pedresearch.org/cgi/content/abstract/52/6/879>

There is evidence that fetal growth restriction is associated with impaired nephrogenesis and reduced numbers of mature nephrons at birth. It has been proposed that such impairment of renal growth may contribute to increased blood pressure in later life. Although prostaglandins (PG) play a key role in kidney development, it is unknown whether a poor fetal substrate supply alters the synthesis or actions of PG within the fetal kidney. Using real-time reverse transcriptase PCR, we have measured the effect of chronic placental restriction (PR) on the renal expression of PG endoperoxide G/H synthase-2 (PGHS-2), PGE2 receptors EP2 and EP4, and renin mRNA in the sheep fetus in late gestation. Restriction of placental growth reduced fetal body weight (PR: 3.2 $\pm$ 0.2 kg, control: 4.8 $\pm$ 0.2 kg) and total kidney weight (PR: 19.7 $\pm$ 1.8 g, control: 25.1 $\pm$ 1.3 g). Mean fetal arterial PO₂ was reduced by PR (PR: 15.03 $\pm$ 0.67 mm Hg, control: 21.3 $\pm$ 0.87 mm Hg). Renal PGHS-2 mRNA was increased in the PR group (PR: 2.26 $\pm$ 0.38, control: 1.20 $\pm$ 0.31) and was inversely related to mean fetal arterial PO₂ in the PR and control groups [PGHS-2: -0.17 (PO₂) + 4.69, r^2 = 0.26]. PR also increased renal EP2 (PR: 1.57 $\pm$ 0.24, control: 0.82 $\pm$ 0.13) but not EP4 mRNA. Renin mRNA was directly related to renal EP2 [renin = 0.37 (EP2) + 0.97, r^2 = 0.29] and EP4, [renin = 0.75 (EP4) + 0.44, r^2 = 0.38] mRNA expression. Thus, the restriction of placental growth and associated chronic hypoxemia appear to increase the renal capacity to synthesize and respond to PG, which may play an important role in maintaining renin mRNA expression in the growth-restricted fetus.

Williams, S. J., D. M. Olson, et al. (2004). "Cortisol Infusion Decreases Renin, But Not PGHS-2, EP2, or EP4 mRNA Expression in the Kidney of the Fetal Sheep at Days 109-116." Pediatr. Res. **55**(4): 637-644.

<http://www.pedresearch.org/cgi/content/abstract/55/4/637>

Renal prostaglandins (PG), renin, and cortisol are necessary for normal kidney development and function during fetal life. We examined the effects of cortisol infusion before completion of nephrogenesis (d 109-116 gestation; 2.0-3.0 mg hydrocortisone succinate/24 h) on the renal mRNA expression of PGHS-2, the PGE2 receptors, EP2 and EP4, and renin in fetal sheep. Cortisol infusion raised plasma cortisol levels to 42.8 $\pm$ 6.0 nmol/L compared with saline infusion levels of 1.5 $\pm$ 0.5 nmol/L (p < 0.001), but had no effect on fetal body weight, proportional kidney mass, or blood gases. Cortisol decreased significantly the relative expression of renin mRNA (saline: 0.93 $\pm$ 0.06 units; cortisol: 0.32 $\pm$ 0.03 units, p < 0.05), however it had no effect upon the expression of PGHS-2, EP2, or EP4 mRNA in fetal sheep kidney. Although there is substantial evidence that PGE2 acting through either the EP2 or EP4 receptor stimulates renin synthesis in the adult kidney, our results have demonstrated that before the completion of nephrogenesis, cortisol down-regulation of renin mRNA expression is independent of any change in the expression of PGHS-2, EP2, or EP4 mRNA expression. During nephrogenesis, the insensitivity of PGHS-2, EP2, and EP4 expression to down-regulation by cortisol may permit continued PG regulation of renal development and urine formation.

Wright, R. O., H. Hu, et al. (2003). "Apolipoprotein E Genotype Predicts 24-Month Bayley Scales Infant Development Score." *Pediatr. Res.* **54**(6): 819-825.

<http://www.pedresearch.org/cgi/content/abstract/54/6/819>

Apolipoprotein E (APOE) regulates cholesterol and fatty acid metabolism, and may mediate synaptogenesis during neurodevelopment. To our knowledge, the effects of APOE4 isoforms on infant development have not been studied. This study was nested within a cohort of mother-infant pairs living in and around Mexico City. A multiple linear regression model was constructed using the 24-mo Mental Development Index (MDI) of the Bayley Scale as the primary outcome and infant APOE genotype as the primary risk factor of interest. Regression models stratified on APOE genotype were constructed to explore effect modification. Of 311 subjects, 53 (17%) carried at least one copy of the APOE4 allele. Mean (SD) MDI scores among carriers with at least one copy of APOE4 were 94.1 (14.3) and among E3/E2 carriers were 91.2 (14.0). After adjustment for covariates, APOE4 carrier status was associated with a 4.4 point (95% confidence interval: 0.1-8.7; $p = 0.04$) higher 24-mo MDI. In the stratified regression models, the negative effects for umbilical cord blood lead level on 24-mo MDI score was approximately 4-fold greater among APOE3/APOE2 carriers than among APOE4 carriers. These results suggest that subjects with the E4 isoform of APOE may have advantages over those with the E2 or E3 isoforms with respect to early life neuronal/brain development.

Zylla, D., Y. Li, et al. (2003). "CCR5 Expression and {beta}-Chemokine Production During Placental Neonatal Monocyte Differentiation." *Pediatr. Res.* **53**(5): 853-858.

<http://www.pedresearch.org/cgi/content/abstract/53/5/853>

The stage of maturation of monocytes affects their susceptibility to HIV infection. The {beta}-chemokines and their receptor CCR5 play a crucial role in inflammatory reactions and HIV infection. We therefore examined the correlation between the expression of CCR5 and {beta}-chemokine production and the susceptibility to HIV infection during cord monocyte (CM) differentiation into macrophages. CM and CM-derived macrophages (CMDM) were examined for {beta}-chemokine and CCR5 expression. The susceptibility of the CM cultured in vitro at different time points to HIV infection was also determined. Although the levels of CCR5 mRNA expression in freshly isolated CM are comparable to those in CMDM, CM had significantly lower levels of CCR5 protein on the cell surface than CMDM did. Steady increase of CCR5 protein expression on the cell surface was observed during CM differentiation into macrophages. The CCR5 expression correlated with the increased susceptibility to HIV infection by CMDM. Although there was no significant difference in endogenous {beta}-chemokine production between CM and CMDM, HIV infection of CMDM significantly enhanced production of macrophage inflammatory protein-1{alpha} and -1{beta}. CCR5 receptor plays a critical role in HIV infection of neonatal blood monocyte/macrophages.

Pediatric Neurology (6)

Peng, C.-T., I. C. Chou, et al. (2004). "Association of the nicotinic receptor beta 2 subunit and febrile

seizures." Pediatric Neurology **30**(3): 186.

<http://www.sciencedirect.com/science/article/B6TBD-4BG3SP9-3/2/d47d1ff33d6968d3236828593bc32240>

The nicotinic acetylcholine receptors are members of a superfamily of ligand-gated ion channels that mediate fast signal transmission at synapses. Mutations in neuronal nicotinic acetylcholine receptor beta 2 subunit have been associated with autosomal dominant nocturnal frontal lobe epilepsies. A major challenge is to establish whether the monogenic epilepsy gene also contributes to the common epilepsies. Because febrile seizures represent the majority of childhood seizures, and a genetic predisposition, we investigated the possibility that the nicotinic acetylcholine receptor beta 2 subunit might be involved in the etiology of febrile seizures. Children were divided into two groups: those with febrile seizures (group 1; N = 104) and control patients (group 2; N = 83). Polymerase chain reaction was used to identify the G/C and T/C polymorphisms of the nicotinic acetylcholine receptor beta 2 subunit gene, which is mapped on chromosome 1. Genotypes and allelic frequencies for nicotinic acetylcholine receptor beta 2 subunit gene polymorphisms in both groups were compared. The results indicated that genotypes and allelic frequencies in both groups were not significantly different. These data suggest that nicotinic acetylcholine receptor beta 2 subunit polymorphisms are not a useful marker for prediction of the susceptibility to febrile seizures.

Skyllouriotis, M. L., M. Marx, et al. (1997). "Myoadenylate deaminase deficiency, hypertrophic cardiomyopathy and gigantism syndrome." Pediatric Neurology **17**(1): 61.

<http://www.sciencedirect.com/science/article/B6TBD-3RJGTYN-9/2/9a98fdff93aa4ab4c9b77bf6a1575ed5>

We report a 20-year-old man with gigantism syndrome, hypertrophic cardiomyopathy, muscle weakness, exercise intolerance, and severe psychomotor retardation since childhood. Histochemical and biochemical analysis of skeletal muscle biopsy revealed myoadenylate deaminase deficiency; molecular genetic analysis confirmed the diagnosis of primary (inherited) myoadenylate deaminase deficiency. Plasma, urine, and muscle carnitine concentrations were reduced. -Carnitine treatment led to gradual improvement in exercise tolerance and cognitive performance; plasma and tissue carnitine levels returned to normal, and echocardiographic evidence of left ventricular hypertrophy disappeared. The combination of inherited myoadenylate deaminase deficiency, gigantism syndrome and carnitine deficiency has not previously been described.

Suzuki, Y., T. Wada, et al. (1998). "Phenotypic variability in a family with a mitochondrial DNA T8993C mutation." Pediatric Neurology **19**(4): 283.

<http://www.sciencedirect.com/science/article/B6TBD-3V8RF5B-S/2/8d32c58e2ecac6afa716357672613c1d>

Two patients are described in a family with a mitochondrial DNA T8993C point mutation. Patient 1, the proband, was a 4-year-old male, and his clinical features were consistent with those of Leigh syndrome, including lactic acidosis, motor development delay, and symmetric basal ganglia lesions on magnetic resonance imaging (MRI). His mental development was delayed mildly, but he has not demonstrated neurologic deterioration. Patient 2 was his maternal aunt. She developed her first neurologic sign at 18 months of age, thereafter her development ceased and regressed. She had lost her head control and become bedridden by 4 years of age and died at 20

years of age, demonstrating a more severe clinical course than that of Patient 1. Analysis of mitochondrial DNA from peripheral leukocytes of Patient 1 revealed a T8993C mutation of 99%. Patient 2 was demonstrated to have the same mutation at high abundance (99%) in the frozen myocardium and in the formaldehyde preserved spinal cord, with only 18% mutant mitochondrial DNA present in the formaldehyde preserved sciatic nerve. The mother of Patient 1, who was phenotypically normal (sister of Patient 2), had 35% mutant mitochondrial DNA in peripheral leukocytes. The authors' findings suggest that T8993C phenotypes are highly variable and that the proportion of the mutant mitochondrial DNA may vary among tissues and not correlate well with clinical severity.

Teach, S. J., H. L. Wallace, et al. (1999). "Human herpesviruses types 6 and 7 and febrile seizures." Pediatric Neurology **21**(4): 699.

<http://www.sciencedirect.com/science/article/B6TBD-3XRP7X4-2/2/582497dfd5639848b25d952edabfb610>

The frequency was studied with which human herpesviruses types 6 and 7 (HHV-6 and HHV-7) occur in the cerebrospinal fluid (CSF) of patients with febrile seizures and matched control patients. CSF samples were prospectively collected from a case series of patients with febrile seizures and from age-, sex-, and race-matched control patients without febrile seizures, all of whom were evaluated in the emergency department of an urban, tertiary care, pediatric medical center. Using polymerase chain reaction, the samples were examined for the presence of viral DNA from HHV-6, HHV-7, herpes simplex viruses types 1 and 2 (HSV-1 and HSV-2), and cytomegalovirus (CMV). CSF from a subset of both groups was also examined for RNA from enteroviruses. During the 7-month, 2-week collection period, a total of 174 patients were evaluated for fever and seizures. Of these, 23 (13.2%) met the study criteria. Their mean age was 1.4 +/- 0.7 years. Sixteen (70%) of the 23 were male. The 23 patients were matched to 21 control subjects. None of the samples from the patients or control subjects had polymerase chain reaction evidence of HHV-6, HHV-7, HSV-1, or HSV-2. All samples from the patients were negative for CMV. One control subject was positive for CMV. The 10 patients and seven control subjects tested for enteroviral RNA were negative. Neither HHV-6 nor HHV-7 appears to be present in the CSF of patients with febrile seizures. What role, if any, they have in the pathogenesis of febrile seizures merits further study.

Tsai, F.-J., I. C. Chou, et al. (2002). "Interleukin-4 intron 3 polymorphism is not related to susceptibility to febrile seizures." Pediatric Neurology **27**(4): 271.

<http://www.sciencedirect.com/science/article/B6TBD-475WPXC-4/2/2ea5e0618eeefe7aed2fc502455b8382>

Interleukin-4 (IL-4) is a cytokine with anti-inflammatory properties. This study was undertaken to investigate whether IL-4 intron 3 gene polymorphism could be used as markers of susceptibility to febrile seizures and epilepsy of children. Children were divided into three groups: group 1, febrile seizures (n = 51); group 2, epilepsy (n = 43); and group 3, normal control group (n = 83). Polymorphisms for IL-4 intron 3 were detected by polymerase chain reaction. Genotypes and allelic frequencies for IL-4 intron 3 gene polymorphism in three groups were compared. We found that proportions of different IL-4 intron 3 polymorphisms in three groups were nonsignificantly different. Proportions of RP1 homozygote/heterozygote/RP2 homozygote for IL-4 intron 3 in three groups were as follows: group 1, 56.9/41.2/1.9%; group 2, 62.8/32.6/4.6%; and group 3, 62.7/33.7/3.6%. The proportion of RP1/RP2 for IL-4 intron 3 in three groups were as follows: group 1, 77.5/22.5%, group 2, 79.1/20.9%, and group 3, 79.5/20.5%. We concluded that the association of IL-4 polymorphisms with febrile seizures and epilepsy of children does not exist. IL-

4 intron 3 polymorphism is not a useful marker for prediction of the susceptibility of febrile seizure and epilepsy of children.

Wisniewski, K. E., N. Zhong, et al. (1998). "Studies of atypical JNCL suggest overlapping with other NCL forms." *Pediatric Neurology* **18**(1): 36.

<http://www.sciencedirect.com/science/article/B6TBD-3SJMwj6-4/2/9a4855a9052a8e0470d5541bf8783a33>

In the United States, juvenile neuronal ceroid-lipofuscinosis (JNCL) is the most common form of NCL. This study analyzed 191 cases, diagnosed on the basis of age-at-onset, clinical symptomatology, and pathologic findings. Twenty percent (40/191) of these cases from 24/120 families manifested atypical clinical symptomatology and/or pathologic findings (typical revealed fingerprints and atypical revealed mixed inclusions, or only curvilinear or granular profiles) and, therefore, represent variant forms of JNCL. Those patients in the study with typical JNCL were a uniform group of cases, whereas the atypical were heterogeneous and were divided into 8 subgroups based on the clinicopathologic findings. Forty-three families were analyzed (27 typical, 16 atypical) for the common 1.02 kb deletion and several pedigrees for novel mutations. In typical JNCL the common 1.02 kb deletion in both alleles (homozygous) were observed in 23/27, and only 1 allele (heterozygous) was exhibited in 4/27 families. In atypical JNCL families, 5/16 were heterozygous for the common 1.02 kb deletion. None of the remaining 11/16 families had the common 1.02 kb deletion in either allele, but in 9/11 cases the palmitoyl-protein thioesterase (PPT) levels were deficient. In cases where the mutation in CLN3 gene has not been identified, several possibilities may exist. The phenotype may be caused by a yet undefined mutation in CLN3 or may be due to overlapping with other forms of NCL.

Pediatrics (4)

Ronchetti, R., M. P. Villa, et al. (2002). "Nasal Cellularity in 183 Unselected Schoolchildren Aged 9 to 11 Years." *Pediatrics* **110**(6): 1137-1142.

<http://pediatrics.aappublications.org/cgi/content/abstract/110/6/1137>

Objective. Although rhinitis is extremely frequent in children, methods for assessing the severity of nasal inflammation produce results with wide variability and hence weak clinical significance. We designed this epidemiologic investigation to define the clinical usefulness of assessing nasal cellularity in children. **Methods.** We studied 183 of 203 eligible unselected schoolchildren who were aged 9 to 11 years and whose parents gave informed consent and completed a questionnaire on the history of atopic and respiratory symptoms. In all children, nasal swabs were obtained from both nostrils and eluted in saline and slides were prepared from cytospin preparations for staining and white cell counts. Children also underwent determination of nasal volume, skin prick tests with 7 common local allergens, flow volume curves, and nitric oxide measurement in expired air. Blood samples were drawn for the measurement of total immunoglobulin E, eosinophil percentage, and detection of Chlamydia pneumoniae antibodies. C pneumoniae DNA was also sought in eluates from nasal swabs. The percentage, standard deviations, and percentiles of the various nasal white cell populations were determined. **Results.** No correlation of the percentage of these cells was found with the history of allergies or

respiratory disease or with functional or laboratory finding. Repeat nasal swabs obtained 1 month after the initial examination in 31 children (20 with neutrophils higher and 11 lower than 14%) in 77.4% of the cases confirmed the previous (high or normal) result. Twelve of the 16 eligible children with persistently high nasal neutrophil counts completed a 15-day cycle of intranasal flunisolide therapy (200 {micro}g twice a day). Therapy significantly reduced nasal neutrophil percentage and increased nasal volume. Conclusions. Increased nasal neutrophils, although related neither to the clinical history nor to laboratory variables, are a common important finding in children. A 15-day cycle of intranasal flunisolide is sufficient to restore normal nasal neutrophilia.

Wine, E., S. S. Reif, et al. (2004). "Pediatric Crohn's Disease and Growth Retardation: The Role of Genotype, Phenotype, and Disease Severity." Pediatrics **114**(5): 1281-1286.

<http://pediatrics.aappublications.org/cgi/content/abstract/114/5/1281>

Background. Delayed growth is a well-established feature of pediatric Crohn's disease. Several factors have been shown to affect growth, including disease location, severity, and treatment. The recently discovered NOD2 gene has been correlated to ileal location of Crohn's disease and subsequently could affect growth through the resulting phenotype or as an independent risk factor. The aim of our study was to determine if growth retardation is affected by genotype independently of disease location or severity. Methods. Genotyping for 3 NOD2 single-nucleotide polymorphisms was performed in 93 patients with detailed growth records. Parameters including disease location, disease severity, and NOD 2 genotype and their effect on z scores for height and weight at disease onset and during follow-up were analyzed. Results. NOD2 mutations were correlated with ileal location but not with disease severity or growth retardation. Ileal involvement was significantly associated with height retardation at disease onset and the lowest z score during follow-up. Use of steroids affected weight but not height. Regression models for growth variables revealed that the strongest association with impaired growth is with disease severity (weight- and height-failure odds ratios: 6.17 and 4.52, respectively). Conclusions. Severity of disease is correlated with growth failure for both height and weight. Location of disease is a weaker predictor of disordered growth and is correlated with growth retardation but not growth failure. The NOD2 genotype was not correlated with growth retardation or growth failure.

Winther, B., F. G. Hayden, et al. (2002). "Viral Respiratory Infection in Schoolchildren: Effects on Middle Ear Pressure." Pediatrics **109**(5): 826-832.

<http://pediatrics.aappublications.org/cgi/content/abstract/109/5/826>

Objective. To evaluate the effect of uncomplicated viral respiratory infections (colds) on middle ear pressure in healthy school-aged children. Methods. Children (ages 2-12) with normal tympanograms before onset of illness had bilateral tympanometry daily except weekends for 2 weeks after the onset of a cold. Nasopharyngeal secretion obtained at onset of illness was cultured for bacterial pathogens of otitis media using selective agars and tested for rhinovirus, coronavirus, respiratory syncytial virus, influenza A and B, and parainfluenza 1-3 by reverse transcriptase polymerase chain reaction technology. Tympanometry was designated as abnormal with peak pressure of ≤ -100 daPa or ≥ 50 daPa and/or a compliance peak of <0.2 cm³. Results. Eighty-six colds were studied, 82 in schoolchildren (5-12 years old) and 4 in 2- to 3-year-olds. Abnormal negative middle ear pressure occurred at least once during the 2 weeks after onset in 57 (66%) of the 86 colds. Tympanometry was abnormal in the first week after onset in 50 (88%) of the 57 colds and was abnormal on a single day in 17 (30%) of the 57. The middle ear pressure abnormalities were intermittent and shifted from one ear to the other ear from day to day. Reverse transcriptase polymerase chain reaction was positive for a respiratory virus in 56 (65%) of the 86 illnesses. Rhinovirus was found in 48% and respiratory syncytial virus in 14%.

Pathogenic bacteria (*Streptococcus pneumoniae*, *Haemophilus influenzae*, or *Moraxella catarrhalis*) were detected in nasopharyngeal secretion in 29 (34%) of the 86 colds; the bacteria were in high titer ($\geq 10^3$ cfu/mL) in 26 of the 29 positive specimens. None developed illness that required a visit to a physician. Age, detection of a respiratory virus, and presence of bacterial pathogen in the nasopharyngeal secretion had a negligible effect on the occurrence of abnormal tympanometry. Occurrence of negative middle ear pressure in winter-spring colds was significantly greater than in fall colds for unexplained reasons. Conclusions. Transient negative middle ear pressure occurred in two thirds of uncomplicated colds in healthy children. This negative pressure, which may facilitate secondary viral or bacterial otitis media, seems to result from viral infection of the nasopharynx and distal tube causing bilateral eustachian tube dysfunction. Tympanometry provides an objective measure of the potential beneficial effects of investigational treatments on the risk of eustachian tube dysfunction/otitis media.

Yasuda, A., H. Kimura, et al. (2003). "Evaluation of Cytomegalovirus Infections Transmitted via Breast Milk in Preterm Infants With a Real-Time Polymerase Chain Reaction Assay." *Pediatrics* **111**(6): 1333-1336.

<http://pediatrics.aappublications.org/cgi/content/abstract/111/6/1333>

Objective. Preterm infants are at greater risk of symptomatic cytomegalovirus (CMV) infection than term infants. Breast milk is the main source of perinatal CMV infections. This study evaluated the kinetics of CMV load in breast milk and the rate of postnatal CMV transmission via breast milk from mothers to their preterm infants. **Methods.** This was a prospective study of 30 mothers and their 43 preterm infants. The infants either had a gestational age of <34 weeks or weighed <2000 g at birth. Breast milk, serum, and urine samples were collected every 2 weeks until discharge, and screened for CMV infection using a real-time PCR assay. Most of the breast milk had been preserved at -20{degrees}C before feeding to the preterm infants. **Results.** Twenty-four mothers (24 of 30, 80%), who had 34 preterm infants, were CMV immunoglobulin G positive. Twenty-one (87.5%) of the 24 seropositive mothers, who had 30 preterm infants, had detectable CMV deoxyribonucleic acid (DNA) in breast milk during the study period. Most breast milk became positive for CMV DNA 2 weeks after delivery. Viral DNA copy numbers increased until they peaked at 4 to 6 weeks. Afterward, the CMV DNA copy numbers decreased. Of the 30 infants who were fed CMV DNA-positive breast milk, CMV infection was confirmed in 3 infants. However, they had no clinical symptoms of CMV infection. **Conclusions.** Despite the high rate of CMV DNA in breast milk, symptomatic infections in the preterm infants did not occur. These results might be associated with the method of breast milk preservation and the population we studied. CMV infections transmitted via breast milk feeding did not have much impact on preterm infants in our institutes.

Peptides (22)

Ali, N. S., M. Keller-Wood, et al. (2005). "Ontogenetic changes in the extra-pituitary expression of pro-opiomelanocortin in the developing ovine fetus." *Peptides* **26**(2): 401.

<http://www.sciencedirect.com/science/article/B6T0M-4DTKP91-1/2/ce50832021808cd8eb26fe8a6d7252a2>

Previous studies performed in this laboratory have demonstrated that the fetal lung contains immunoreactive adrenocorticotropin (irACTH), and that the lung both clears and secretes irACTH under basal and stimulated conditions. Furthermore, we have demonstrated that the irACTH in fetal lung is accounted for by proopiomelanocortin (POMC), and that there is an evidence of post-translational processing that is distinct from the pattern of processing typical of the anterior pituitary. The present study was designed to test the hypothesis that POMC is synthesized in the fetal lung, and that there is decreased synthesis in the late-gestation ovine fetal lung. Lungs were collected from fetal sheep at 80, 100, 120, 130, and 145 days gestation (n = 4/group; term = 147 days). POMC mRNA was measured using reverse transcription and real-time polymerase chain reaction with probe and primers designed in this laboratory. The greatest abundance of POMC mRNA was in the 80-days fetal sheep, and the relative abundance decreased as a function of fetal gestational age. POMC protein was measured using immunoblot analysis in lungs from 80, 120, and 145-days fetal sheep. The pattern of POMC protein abundance was consistent with that of the mRNA (highest at 80 days, lowest at 145 days). The POMC immunoblot revealed specific staining of a peptide with molecular weight of 27 kDa and another peptide with a molecular weight slightly higher than that of native POMC (32 kDa). For comparison, we measured POMC mRNA in skeletal muscle and small intestine. We found POMC expression in both fetal tissues, but no statistically significant ontogenetic pattern of expression. We conclude that POMC is synthesized in the ovine fetal lung, and that the rate of synthesis decreases as the fetus matures in utero. We speculate that the decreasing abundance of POMC mRNA and protein reflects decreased release of POMC and POMC-related peptides into the fetal bloodstream.

Borowsky, B., M. W. Walker, et al. (1998). "Cloning and characterization of the human galanin GALR2 receptor." Peptides **19**(10): 1771.

<http://www.sciencedirect.com/science/article/B6T0M-3VJ38VT-M/2/ffa7064c746ce9f40935efeb9efb729c>

We present the molecular cloning and characterization of the human galanin receptor, hGALR2. hGALR2 shares 85%, 39%, and 57% amino acid identities to rGALR2, hGALR1, and hGALR3, respectively. hGALR2, along with rGALR2, can be distinguished from the other cloned galanin receptors by a tolerance for both N-terminal extension and C-terminal deletion of galanin, as well as by a primary signaling mechanism involving phosphatidyl inositol hydrolysis and calcium mobilization. By RT-PCR, GALR2 mRNA was abundant in human hippocampus, hypothalamus, heart, kidney, liver, and small intestine. A weak GALR2 mRNA signal was detected in human retina, and no signal was detected in cerebral cortex, lung, spleen, stomach, or pituitary.

Chauhan, M., C. S. Thota, et al. (2003). "Evidence for the existence of a new receptor for CGRP, which is not CRLR." Peptides **24**(1): 65.

<http://www.sciencedirect.com/science/article/B6T0M-47HC77K-1/2/6d6a557a6fb52817aa948508306d78c6>

Receptors for calcitonin gene-related peptide (CGRP), a neuropeptide known to be the most potent vasodilator, are abundantly expressed in cerebellum. A monoclonal antibody to cerebellar CGRP receptors specifically detects a 66 kDa protein from rat cerebellum and other rat and human tissues, but not from SK-N-MC cells which express calcitonin receptor-like receptor (CRLR), a recently described component of CGRP receptors. In contrast, mRNA expression for CRLR was abundant in SK-N-MC cells, but it was undetectable in rat cerebellum. Furthermore, the antibody could not detect any immunoreactive protein in HEK 293 cells transiently transfected with CRLR and receptor activity-modifying protein 1 (RAMP1) indicating the possible existence of another CGRP receptor, which does not involve CRLR. Due to the absence of biochemical or

structural data on the existence of a CGRP2 receptor and the new data provided in this paper, we suggest to identify the two CGRP receptors as CGRP-A and CGRP-B.

Costain, W. J. and R. K. Mishra (2003). "PLG regulates hnRNP-L expression in the rat striatum and pre-frontal cortex: identification by ddPCR." Peptides **24**(1): 137.

<http://www.sciencedirect.com/science/article/B6T0M-47HC77K-3/2/ec2ba7742b400c43c98204f82b069457>

Central dopaminergic systems are implicated in schizophrenia and Parkinson's disease, and are known to be modulated by the endogenous tripeptide Pro-Leu-Gly-NH₂ (PLG or MIF-1, melanocyte-stimulating hormone release inhibiting factor-1). Differential display polymerase chain reaction (ddPCR) was utilized to identify genes that are regulated by protracted PLG treatment (20 mg/kg, i.p. for 28 days) in male Sprague-Dawley rats. A total of 2400 genes were screened and 3 down-regulated bands were identified in the PLG-treated samples. Sequencing analysis revealed a total of six unique cDNA species. One fragment possessed a high degree of homology with *Mus musculus* hnRNP-L (protein L) mRNA (GenBank #AB009392) (termed PRG1: PLG regulated gene 1). Elongation of the PRG1 cDNA, by RACE-PCR, provided an 835 bp sequence with 95% homology to AB009392 over a 743 bp span. Open reading frame analysis provided a putative amino acid sequence consistent with the identity of PRG1 as rat hnRNP-L. Northern hybridization experiments with PRG1 revealed a 2.3 kb mRNA species that was decreased by 65% in the PLG-treated tissue. Western blot analysis revealed significantly decreased hnRNP-L levels in the striatum and pre-frontal cortex (but not the nucleus accumbens) by 71 and 61%, respectively of PLG-treated animals. The identification of altered expression of hnRNP-L following PLG treatment provides insight into the long-term effects of PLG and may provide insight into its molecular mechanism of action.

Ellacott, K. L. J., E. L. Donald, et al. (2005). "Characterization of a naturally-occurring polymorphism in the UHR-1 gene encoding the putative rat prolactin-releasing peptide receptor." Peptides **26**(4): 675.

<http://www.sciencedirect.com/science/article/B6T0M-4F7H5FX-1/2/19c9003ce8942903b6eddbee2feea400>

The rat orphan receptor UHR-1 and its human orthologue, GPR10, were first isolated in 1995. The ligand for this receptor, prolactin-releasing peptide (PrRP), was identified in 1998 by reverse pharmacology and has subsequently been implicated in a number of physiological processes. As supported by its localization and regulation in the hypothalamus and brainstem, we have shown previously that PrRP is involved in energy homeostasis. Here we describe a naturally occurring polymorphism in the UHR-1 gene that results in an ATG to ATA change at the putative translational initiation site. The presence of the polymorphism abolished the binding of ¹²⁵I PrRP in rat brain slices but did not affect the ability of PrRP to reduce fast-induced food intake. Together this data suggest that PrRP may be exerting its feeding effects through a receptor other than UHR-1.

Fay, M. J., J. Du, et al. (1996). "Evidence for expression of vasopressin V2 receptor mRNA in human lung." Peptides **17**(3): 477.

<http://www.sciencedirect.com/science/article/B6T0M-3VYTDF7-K/2/570e1518649e3db3cd26422d5bbc8cf6>

Studies using fetal sheep, goats, and guinea pigs indicate that vasopressin may play a role in preparing the fetal lung for the transition from a uterine to an air-breathing environment by slowing lung liquid secretion. The mechanism of vasopressin action is believed to occur through V2 receptors with subsequent activation of amiloride-sensitive sodium channels. However, the presence of the V2 receptor in human lung has not yet been documented. In the present study, expression of the vasopressin V2 receptor in fetal and adult human lung was examined using reverse transcription-polymerase chain reaction (RT-PCR), Northern blot analysis, and DNA sequencing. Using RT-PCR and primer pairs specific for the human V2 receptor, PCR products of the predicted sizes of 512 and 862 bp were obtained from adult human lung. DNA sequencing of the cloned PCR products revealed exact identity with the published sequence for the V2 receptor. Northern blot analysis revealed the expression of a ~ 1.9 kb mRNA in adult human lung as well as in kidney, but not in fetal human lung at 22-24 weeks of gestation. However, using the more sensitive RT-PCR assay the 862-bp product was successfully amplified from human fetal lung, although the data indicate the mRNA for this receptor is expressed in lower levels than in adult human lung or kidney. Using RT-PCR and primers specific for the rat V2 receptor, a PCR product of the predicted size of 461 bp was amplified from adult rat lung and kidney, despite an earlier report that this receptor mRNA is absent from the lung of this species. The role for the V2 receptor in adult human lung is unknown at this time, but, as in the human kidney and lungs of fetal sheep, goats, and guinea pigs, this receptor may play a role in fluid balance.

Guerrero, F. D. (1997). "Transcriptional Expression of a Putative Tachykinin-like Peptide Receptor Gene From Stable Fly." *Peptides* **18**(1): 1.

<http://www.sciencedirect.com/science/article/B6T0M-3S3DS2M-1/2/244b8cdde6e4cb0220515d581a48701a>

Guerrero, F. D. Transcriptional expression of a putative tachykinin-like peptide receptor gene from stable fly. *Peptides* 18(1) 1-5, 1997.--STKR is a 4118 bp clone from a stable fly, *Stomoxys calcitrans*, cDNA library which encodes a protein with significant amino acid identity to tachykinin-like peptide receptors. Ribonuclease protection assays and RT-PCR were utilized to examine the transcriptional expression of STKR from various life stages of the stable fly. STKR expression was detectable in all stages, but was most abundant in isolated adult fly gut and lowest in developing embryos.

Hagner, S., R. V. Haberberger, et al. (2002). "Expression and distribution of calcitonin receptor-like receptor in human hairy skin." *Peptides* **23**(1): 109.

<http://www.sciencedirect.com/science/article/B6T0M-44YWMXH-H/2/9805a4d74509ffeabf5d436347bf78b4>

Calcitonin gene-related peptide and adrenomedullin exert potent effects in skin but their cellular targets are unknown. This study aimed to identify the cellular location of calcitonin receptor-like receptor (CRLR) which is pharmacologically identical to CGRP receptor-1, a putative molecular target of CGRP and adrenomedullin. RT-PCR analysis of human hairy skin revealed the presence of CRLR mRNA and immunohistochemical analysis, employing a previously characterized polyclonal antibody raised to CRLR, provided novel evidence of the cellular distribution of CRLR. Extensive and specific CRLR-immunostaining was detected in arteriolar smooth muscle and venular endothelium and is consistent with CGRP's putative role in neurogenic inflammation. Novel targets for CGRP and/or adrenomedullin were identified, including capillary endothelium, hair follicles and sweat glands.

Hobara, N., A. Nakamura, et al. (2004). "Distribution of adrenomedullin-containing perivascular nerves in the rat mesenteric artery." Peptides **25**(4): 589.

<http://www.sciencedirect.com/science/article/B6T0M-4C59XM3-1/2/f6fc45f7a19ea6914955d0dec6ce8e00>

Distribution of adrenomedullin (AM)-containing perivascular nerve fibers was studied in rat mesenteric arteries. Many fibers containing AM-like immunoreactivity (LI) were observed in the adventitia. AM-LI fibers were abolished by cold storage denervation or capsaicin but not 6-hydroxydopamine. Double immunostainings showed colocalization of AM-LI with calcitonin gene-related peptide (CGRP)-LI. The dorsal root ganglia had many AM-positive cells and AM mRNA detected by RT-PCR. Electron microscopy study revealed high proportions of immunogold labeling for AM and colocalization of both AM-LI and CGRP-LI in unmyelinated nerve axons. These results suggest that AM-containing perivascular nerves are distributed in the rat mesenteric artery.

Ignar, D. M., J. L. Andrews, et al. (2003). "Regulation of TNF-[alpha] secretion by a specific melanocortin-1 receptor peptide agonist." Peptides **24**(5): 709.

<http://www.sciencedirect.com/science/article/B6T0M-4903R2N-4/2/b70e427fee18446a7646c18b9022f833>

The lack of specific pharmacological tools has impeded the evaluation of the role of each melanocortin receptor (MCR) subtype in the myriad physiological effects of melanocortins. 154N-5 is an octapeptide (MFRdWFKPV-NH₂) that was first identified as an MC1R antagonist in *Xenopus melanophores* [J. Biol. Chem. 269 (1994) 29846]. In this manuscript, we show that 154N-5 is a specific agonist for human and murine MC1R. The peptide has negligible activity at MC3R and MC4R and is 25-fold less potent and a weak agonist at MC5R. 154N-5 was tested in both a cellular and an animal model of tumor necrosis factor-[alpha] (TNF-[alpha]) secretion. The inhibitory efficacy of 154N-5 on TNF-[alpha] secretion in both models was similar to the nonselective agonist NDP-[alpha]-melanocyte stimulating hormone (NDP-[alpha]MSH), thus, we conclude that inhibition of TNF-[alpha] secretion by melanocortin peptides is mediated by MC1R. 154N-5 is a valuable new tool for the evaluation of specific contribution of MC1R agonism to physiological and pathological processes.

Lucas, G. A., L. R. White, et al. (1996). "Relaxation of Human Temporal Artery by Endothelin ETB Receptors." Peptides **17**(7): 1139.

<http://www.sciencedirect.com/science/article/B6T0M-3RSF7NG-9/2/2f04a193c99de876519905a4763ed6fe>

LUCAS, G.A., L.R. WHITE, R. JUUL, J. CAPPELEN, J. AASLY AND L. EDVINSSON. Relaxation of human temporal artery by endothelin ETB receptors. PEPTIDES 17(7) 1139-1144, 1996.-- Endothelin receptors have been characterized in human temporal artery by molecular biological methods and in vitro pharmacology. Reverse transcriptase-polymerase chain reaction was used to detect mRNA encoding ETA and ETB receptors in normal and endothelium-denuded arteries. Vasomotor response experiments with a specific ETA antagonist (FR 139317) suggested the presence of ETA subtypes. Marked ETB-mediated relaxation was obtained with ET-3 when ETA activity was blocked in precontracted arteries. Relaxation was significantly reduced by bosentan,

indomethacin, and a nitric oxide synthase inhibitor. It may be speculated that the relaxant activity is mediated through ETB1 receptors. Copyright (c) 1996 Elsevier Science Inc.

Lundell, I., H. Eriksson, et al. (2001). "Cloning and characterization of the guinea pig neuropeptide Y receptor Y5." Peptides **22**(3): 357.

<http://www.sciencedirect.com/science/article/B6T0M-42NY293-9/2/dbeff0d509b12c589263ad44a293ca20>

The Y5 receptor has been postulated to be the main receptor mediating NPY-induced food intake in rats, based on its pharmacological profile and mRNA distribution. To further characterize this important receptor subtype, we isolated the Y5 gene in the guinea pig, a widely used laboratory animal in which all other known NPY receptors (Y1, Y2, Y4, y6) [2, 13, 33 and 37] have recently been cloned by our group. Our results show that the Y5 receptor is well conserved between species; guinea pig Y5 displays 96% overall amino acid sequence identity to human Y5, the highest identity reported for any non-primate NPY receptor orthologue, regardless of subtype. Thirteen of the twenty substitutions occur in the large third cytoplasmic loop. The identities between the guinea pig Y5 receptor and the dog, rat, and mouse Y5 receptors are 93%, 89%, and 89% respectively. When transiently expressed in EBNA cells, the guinea pig Y5 receptor showed a high binding affinity to iodinated porcine PYY with a dissociation constant of 0.41 nM. Competition experiments showed that the rank order of potency for NPY-analogues was PYY = NPY = NPY2-36 > gpPP > rPP >> NPY 22-36. Thus the pharmacological profile of the guinea pig Y5 receptor agrees well with that reported for the Y5 receptor from other cloned species.

Mowa, C. N., S. Usip, et al. (2003). "The effects of pregnancy and estrogen on the expression of calcitonin gene-related peptide (CGRP) in the uterine cervix, dorsal root ganglia and spinal cord." Peptides **24**(8): 1163.

<http://www.sciencedirect.com/science/article/B6T0M-49W2135-1/2/c6c6d92ccdf1492e67144bc6f2d0e029>

Before parturition the uterine cervix undergoes a ripening process ("softens" and dilates) to allow passage of the fetus at term. The exact mechanism(s) responsible for cervical ripening are unknown, though a role for peptidergic sensory neurons is emerging. Previous work demonstrated that administration of substance P (SP) to ovariectomized rats caused events associated with cervical ripening, that production of SP in cervix-related dorsal root ganglion (DRG) is estrogen responsive, and that release of SP from neurons terminating in the cervix and spinal cord peaks prior to parturition. The present study was designed to test the hypothesis that calcitonin gene-related peptide (CGRP), a neuropeptide co-stored with SP in many sensory neurons, undergoes changes with pregnancy and hormonal environment. Immunohistochemistry, in situ hybridization, reverse transcriptase-polymerase chain reaction (RT-PCR) and radioimmunoassay (RIA) were used to investigate CGRP in L6-S1 DRG, spinal cord and cervix during pregnancy and the role of estrogen in CGRP synthesis. CGRP-immunoreactive primary sensory neurons expressed estrogen receptors (ER-[alpha] and ER-[beta]). In the cervix, CGRP concentrations decreased, but in the L6-S1 DRG and the spinal cord segments, CGRP levels increased, with peak effects observed at day 20 of gestation. CGRP mRNA synthesis increased in DRG over pregnancy. Sensory neurons of ovariectomized rats treated with estrogen showed increased CGRP mRNA synthesis in a dose-related manner, an effect blocked by the ER antagonist ICI 182 780. From these results, we postulate that synthesis of CGRP in L6-S1 DRG and utilization in the cervix increase over pregnancy and this synthesis is under influence of the estrogen-ER system. Collectively, these data are consistent with the hypothesis that CGRP plays a role in cervical ripening and, consequently in the birth process.

Mowa, C. N., S. Usip, et al. (2003). "Substance P in the uterine cervix, dorsal root ganglia and spinal cord during pregnancy and the effect of estrogen on SP synthesis." Peptides **24**(5): 761.

<http://www.sciencedirect.com/science/article/B6T0M-4909MCT-6/2/8f3180b6dd915b68ee6fc6023ebb8432>

Prior to parturition the non-pliable uterine cervix undergoes a ripening process ("softens" and dilates) to allow a timely passage of the fetus at term. The exact mechanism(s) triggering and involved in cervical ripening are unknown, though evidence for a role for sensory neurons and their contained neuropeptides is emerging. Moreover, an apparent increase in neuropeptide immunoreactive nerves occurs in the cervix during pregnancy, maternal serum estrogen levels rise at term and uterine cervix-related L6-S1 dorsal root ganglia (DRG) sensory neurons express estrogen receptor (ER) and neuropeptides. Thus, we sought to test the hypothesis that the neuropeptide substance P (SP) changes biosynthesis and release over pregnancy, that estrogen, acting via the ER pathway, increases synthesis of SP in DRG, and that SP is utilized in cervical ripening at late pregnancy. Using immunohistochemistry, in situ hybridization, reverse transcriptase-polymerase chain reaction (RT-PCR) and radioimmunoassay (RIA), we investigated coexpression of ER-[alpha]/[beta] and SP; differential expression of ER-[alpha] and -[beta] mRNA in DRG neurons; SP synthesis in DRG; and changes in SP concentration in the cervix, DRG and spinal cord over pregnancy. In addition, the effect of exogenous estrogen on SP synthesis in L6-S1 DRG of ovariectomized rats was examined. SP-immunoreactive neurons expressed ER-[alpha] and ER-[beta]. SP synthesis (expressed as [beta]-PPT mRNA label) was prominent in small DRG neurons. SP concentration increased in the L6-S1 DRG and spinal cord segments, with a peak at Day 20 of gestation, but decreased in the cervix during the first two trimesters, with a rise over the last trimester to Day 10 levels. SP and ER-[alpha] mRNA synthesis increased in DRG over pregnancy but ER-[beta] mRNA levels were largely unchanged. When ovariectomized rats were treated with exogenous estrogen, SP mRNA synthesis in the DRG increased in a dose-related manner, an effect blocked by ER blocker ICI 182 780. From these results, we postulate that synthesis of SP in L6-S1 DRG and utilization in the cervix increase over pregnancy and this synthesis is under influence of the estrogen-ER system, most likely ER-[alpha]. We postulate that SP may play a role in cervical ripening and, consequently in the birth process.

Sithigorngul, P., J. C. Nanda, et al. (2003). "A strategy for isolating rare peptides: isolation and sequencing of a large peptide present in a single neuron of the nematode *Ascaris suum*." Peptides **24**(7): 1025.

<http://www.sciencedirect.com/science/article/B6T0M-49D25Y3-1/2/07cab36acbfd65ed3dbae9657b29154>

Monoclonal antibody G15-6A was generated by immunizing mice with *Ascaris* head extracts. It recognizes an antigen present in a single neuron, with a cell body in the dorsal rectal ganglion, that projects along the ventral cord to the nerve ring. *Ascaris* extracts were fractionated by HPLC and ammonium sulfate precipitation, and fractions assayed by dot-blotting with antibody G15-6A. A single immunoreactive polypeptide was purified; mass spectrometry showed a molecular weight of 11,542 Da. Partial N-terminal sequencing, followed by cloning of the transcript encoding the peptide, revealed a predicted peptide product comprising 109 amino acids, and a molecular mass of 11,863 Da. The N-terminus of the predicted peptide includes four more amino acids than are found in the isolated product.

Takahashi, A., K. Tsuchiya, et al. (2004). "Possible involvement of melanin-concentrating hormone in food intake in a teleost fish, barfin flounder." Peptides **25**(10): 1613.

<http://www.sciencedirect.com/science/article/B6T0M-4D9DFRJ-1/2/ce69d119678dcde9375b736cbe8b95aa>

We investigated the involvement of MCH in food intake in barfin flounder. The structure of barfin flounder MCH was determined by cDNA cloning and mass spectrometry. In fasted fish, the MCH gene expression and the number of MCH neurons in the brain were greater than controls. In white-reared fish, the MCH gene expression and the number of MCH neurons in the brain were greater than black-reared fish. Furthermore, white-reared fish grew faster than black-reared fish. These results indicate that a white background stimulated production of MCH and MCH, in turn, enhanced body growth, probably by stimulating food intake.

Tamakawa, H., A. Miyata, et al. (1998). "The augmentation of pituitary adenylate cyclase-activating polypeptide (PACAP) in streptozotocin-induced diabetic rats." Peptides **19**(9): 1497.

<http://www.sciencedirect.com/science/article/B6T0M-3V8KBK5-4/2/bf0fd690b378e25f0714c73f9d137c55>

Pituitary adenylate cyclase activating polypeptide (PACAP), which was isolated from ovine hypothalamic extract, has been shown to have a physiological role in the regulation of insulin or islet functions. In streptozotocin (STZ)-induced diabetic rats, we examined the content of PACAP immunoreactivity and gene expression of three specific receptors. Four weeks after administration of STZ (50 mg/kg), plasma glucose levels increased 3.3-fold, and plasma insulin levels decreased to one-tenth as compared with the control. The content of PACAP immunoreactivity in the pancreas potentially increased by 30%, but the content of vasoactive intestinal polypeptide (VIP) immunoreactivity was not changed. In the other tissues, the content of PACAP immunoreactivity did not significantly change except in the hypothalamus, which showed a 10% increment. In the expression level of PACAP/VIP receptors, semi-quantitative RT-PCR analysis revealed that VIP1/PACAP receptor mRNA significantly increased as compared with the other two types of receptors in the pancreas of STZ-induced diabetic rats. These findings suggest that PACAP and VIP1/PACAP receptor might be involved in the pathophysiology of diabetes mellitus.

Uddman, R., S. Moller, et al. (2002). "Neuropeptide Y Y1 and neuropeptide Y Y2 receptors in human cardiovascular tissues." Peptides **23**(5): 927.

<http://www.sciencedirect.com/science/article/B6T0M-45H99JJ-M/2/2ed223bba6c73377d119d2ce931ccd39>

mRNA encoding the human NPY Y1 and NPY Y2 receptors were detected in cerebral, meningeal, and coronary arteries using reverse transcriptase-polymerase chain reaction (RT-PCR). In addition, the trigeminal and superior cervical ganglia were positive for both receptors. In some arteries and in SK-N-MC cells only mRNA encoding the NPY Y1 was detected. Besides the expected NPY Y1 PCR products, an additional 97 bp longer amplicon originating from an alternative splicing event was found in most tissues studied. Antibodies directed against the NPY Y1 receptor revealed immunostaining mainly in the smooth muscle layer of blood vessels whereas antibodies against the NPY Y2 receptor showed immunostaining in nerve cell bodies.

Vilim, F. S., V. Alexeeva, et al. (2001). "Cloning, expression and processing of the CP2 neuropeptide precursor of Aplysia." Peptides **22**(12): 2027.

<http://www.sciencedirect.com/science/article/B6T0M-44V0PX6-D/2/f075162c335694ebf63a1e6b77bbc5a0>

The cDNA sequence encoding the CP2 neuropeptide precursor is identified and encodes a single copy of the neuropeptide that is flanked by appropriate processing sites. The distribution of the CP2 precursor mRNA is described and matches the CP2-like immunoreactivity described previously. Single cell RT-PCR independently confirms the presence of CP2 precursor mRNA in selected neurons. MALDI-TOF MS is used to identify additional peptides derived from the CP2 precursor in neuronal somata and nerves, suggesting that the CP2 precursor may give rise to additional bioactive neuropeptides.

Wahbi, K., J. P. Magaud, et al. (2001). "Coding region of the sorbin gene in different species." Peptides **22**(12): 2045.

<http://www.sciencedirect.com/science/article/B6T0M-44V0PX6-G/2/4eff1dc146f2ce0d05bbb6c3140f6f7a>

The coding region of 153 amino-acid sorbin, isolated from porcine intestine has been cloned and sequenced in pig, human and rat. The coding region includes 459 bases comprising the 5' region of 24 bases, the middle region named "sorbin-like sequence" (25-432) and the 3' region (433-459). The peptidic C-terminal segment presents the biological activity: absorption of water and electrolytes from the intestine and gall-bladder. The cDNA homology between the three species was 95%. Three forms of mRNA were found, two major forms (6.5 and 8 Kb) and one minor (4.5 Kb).

Zhong, Q., R. J. Bollag, et al. (2000). "Glucose-dependent insulinotropic peptide signaling pathways in endothelial cells." Peptides **21**(9): 1427.

<http://www.sciencedirect.com/science/article/B6T0M-41KP2XJ-J/2/75771a71c2578b320007de0663d2334a>

Glucose-dependent insulinotropic peptide (GIP) potentiates glucose-induced insulin secretion. In addition, GIP has vasoconstrictive or vasodilatory properties depending on the vascular bed affected. In order to assess whether this effect could be related to differences in GIP receptor expression, several different endothelial cell types were examined for GIP receptor expression. GIP receptor splice variants were detected and varied depending on the endothelial cell type. Furthermore, stimulation of these cells with GIP led to cell type dependent differences in activation of the calcium and cAMP signaling pathways. To our knowledge this is the first physiological characterization of receptors for GIP in endothelial cells.

Zhou, Q., P.-A. Frandberg, et al. (2003). "Substance P(1-7) affects the expression of dopamine D2 receptor mRNA in male rat brain during morphine withdrawal." Peptides **24**(1): 147.

<http://www.sciencedirect.com/science/article/B6T0M-47MKCMH-2/2/4d0662deac9dac8418ffe2960286e9ac>

Previous studies have confirmed an important role of the undecapeptide substance P (SP) in opioid reward and dependence. It is further shown that the SP N-terminal metabolite SP1-7 may attenuate the intensity of opioid withdrawal in mice. In this study we have investigated the effect of the heptapeptide fragment on the expression of the brain dopamine D2 receptor mRNA and on the withdrawal reaction, as well, in morphine-dependent rats. Male Sprague-Dawley rats were randomly distributed into two groups. Guide cannula was implanted and aimed at the lateral ventricle and animals were subsequently made opioid dependent by two daily injections of morphine (10 mg/kg) for 7 days. Half an hour before naloxone challenge (2 mg/kg) one group of rats received an injection of SP1-7 (28 nmol per rat) and the other, serving as control, was injected with saline through the cannula. Animals were decapitated 4 h following SP1-7 or saline injections. The results indicated that the level of the dopamine D2 receptor transcript was significantly reduced by SP1-7 in nucleus accumbens and frontal cortex but not altered in the striatum. In behavioral tests it was found that the heptapeptide attenuated several somatic withdrawal symptoms. The observed reduction in the receptor transcript in nucleus accumbens and frontal cortex is suggested to reflect an increased dopamine activity in these areas, which in turn may counteract the withdrawal reaction.

Pharmacological Research (2)

Dan, K., K. Miyashita, et al. (2002). "THE MEMORY EFFECT OF HETEROPOLYOXOTUNGSTATE (PM-19) PRETREATMENT ON INFECTION BY HERPES SIMPLEX VIRUS AT THE PENETRATION STAGE." Pharmacological Research **46**(4): 357.

<http://www.sciencedirect.com/science/article/B6WP9-46VJCDW-C/2/a577a8f7a4f4f6819c1c7b60d3b25e32>

The keggin-type heteropolyoxotungstate $K_7[Pt_{12}W_{10}O_{40}].6H_2O$ (PM-19) is a potent polyoxometalate (PM) inhibitor of the replication of herpes simplex virus (HSV). Pretreatment of Vero cells with PM-19 prior to HSV-2 infection enhanced the antiviral potency of PM-19 almost 10-fold compared with treatment of the cells only after infection. The pretreatment effect of PM-19 is called 'the memory effect'. The memory effect was reflected by inhibition of plaque formation and decrease of intracellular virus DNA quantity, and was strongest when PM-19 was present during the penetration stage of HSV-2 infection. The effect was maintained under conditions of fusion induced by polyethyleneglycol treatment. This suggests that PM-19 does not act at the fusion stage of infection. Using the infectious center assay method, it was clarified that a second round of infection was inhibited by about 30% in the presence of PM-19 at the penetration stage compared with the virus control in nontreated cells. The inhibition was enhanced to about 60% by PM-19 pretreatment prior to infection. This suggests that PM-19 pretreatment of the cells protects them against HSV-2 infection.

Hernandez, E., A. Correa, et al. (2002). "PENTOXIFYLLINE DIMINISHED ACETALDEHYDE-INDUCED COLLAGEN PRODUCTION IN HEPATIC STELLATE CELLS BY DECREASING INTERLEUKIN-6 EXPRESSION." Pharmacological Research **46**(5): 435.

<http://www.sciencedirect.com/science/article/B6WP9-473VN84-C/2/3f0afb919ff5896e2eb79facf97d270f>

The effect of pentoxifylline (PTX), a methylxanthine derivative, on collagen induction and secretion and on the production of mRNA of two fibrogenic cytokines: interleukin-6 and transforming growth factor- β 1 (IL-6 and TGF- β 1) in a rat hepatic stellate cell line (CFSC-2G) exposed to acetaldehyde was studied. CFSC-2G cells were treated with 175 μ M acetaldehyde for 24 h. The cells were then exposed to a medium containing 200 μ M PTX. Collagen secretion, increased 2.6 times in acetaldehyde treated cells. Cells exposed to acetaldehyde and treated with PTX diminished collagen secretion to control values and decreased α 1(I) collagen mRNA by 15%. Reverse transcriptase-polymerase chain reaction (RT-PCR) assays of TGF- β 1 mRNA showed no variation in different experimental conditions. However, PTX induced a decrease of 32% in IL-6 mRNA in acetaldehyde-treated cells. CFSC-2G cells treated with anti-IL-6 monoclonal antibody, 15 min before acetaldehyde was added, did not present an increase in α 1(I) collagen mRNA. These results show that PTX inhibits the expression of α 1(I) collagen via the inhibition of IL-6 in acetaldehyde treated cells. The effect herein reported on IL-6 and α 1(I) collagen mRNA adds to the previously described effect of PTX, which could be useful in the fibrogenic process induced by acetaldehyde.

Physiol Genomics (13)

Barber, R. D., D. W. Harmer, et al. (2005). "GAPDH AS A HOUSEKEEPING GENE: ANALYSIS OF GAPDH mRNA EXPRESSION IN A PANEL OF 72 HUMAN TISSUES." Physiol Genomics: 00025.2005.

<http://physiolgenomics.physiology.org/cgi/content/abstract/00025.2005v1>

Quantitative gene expression data are often normalised to the expression levels of control or so-called 'housekeeping' genes. An inherent assumption in the use of housekeeping genes is that expression of the genes remains constant in the cells or tissues under investigation. Although exceptions to this assumption are well documented, housekeeping genes are of value in fully characterised systems. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is one of the most commonly used housekeeping genes used in comparisons of gene expression data. To investigate the value of GAPDH as a housekeeping gene in human tissues, the expression of GAPDH mRNA was measured in a panel of 72 different pathologically normal human tissue types. Measurements were obtained from 371,088 multiplexed, quantitative real-time reverse transcription polymerase chain reactions (Q-RT-PCRs) with specific target genes. Significant differences in the expression levels of GAPDH mRNA were observed between tissue types, and between donors of the same tissue. A 15-fold difference in GAPDH mRNA copy numbers was observed between the highest and lowest expressing tissue types, skeletal muscle and breast, respectively. No specific effect of either age or gender was observed on GAPDH mRNA expression. These data provide an extensive analysis of GAPDH mRNA expression in human tissues, and confirm previous reports of the marked variability of GAPDH expression between tissue types. These data establish comparative levels of expression, and can be used to add value to gene expression data in which GAPDH is used as the internal control.

Correia, M. J., T. G. Wood, et al. (2004). "Molecular characterization of an inward rectifier channel (IKir) found in avian vestibular hair cells: cloning and expression of pKir2.1." Physiol Genomics **19**(2): 155-169.

<http://physiolgenomics.physiology.org/cgi/content/abstract/19/2/155>

A fast inwardly rectifying current has been observed in some of the sensory cells (hair cells) of the inner ear of several species. While the current was presumed to be an IKir current, contradictory evidence existed as to whether the cloned channel actually belonged to the Kir2.0 subfamily of potassium inward rectifiers. In this paper, we report for the first time converging evidence from electrophysiological, biochemical, immunohistochemical, and genetic studies that show that the Kir2.1 channel carries the fast inwardly rectifying currents found in pigeon vestibular hair cells. Following cytoplasm extraction from single type II and multiple pigeon vestibular hair cells, mRNA was reverse transcribed, amplified, and sequenced. The open reading frame (ORF), consisting of a 1,284-bp nucleotide sequence, showed 94, 85, and 83% identity with Kir2.1 subunit sequences from chick lens, Kir2 sequences from human heart, and a mouse macrophage cell line, respectively. Phylogenetic analyses revealed that pKir2.1 formed an immediate node with hKir2.1 but not with hKir2.2-2.4. Hair cells (type I and type II) and supporting cells in the sensory epithelium reacted positively with a Kir2.1 antibody. The whole cell current recorded in oocytes and CHO cells, transfected with pigeon hair cell Kir2.1 (pKir2.1), demonstrated blockage by Ba²⁺ and sensitivity to changing K⁺ concentration. The mean single-channel linear slope conductance in transfected CHO cells was 29 pS. The open dwell time was long ([~]300 ms at -100 mV), and the closed dwell time was short ([~]34 ms at -100 mV). Multistates ranging from 3-6 were noted in some single-channel responses. All of the above features have been described for other Kir2.1 channels. Current clamp studies of native pigeon vestibular hair cells illustrated possible physiological roles of the channel and showed that blockage of the channel by Ba²⁺ depolarized the resting membrane potential by [~]30 mV. Negative currents hyperpolarized the membrane [~]20 mV before block but [~]60 mV following block. RT-PCR studies revealed that the pKir2.1 channels found in pigeon vestibular hair cells were also present in pigeon vestibular nerve, vestibular ganglion, lens, neck muscle, brain (brain stem, cerebellum and optic tectum), liver, and heart.

Culp, D. J., L. R. Latchney, et al. (2004). "The gene encoding mouse Muc19: cDNA, genomic organization and relationship to Smgc." *Physiol Genomics* **19**(3): 303-318.

<http://physiolgenomics.physiology.org/cgi/content/abstract/19/3/303>

We previously demonstrated expression of full-length transcripts for sublingual mucin apoprotein, Muc19, of [~]24 kb (Fallon MA, Latchney LR, Hand AR, Johar A, Denny PA, Georgel PT, Denny PC, and Culp DJ. *Physiol Genomics* 14: 95-106, 2003). We now describe the complete sequence and genomic organization of the apomucin encoded by 43 exons. Southern analyses indicate a central exon of [~]18 kb containing 36 tandem repeats, each encoding 163 residues rich in serine and threonine. Full-length transcripts are an estimated 22,795 bp in length that span 106 kb of genomic DNA. The transcriptional start site is 24 bp downstream of a TATA box and 42 bp upstream of the conceptual translational start codon. The putative apoprotein has an estimated mass of 693.4 kDa and contains 7,524 amino acids (80% serine, threonine, glycine, alanine, and proline). We present a model for rat Muc19 transcripts and compare the conceptually translated Muc19 proteins for mouse, rat, pig, and the 3' end of human Muc19. Conserved among these apoproteins are a signal peptide, a large tandem repeat region, von Willebrand factor type C and D domains, a trypsin inhibitor-like Cys-rich domain, and a COOH-terminal cystine knot-like domain. Southern blot analyses indicate transcripts for Muc19 and Smgc (submandibular gland protein C) are splice variants of a larger gene, Muc19/Smgc. Comparative Northern analyses between the major salivary glands demonstrate highly selective Muc19 expression in neonatal and adult sublingual glands, whereas Smgc is expressed in neonatal submandibular and sublingual glands. Regulation of Muc19/Smgc gene expression is discussed with respect to alternative splicing and mucous cell cytodifferentiation.

Fertuck, K. C., J. E. Eckel, et al. (2003). "Identification of temporal patterns of gene expression in the uteri of immature, ovariectomized mice following exposure to ethynylestradiol." Physiol Genomics **15**(2): 127-141.

<http://physiolgenomics.physiology.org/cgi/content/abstract/15/2/127>

Estrogen induction of uterine wet weight provides an excellent model to investigate relationships between changes in global gene expression and well-characterized physiological responses. In this study, time course microarray GeneChip data were analyzed using a novel approach to identify temporal changes in uterine gene expression following treatment of immature ovariectomized C57BL/6 mice with 0.1 mg/kg 17{alpha}-ethynylestradiol. Functional gene annotation information from public databases facilitated the association of changes in gene expression with physiological outcomes, which allowed detailed mechanistic inferences to be drawn regarding cell cycle control and proliferation, transcription and translation, structural tissue remodeling, and immunologic responses. These systematic approaches confirm previously established responses, identify novel estrogen-regulated transcriptional effects, and disclose the coordinated activation of multiple modes of action that support the uterotrophic response elicited by estrogen. In particular, it was possible to elucidate the physiological significance of the dramatic induction of arginase, a classic estrogenic response, by elucidating its mechanistic relevance and delineating the role of arginine and ornithine utilization in the estrogen-stimulated induction of uterine wet weight.

Hahn, C. N., Z. J. Su, et al. (2005). "EXPRESSION PROFILING REVEALS FUNCTIONALLY IMPORTANT GENES AND COORDINATELY REGULATED SIGNALLING PATHWAY GENES DURING IN VITRO ANGIOGENESIS." Physiol Genomics: 00278.2004.

<http://physiolgenomics.physiology.org/cgi/content/abstract/00278.2004v1>

Angiogenesis is a complex multicellular process requiring the orchestration of many events including migration, alignment, proliferation, lumen formation, remodeling and maturation. Such complexity indicates that not only individual genes but also entire signalling pathways will be crucial in angiogenesis. In order to define an angiogenic blueprint of regulated genes, we utilized our well characterized 3D collagen gel model of in vitro angiogenesis, in which the majority of cells synchronously progress through defined morphological stages culminating in the formation of capillary tubes. We developed a comprehensive three tiered approach using microarray analysis which has allowed identification of genes known to be involved in angiogenesis, genes hitherto unlinked to angiogenesis as well as novel genes, and has proven especially useful for genes where the magnitude of change is small. Of interest is the ability to recognize complete signalling pathways which are regulated and genes clustering into ontological groups implicating the functional importance of particular processes. We have shown that consecutive members of the mitogen activated protein kinase (MAPK) and leukemia inhibitory factor (LIF) signalling pathways are altered at the mRNA level during in vitro angiogenesis. Thus, at least for the MAPK pathway, mRNA changes as well as the phosphorylation changes of these gene products may be important in the control of blood vessel morphogenesis. Furthermore, in this study, we demonstrate the power of virtual northern blot analysis, as an alternative to quantitative reverse transcription polymerase chain reaction (Q-RT-PCR), for measuring the magnitudes of differential gene expression.

Ivanov, A. I., A. A. Steiner, et al. (2005). "Expression of Eph receptors and their ligands, ephrins, during lipopolysaccharide fever in rats." Physiol Genomics **21**(2): 152-160.

<http://physiolgenomics.physiology.org/cgi/content/abstract/21/2/152>

Erythropoietin-producing hepatocellular (Eph) receptor tyrosine kinases and their ligands, ephrins, are involved in embryogenesis and oncogenesis by mediating cell adhesion and migration. Although ephrins can be induced by bacterial LPS in vitro, whether they are involved in inflammation in vivo is unknown. Using differential mRNA display, we found that a febrigenic dose of LPS (50 {micro}g/kg iv) induces a strong transcriptional upregulation of ephrin-A1 in rat liver. We confirmed this finding by real-time RT-PCR. We then quantified the mRNA expression of different ephrins and Eph receptors at phases 1-3 of LPS fever in different organs. Febrile phases 2 (90 min post-LPS) and 3 (300 min) were characterized by robust upregulation (up to 16-fold) and downregulation (up to 21-fold) of several ephrins and Eph receptors. With the exception of EphA2, which showed upregulation in the brain at phase 2, expressional changes of Eph receptors and ephrins were limited to the LPS-processing organs: liver and lung. Characteristic, counter-directed changes in expressional regulation of Eph receptors and their corresponding ligands were found: upregulation of EphA2, downregulation of ephrin-A1 in the liver and lung at phase 2; downregulation of EphB3, upregulation of ephrin-B2 in the liver at phase 2; downregulation of EphA1 and EphA3, upregulation of ephrins-A1 and -A3 in liver at phase 3. In the liver, transcriptional changes of EphA2 and EphB3 at phase 2 were confirmed at protein level. These coordinated, phase-specific responses suggest that different sets of ephrins and Eph receptors may be involved in cellular events (such as disruption of tissue barriers and leukocyte transmigration) underlying different stages of systemic inflammatory response to LPS.

Lefebvre-Lavoie, J., J. G. Lussier, et al. (2005). "Profiling of Differentially Expressed Genes in Wound Margin Biopsies of Horses, Using Suppression Subtractive Hybridization." Physiol Genomics: 00018.2005.

<http://physiolgenomics.physiology.org/cgi/content/abstract/00018.2005v1>

Disturbed gene expression may disrupt the normal process of repair and lead to pathologic situations resulting in excessive scarring. To prevent and treat impaired healing it is necessary to first define baseline gene expression during normal repair. The objective of this study was to compare gene expression in normal intact skin (IS) and wound biopsies (WM), using suppression subtractive hybridization (SSH) to identify genes differentially expressed during wound repair in horses. Tissue samples included both normal intact skin and biopsies from 7 day old wounds. IS cDNAs were subtracted from WM cDNAs to establish a subtracted (WM-IS) cDNA library. 226 non-redundant cDNAs were identified. Detection of genes previously shown to be expressed 7 days following trauma, including COL1A2, ANXA2, COL6A3, ACTB, FGF-7, LAMR1, MMP1, SPARC and TIMP-2, supported the validity of the experimental design. An RTPCR assay confirmed an increase or induction of the cDNAs of specific genes (COL1A2, MMP1, DSPG2, CD68, CD163 and ADAM9) within wound biopsies. Among these, COL1A2 and MMP1 had previously been documented in horses. 68.8% of the cDNAs had not previously been attributed a role during wound repair, of which SSAT, SERPINB10 and SNX9 were highly expressed and whose known functions in other processes made them potential candidates in regulating the proliferative response to wounding. In conclusion, we have identified novel genes that are differentially expressed in equine wound biopsies and which may modulate repair. Future experiments must correlate changes in mRNA levels for precise molecules with spatio-temporal protein expression within tissues.

McDanel, T. G., D. L. Hancock, et al. (2004). "Altered mRNA abundance of ASB15 and four other genes in skeletal muscle following administration of {beta}-adrenergic receptor agonists." Physiol Genomics **16**(2): 275-283.

<http://physiolgenomics.physiology.org/cgi/content/abstract/16/2/275>

{beta}-Adrenergic receptor agonists (BA) stimulate skeletal muscle growth. However, downstream signaling pathways that facilitate this effect remain poorly defined. Objectives of this study were to identify genes differentially expressed after administration of a novel BA and to evaluate the expression of one of those genes in additional models of skeletal muscle growth. Differentially expressed gene fragments were identified through differential display of skeletal muscle biopsies from five steers 24 h after administration of the BA. Five gene fragments designated DD53, DD143, DD163, DD209, and DD214 were identified. Tissue distribution of these genes was evaluated by RT-PCR. While DD53, DD163, DD209, and DD214 were expressed across tissues, DD143 mRNA expression was most abundant in skeletal muscle. DD143, later identified as bovine ASB15, was evaluated in rats following administration of anabolic compounds. Thirteen 7-wk-old female rats were randomly assigned to each of four treatment groups including: control, clenbuterol, trenbolone acetate (TBA), and growth hormone (GH). Changes in rat Asb-15 mRNA were measured at 30 min, 12 h, and 24 h following intraperitoneal injections of each compound. Clenbuterol treatment decreased Asb-15 mRNA in skeletal muscle at 12 and 24 h ($P < 0.01$) and also decreased mRNA in lung at 12 h ($P < 0.05$). TBA and GH treatments did not alter Asb-15 mRNA in any of the tissues evaluated ($P > 0.10$). These results are the first to associate an Asb gene family member with muscle growth or BA administration and suggest a potential role for ASB15 in {beta}-agonist-induced skeletal muscle hypertrophy.

Orloff, M. S., S. K. Iyengar, et al. (2005). "Variants in the Wilms' tumor gene are associated with focal segmental glomerulosclerosis in the African American population." *Physiol Genomics* **21**(2): 212-221.

<http://physiolgenomics.physiology.org/cgi/content/abstract/21/2/212>

Wilms' tumor gene (WT1) is important for nephrogenesis and gonadal growth. WT1 mutations cause Denys-Drash and Frasier syndromes, which are characterized by glomerular scarring. To test whether genetic variations in WT1 and WIT1 (gene immediately 5' to WT1) associate with focal segmental glomerulosclerosis (FSGS), patients with biopsy-proven idiopathic and HIV-1-associated FSGS were enrolled in a multicenter study. We genotyped SNP rs6508 located in WIT1 exon 1, three SNPs (rs2301250, rs2301252, rs2301254) in the promoter shared by WT1 and WIT1, rs2234590 in exon 6, rs2234591 in intron 6, rs16754 in exon 7, and rs1799937 in intron 9 of WT1. Cases ($n = 218$) and controls ($n = 281$) were compared in the African American population. Stratification by HIV-1 infection status showed that SNPs rs6508, rs2301254, and rs1799937 were significantly associated with FSGS [rs6508 odds ratio (OR) 1.82, $P = 0.006$; rs2301254 OR 1.65, $P = 0.049$; rs1799937 OR 1.91, $P = 0.005$] in the non-HIV-1 group and rs2234591 (OR 0.234, $P = 0.011$) in the HIV-1 group. Haplotype analyses in the population revealed that seven SNPs were associated with FSGS; five SNPs had the highest contingency score [$-\log_{10}(P \text{ value}) = 13.57$] in the HIV-1 group. This association could not be explained by population substructure. We conclude that SNPs in WT1 and WIT1 genes are significantly associated with FSGS, suggesting that variants in these genes may mediate pathogenesis by altering WT1 function. Furthermore, HIV-1 infection status interacts with genetic variations in both genes to influence this phenotype. We speculate that nephropathy liability alleles in WT1 pathway genes cause podocyte dysfunction and glomerular scarring.

Rise, M. L., S. R. M. Jones, et al. (2004). "Microarray analyses identify molecular biomarkers of Atlantic salmon macrophage and hematopoietic kidney response to *Piscirickettsia salmonis* infection." *Physiol Genomics* **20**(1): 21-35.

<http://physiolgenomics.physiology.org/cgi/content/abstract/20/1/21>

Piscirickettsia salmonis is the intracellular bacterium that causes salmonid rickettsial septicemia, an infectious disease that kills millions of farmed fish each year. The mechanisms used by *P. salmonis* to survive and replicate within host cells are not known. *Piscirickettsiosis* causes severe necrosis of hematopoietic kidney. Microarray-based experiments with QPCR validation were used to identify Atlantic salmon macrophage and hematopoietic kidney genes differentially transcribed in response to *P. salmonis* infection. Infections were confirmed by microscopy and RT-PCR with pathogen-specific primers. In infected salmon macrophages, 71 different transcripts were upregulated and 31 different transcripts were downregulated. In infected hematopoietic kidney, 30 different transcripts were upregulated and 39 different transcripts were downregulated. Ten antioxidant genes, including glutathione S-transferase, glutathione reductase, glutathione peroxidase, and cytochrome b558 {alpha}- and {beta}-subunits, were upregulated in infected macrophages but not in infected hematopoietic kidney. Changes in redox status of infected macrophages may allow these cells to tolerate *P. salmonis* infection, raising the possibility that treatment with antioxidants may reduce hematopoietic tissue damage caused by this rickettsial infection. The downregulation of transcripts involved in adaptive immune responses (e.g., T cell receptor {alpha}-chain and C-C chemokine receptor 7) in infected hematopoietic kidney but not in infected macrophages may contribute to infection-induced kidney tissue damage. Molecular biomarkers of *P. salmonis* infection, characterized by immune-relevant functional annotations and high fold differences in expression between infected and noninfected samples, may aid in the development of anti-*piscirickettsial* vaccines and therapeutics.

Villablanca, A. C., K. A. Lewis, et al. (2003). "Differential regulation of gene expression by ovariectomy in mouse aorta." *Physiol Genomics* **12**(3): 175-185.

<http://physiolgenomics.physiology.org/cgi/content/abstract/12/3/175>

The purpose of this study was to investigate the effects of ovarian hormones on gene expression in the vascular wall. Our approach employed an RT-PCR-based cloning strategy of DNA differential display analysis and verification/confirmation of differential expression by semi-quantitative PCR and real-time PCR. mRNA analysis of normal aortas from intact and ovariectomized female C57BL/6J mice, showed altered expression of 20 genes with significant (>70%) sequence homology to known genes. Eight were selected for further study based on the genes' known function and potential relevance to vascular physiology. Differential expression of mRNA for three genes was confirmed by both semi-quantitative and real-time RT-PCR using gene-specific primers. Ovariectomy downregulated expression of elongation factor-1{alpha} (3.5-fold), ganglioside-induced differentiation associated protein (8.2-fold), and NADH:ubiquinone oxidoreductase (3.8-fold). Thus, in normal mouse aortas, ovariectomy resulted in significant differential downregulation of a number of vascular genes important to vascular cell growth and angiogenesis, cellular differentiation, and mitochondrial energy metabolism, respectively. These studies have implications for our understanding of hormonal regulation of vascular gene expression and the therapeutic targeting of specific vascular genetic sequences by female sex steroid hormones.

Wong, C., N. R. Mahapatra, et al. (2003). "The angiotensin II receptor (Agtr1a): functional regulatory polymorphisms in a locus genetically linked to blood pressure variation in the mouse." *Physiol Genomics* **14**(1): 83-93.

<http://physiolgenomics.physiology.org/cgi/content/abstract/14/1/83>

Hypertension is a complex trait with multiple genetic determinants. A previous genome-wide linkage study of systolic blood pressure in a mouse genetic backcross implicated a region of chromosome 13 (LOD = 3.3 at 16.0 cM) as a determinant of blood pressure differences between

a hereditary low blood pressure strain of *Mus musculus* (BPL/1) and *Mus spretus* (SPRET); at this locus, the unexpected effect of the BPL/1 allele was to increase blood pressure. A plausible candidate locus encoding angiotensin II receptor isoform 1a (Agtr1a) is also located at 16.0 cM on chromosome 13. We therefore investigated structural and functional differences at Agtr1a between BPL/1 and SPRET, as well as the BPH/2 strain. Resequencing Agtr1a in the three strains established the exon/intron and proximal promoter structure of the mouse gene. Coding exon 3 spanned 1,960 bp (with 26 SNPs), including the 1,077-bp/359-amino acid ORF (with 5 cSNPs, all of which were synonymous). Promoter sequences revealed a consensus TATA box, conserved G/C-rich regions, and a striking, lengthy simple sequence repeat region, composed of di-, tri-, tetra-, and penta-nucleotide repeats, whose overall length varied markedly among the strains. Twenty-five other SNPs and three single nucleotide deletions differentiated the strains' promoters, six of which were in likely functional promoter motifs. Agtr1a mRNA abundance in the adrenal gland in vivo was greater ($P < 0.05$) in BPL/1 than SPRET, consistent with the predicted effect of the BPL/1 allele to confer higher blood pressure. When Agtr1a promoters were subcloned into luciferase reporter plasmids and transfected into PC12 chromaffin cells, basal promoter expression was higher ($P < 0.001$) in BPL/1 than in SPRET, consistent with the endogenous mRNA results. In summary, Agtr1a on chromosome 13 is highly polymorphic between mouse strains, although the amino acid sequence specified by the ORF is invariant, even across mouse species. We conclude that polymorphisms in the Agtr1a promoter account for differences in gene expression in vivo between BPL/1 and SPRET, in a way consistent with the effects of alleles at this locus on chromosome 13 to affect blood pressure in the mouse genome-wide linkage study.

Wood, R. J., L. Tchack, et al. (2004). "DNA microarray analysis of vitamin D-induced gene expression in a human colon carcinoma cell line." *Physiol Genomics* **17**(2): 122-129.

<http://physiolgenomics.physiology.org/cgi/content/abstract/17/2/122>

The full extent to which 1,25-dihydroxyvitamin D₃ affects gene expression in human intestinal epithelial cells is unknown. We used oligonucleotide arrays to catalog vitamin D-induced changes in gene expression in Caco-2 cells, a human colon carcinoma cell line. Five paired sets of Caco-2 cell cultures were subjected to either control conditions or 1,25-dihydroxyvitamin D (10⁻⁷ mol/l x 24 h), and RNA was analyzed on an Affymetrix cDNA array containing 12,625 human sequences. Only 13 sequences representing 12 distinct genes exhibited statistically significant changes in expression of twofold or greater and were also called as "present" or "marginal" by the array-reading software in all five experiments. Genes regulated by 1,25-dihydroxyvitamin D included two previously known genes (25-hydroxyvitamin D-24-hydroxylase and amphiregulin) and 10 genes (sorcini, Gem, adaptin-γ, TIG1, CEACAM6, carbonic anhydrase XII, junB, ceruloplasmin, and two unidentified sequences) that were novel. We tested and independently confirmed the effect of 1,25-dihydroxyvitamin D on 11 of these genes by RT-PCR. Increased protein expression was tested and confirmed in two of the novel 1,25-dihydroxyvitamin D-regulated genes, ceruloplasmin and sorcini. The known function of these genes suggests that many of them could be involved in the antiproliferative effects of 1,25-dihydroxyvitamin D₃.

Physiological and Molecular Plant Pathology (3)

Little, C. R. and C. W. Magill (2003). "Elicitation of defense response genes in sorghum floral tissues

infected by *Fusarium thapsinum* and *Curvularia lunata* at anthesis." Physiological and Molecular Plant Pathology **63**(5): 271.

<http://www.sciencedirect.com/science/article/B6WPC-4CBV2VF-1/2/bd4e8d3802e7e299d9adb10d621b4d06>

Grain mold of *Sorghum bicolor* is one of the leading constraints for the production of optimum quality sorghum grain worldwide. Differences in mold levels among different varieties grown in the same environment imply that genes play a role in controlling mold severity. In order to determine if genes that function in active defense responses also affect molding, panicles of resistant and susceptible cultivars were inoculated at anthesis with conidial suspensions of *Fusarium thapsinum* and *Curvularia lunata*, the fungi most often found in naturally infected grain. RNA was extracted from the immature floral tissues at various times following inoculation. Levels of mRNA for four known defense-response genes, phenylalanine ammonia lyase (PAL1-1), chalcone synthase (CHS2G), [beta]-1,3-glucanase (GLUC2-1) and chitinase (CHIT25-1) were examined by hybridization to PCR generated clones of the respective genes. Expression of each gene increased rapidly following inoculation with either fungus. Although differences were seen in response to the two pathogens, the general pattern was similar in resistant and susceptible cultivars. The results imply that factors other than the level or timing of active defense responses account for the cultivar differences seen when the plants are challenged at the time of flowering.

Okuda, M., S. Taba, et al. (2003). "The S RNA segment determines symptom differences on *Tetragonia expansa* between two Watermelon silver mottle virus isolates." Physiological and Molecular Plant Pathology **62**(6): 327.

<http://www.sciencedirect.com/science/article/B6WPC-49CSYXY-2/2/94073937026ab563e6b2ce479cb210b5>

The WS-Y isolate of Watermelon silver mottle virus (WSMoV) causes severe necrosis in *Tetragonia expansa*. To determine the RNA segment that induces symptoms, genome reassortants between WS-Y and an isolate causing mild mottle, WS-O, were generated. The origin of each segment in the reassortants was identified by RT-PCR and subsequent restriction enzyme analysis of the amplified fragments. Thirty genome reassortants were isolated from co-infected *T. expansa* plants. The reassortants with the S RNA segment of WS-Y caused severe necrosis, while those with the S RNA segment of WS-O caused a mild mottle; hence, the S RNA determined symptom expression. The incidence of reassortants was disproportional among genotypes. The most frequent genome reassortant possesses the L RNA of WS-Y, the M RNA of WS-O and the S RNA of WS-Y. A similar ratio of genotypes was found in isolates of local lesions on *Chenopodium quinoa*. These results strongly suggested that competition occurred independently between the individual RNA segments in a co-infected *T. expansa* plant, not between isolates.

Skov, J., M. Lemmens, et al. (2004). "Role of a *Fusarium culmorum* ABC transporter (FcABC1) during infection of wheat and barley." Physiological and Molecular Plant Pathology **64**(5): 245.

<http://www.sciencedirect.com/science/article/B6WPC-4F2VS6B-1/2/8e3b8bdc0ed657eb44a45a94194edcd1>

A gene encoding the ATP-binding cassette (ABC) transporter FcABC1 was characterised in *Fusarium culmorum*. It is homologous to two transporters, *Magnaporthe grisea* ABC1 and *Gibberella pulicaris* ABC1, that play a role in plant pathogenesis. Transcripts of FcABC1 were

present during infection of barley roots. A FcABC1 replacement mutant was produced and in a point inoculation experiment of wheat heads, the mutant displayed reduced aggressiveness, indicating that FcABC1 has a function during infection of plant tissue. It is hypothesised that wheat contains an antifungal compound that inhibits the spread of the fungus in the plant and to which FcABC1 confers resistance.

Physiology & Behavior (2)

Gullicksen, P. S., W. P. Flatt, et al. (2002). "Energy metabolism and expression of uncoupling proteins 1, 2, and 3 after 21 days of recovery from intracerebroventricular mouse leptin in rats." Physiology & Behavior **75**(4): 473.

<http://www.sciencedirect.com/science/article/B6T0P-45KWD47-2/2/9156c6770f223f557848ed90853db2be>

Animals tend to maintain a lower body weight for an extended period after leptin administration has ended. This may be due to an enhancement of metabolic rate that persists after treatment withdrawal. Our objectives were to determine the period of leptin influence, when injected intracerebroventricularly (icv), on food intake, body weight, and energy expenditure. Additionally, the relationship between expressions of UCP1, UCP2, and UCP3 in different adipose tissues and heat production (HP) was assessed. Twenty-four adult male Sprague-Dawley rats were injected intracerebroventricularly with either 10 [mu]g mouse leptin or 10 [mu]l vehicle once per day for 4 days. At 24 h after the last injection, one group was killed while the other was placed in calorimetry chambers and monitored for 21 days of recovery. Leptin-injected rats exhibited an overshoot of food intake and respiratory quotient (RQ) during recovery, but body weight remained significantly lower up to 6 days. HP decreased in both groups over time but remained higher in the leptin group through recovery. However, retained energy (RE) was significantly greater than control for about 8 days. Overall, UCP expression was reduced at the end of recovery in parallel with the decline in HP. Brown adipose tissue (BAT) was the most responsive to leptin administration by dramatically changing UCP1 and UCP3 mRNA levels. Our data show that leptin has extended effects on energy expenditure but relieves control on food intake and RQ after treatment withdrawal. This translated into a reduced positive energy balance that slowed body weight recovery.

Suzuki, M., M. Bannai, et al. (2000). "Suppression of copulatory behavior by intracerebroventricular infusion of antisense oligodeoxynucleotide of granulin in neonatal male rats." Physiology & Behavior **68**(5): 707.

<http://www.sciencedirect.com/science/article/B6T0P-40TR8DW-G/2/b180ccb9f09d41f0371c2036f9b39a89>

Sexual dimorphism of the rodent brain is manifested by the epigenetic action of gonadal steroids. Our previous research identified the granulin (grn) precursor gene as a sex steroid-inducible gene, which was shown to be expressed more abundantly in male than female neonates at the mediobasal hypothalamic area. Grn is a 6-kDa polypeptide promoting or inhibiting the growth of epithelial cells and hematocytes in vitro. In this study, effects on male sexual behavior of male were pursued under conditions in which grn gene expression was suppressed during the critical

period. To this end, an antisense oligodeoxynucleotide (ODN) of the *grn* precursor gene was designed, incorporated into inactivated Sendai virus (HVJ)-liposome complexes, and infused into the third ventricle of 2-day-old male rats. Two different control treatments were used: the first consisted of a control sequence ODN that had little homology to known mRNAs; the second of vehicle (HVJ-liposome) alone. After maturation, animals treated with antisense ODN of *grn* displayed significantly lower scores than control males on various parameters assessing sexual behavior; i.e., mount, intromission, and ejaculation. The antisense ODN, however, did not affect body growth or serum concentrations of testosterone and luteinizing hormone. Further, there was no significant difference in the volume of the sexual dimorphic nucleus of the preoptic area between antisense ODN-treated and control animals. It was shown that inadequate expression of the *grn* gene in the brain of male neonatal rats during the critical period suppressed the induction of some type of male sexual behavior, suggesting the *grn* was involved in the process of masculinization of the rat brain.

Plasmid (3)

Forde, A. and G. F. Fitzgerald (2003). "Molecular organization of exopolysaccharide (EPS) encoding genes on the lactococcal bacteriophage adsorption blocking plasmid, pCI658." Plasmid **49**(2): 130.

<http://www.sciencedirect.com/science/article/B6WPF-47XWK13-5/2/0be58c651e7c2bb57a224849b0633c38>

The lactococcal plasmid pCI658 (58 kb) isolated from *Lactococcus lactis* ssp. *cremoris* HO2 encodes the production of a hydrophilic exopolysaccharide (EPS) which consists primarily of galactose and glucuronic acid and which interferes with adsorption of phages o712 and oc2 to cell surface receptors. Examination of the nucleotide sequence of a 21.8-kb region of the plasmid revealed a large genetic cluster consisting of at least 23 putative EPS biosynthetic determinants in addition to the presence of insertion sequences at the 5' and 3' ends. According to homology searches, the genes were organized in specific regions involved in regulation, synthesis and export of the EPS. The predicted products of individual genes exhibited significant homology to exopolysaccharide, capsular polysaccharide (CPS), and lipopolysaccharide (LPS) gene products from a variety of Gram positive and Gram negative bacteria. Evidence of a gene encoding UDP-glucose dehydrogenase is also presented and this is the first description of such a gene in *Lactococcus*.

Lambertsen, L. M., S. Molin, et al. (2004). "Transcriptional regulation of pWW0 transfer genes in *Pseudomonas putida* KT2440." Plasmid **52**(3): 169.

<http://www.sciencedirect.com/science/article/B6WPF-4D7CD1R-4/2/efc386b2d73bff5336a68522af16c8a4>

The conjugative IncP-9 plasmid pWW0 (TOL) carries transfer genes, many of whose functions can be predicted from sequence similarities to the well-studied IncW and IncP-1 plasmids, and that are clustered with the replication and maintenance genes of the plasmid core. In this study we show that the IncP-9 transfer genes are transcribed from at least three promoter regions. The promoters for *traA* and *traD* act divergently from the region found to encode the origin of transfer,

oriT. These promoters regulate expression of traA, B, and perhaps traC in one direction and traD in the other, all of whose gene products are predicted to be involved in relaxosome formation and DNA processing during transfer, and they are repressed by TraA. The third promoter region, upstream of mpfR, is responsible for transcription of mpfR and mpfA to mpfJ, encoding proteins involved in mating pair formation. Transcription from this region is negatively autoregulated by MpfR. Thus the pWW0 transfer genes, like those of the IncP-1 plasmids, are expressed at all times, but kept in control by a negative feed back loop to limit the metabolic burden on the host. Although many of the related mating pair formation systems are, as in pWW0, transcribed divergently from an operon for replication and/or stable inheritance functions, MpfR is not related to the known regulatory proteins of these other transfer systems outside those of the IncP-9 family and indeed the regulators tend to be specific for each plasmid family. This suggests that the general pattern of genetic organisation exhibited by these systems has arisen a number of times independently and must therefore be highly favourable to plasmid survival and spread.

Seubert, A., C. Falch, et al. (2003). "Characterization of the cryptic plasmid pBGR1 from *Bartonella grahamii* and construction of a versatile *Escherichia coli*-*Bartonella* spp. shuttle cloning vector." Plasmid **49**(1): 44.

<http://www.sciencedirect.com/science/article/B6WPF-47PG7XM-1/2/49a68e02e200a866092744847128eb0a>

We report herein the isolation and molecular characterization of pBGR1, the first native plasmid isolated from the genus *Bartonella*. Cloning and sequencing revealed a 2725-base pair (bp) cryptic plasmid comprising two open reading frames of considerable length, which were designated rep and mob. The regions containing rep and mob are separated by 140-bp inverted repeat sequences and display a difference in G + C content from one another. A 1435-bp SacI-BclI fragment containing the rep gene is sufficient to mediate replication in the species *Bartonella henselae* and *Bartonella tribocorum*, while this replicon does not appear to be functional in *Escherichia coli*. The Rep protein of 190 amino acids (aa) shares homology to putative replication proteins of cryptic plasmids of Gram-negative origin, which form a subgroup of the rolling-circle replication proteins of the pSN2 plasmid superfamily of Gram-positive bacteria. The Mob protein of 333 aa is related to mobilization proteins of several cryptic plasmids and is associated with a conserved recombination site A. The tra functions of RP4 can mobilize pBGR1 derivatives in a mob-dependent manner. Mobilizable pBGR1-based *E. coli*-*Bartonella* spp. shuttle vectors were constructed and were shown to be maintained in *B. tribocorum* during in vivo passage in a rat model in the absence of antibiotic selection. The small size and stability of these shuttle cloning vectors should render them particularly valuable for genetic studies in *Bartonella* spp.

PNAS (102)

Aanen, D. K., P. Eggleton, et al. (2002). "The evolution of fungus-growing termites and their mutualistic fungal symbionts." PNAS **99**(23): 14887-14892.

<http://www.pnas.org/cgi/content/abstract/99/23/14887>

We have estimated phylogenies of fungus-growing termites and their associated mutualistic fungi of the genus *Termitomyces* using Bayesian analyses of DNA sequences. Our study shows that

the symbiosis has a single African origin and that secondary domestication of other fungi or reversal of mutualistic fungi to a free-living state has not occurred. Host switching has been frequent, especially at the lower taxonomic levels, and nests of single termite species can have different symbionts. Data are consistent with horizontal transmission of fungal symbionts in both the ancestral state of the mutualism and most of the extant taxa. Clonal vertical transmission of fungi, previously shown to be common in the genus *Microtermes* (via females) and in the species *Macrotermes bellicosus* (via males) [Johnson, R. A., Thomas, R. J., Wood, T. G. & Swift, M. J. (1981) *J. Nat. Hist.* 15, 751-756], is derived with two independent origins. Despite repeated host switching, statistical tests taking phylogenetic uncertainty into account show a significant congruence between the termite and fungal phylogenies, because mutualistic interactions at higher taxonomic levels show considerable specificity. We identify common characteristics of fungus-farming evolution in termites and ants, which apply despite the major differences between these two insect agricultural systems. We hypothesize that biparental colony founding may have constrained the evolution of vertical symbiont transmission in termites but not in ants where males die after mating.

Ajdic, D., W. M. McShan, et al. (2002). "Genome sequence of *Streptococcus mutans* UA159, a cariogenic dental pathogen." *PNAS* **99**(22): 14434-14439.

<http://www.pnas.org/cgi/content/abstract/99/22/14434>

Streptococcus mutans is the leading cause of dental caries (tooth decay) worldwide and is considered to be the most cariogenic of all of the oral streptococci. The genome of *S. mutans* UA159, a serotype c strain, has been completely sequenced and is composed of 2,030,936 base pairs. It contains 1,963 ORFs, 63% of which have been assigned putative functions. The genome analysis provides further insight into how *S. mutans* has adapted to surviving the oral environment through resource acquisition, defense against host factors, and use of gene products that maintain its niche against microbial competitors. *S. mutans* metabolizes a wide variety of carbohydrates via nonoxidative pathways, and all of these pathways have been identified, along with the associated transport systems whose genes account for almost 15% of the genome. Virulence genes associated with extracellular adherent glucan production, adhesins, acid tolerance, proteases, and putative hemolysins have been identified. Strain UA159 is naturally competent and contains all of the genes essential for competence and quorum sensing. Mobile genetic elements in the form of IS elements and transposons are prominent in the genome and include a previously uncharacterized conjugative transposon and a composite transposon containing genes for the synthesis of antibiotics of the gramicidin/bacitracin family; however, no bacteriophage genomes are present.

Angenent, L. T., S. T. Kelley, et al. (2005). "Molecular identification of potential pathogens in water and air of a hospital therapy pool." *PNAS* **102**(13): 4860-4865.

<http://www.pnas.org/cgi/content/abstract/102/13/4860>

Indoor warm-water therapy pool workers in a Midwestern regional hospital were diagnosed with non-tuberculosis pulmonary hypersensitive pneumonitis and *Mycobacterium avium* infections. In response, we conducted a multiseason survey of microorganisms present in this therapy pool water, in biofilms associated with the pool containment walls, and in air immediately above the pool. The survey used culture, microscopy, and culture-independent molecular phylogenetic analyses. Although outfitted with a state-of-the-art UV-peroxide disinfection system, the numbers of bacteria in the therapy pool water were relatively high compared with the potable water used to fill the pool. Regardless of the source, direct microscopic counts of microbes were routinely {approx}1,000 times greater than conventional plate counts. Analysis of clone libraries of small

subunit rRNA genes from environmental DNA provided phylogenetic diversity estimates of the microorganisms collected in and above the pool. A survey of >1,300 rRNA genes yielded a total of 628 unique sequences, the most common of which was nearly identical to that of *M. avium* strains. The high proportion of clones with different *Mycobacterium* spp. rRNA genes suggested that such organisms comprised a significant fraction of microbes in the pool water (to >30%) and preferentially partition into aerosols (to >80%) relative to other waterborne bacteria present. The results of the study strongly validate aerosol partitioning as a mechanism for disease transfer in these environments. The results also show that culture protocols currently used by public health facilities and agencies are seriously inadequate for the detection and enumeration of potential pathogens.

Arking, D. E., A. Krebsova, et al. (2002). "Association of human aging with a functional variant of klotho." PNAS **99**(2): 856-861.

<http://www.pnas.org/cgi/content/abstract/99/2/856>

Mice deficient in Klotho gene expression exhibit a syndrome resembling premature human aging. To determine whether variation in the human KLOTHO locus contributes to survival, we applied two newly characterized polymorphic microsatellite markers flanking the gene in a population-based association study. In a cohort chosen for its homogeneity, Bohemian Czechs, we demonstrated significant differences in selected marker allele frequencies between newborn and elderly individuals ($P < 0.05$). These results precipitated a search for functional variants of klotho. We identified an allele, termed KL-VS, containing six sequence variants in complete linkage disequilibrium, two of which result in amino acid substitutions F352V and C370S. Homozygous elderly individuals were underrepresented in three distinct populations: Bohemian Czechs, Baltimore Caucasians, and Baltimore African-Americans [combined odds ratio (OR) = 2.59, $P < 0.0023$]. In a transient transfection assay, secreted levels of klotho harboring V352 are reduced 6-fold, whereas extracellular levels of the S370 form are increased 2.9-fold. The V352/S370 double mutant exhibits an intermediate phenotype (1.6-fold increase), providing a rare example of intragenic complementation in cis by human single nucleotide polymorphisms. The remarkable conservation of F352 among homologous proteins suggests that it is functionally important. The corresponding substitution, F289V, in the closest human klotho paralog with a known substrate, cBGL1, completely eliminates its ability to cleave p-nitrophenyl-[beta]-D-glucoside. These results suggest that the KL-VS allele influences the trafficking and catalytic activity of klotho, and that variation in klotho function contributes to heterogeneity in the onset and severity of human age-related phenotypes.

Bader, A. G., K. A. Felts, et al. (2003). "Y box-binding protein 1 induces resistance to oncogenic transformation by the phosphatidylinositol 3-kinase pathway." PNAS **100**(21): 12384-12389.

<http://www.pnas.org/cgi/content/abstract/100/21/12384>

Y box-binding protein 1 (YB-1) is a multifunctional protein that can act as a regulator of transcription and of translation. In chicken embryo fibroblasts transformed by the oncoproteins P3k (phosphatidylinositol 3-kinase) or Akt, YB-1 is transcriptionally down-regulated. Expression of YB-1 from a retroviral vector induces a strong cellular resistance to transformation by P3k or Akt but does not affect sensitivity to transformation by other oncoproteins, such as Src, Jun, or Qln. The YB-1-expressing cells assume a tightly adherent, flat phenotype, with YB-1 localized in the cytoplasm, and show a greatly reduced saturation density. Both cap-dependent and cap-independent translation is inhibited in these cells, but the activity of Akt remains unaffected, suggesting that YB-1 functions downstream of Akt. A YB-1 protein with a loss-of-function mutation in the RNA-binding motif no longer binds to the mRNA cap structure, is localized in the

cell nucleus, does not induce the flat cellular phenotype, and fails to interfere with P3k- or Akt-induced oncogenic transformation. This mutant also does not inhibit cap-dependent or cap-independent translation. These results suggest that YB-1 acts like a rapamycin mimic, inhibiting translational events that are required in phosphatidylinositol 3-kinase-driven oncogenic transformation.

Berlin, S. and H. Ellegren (2004). "Chicken W: A genetically uniform chromosome in a highly variable genome." PNAS **101**(45): 15967-15969.

<http://www.pnas.org/cgi/content/abstract/101/45/15967>

The Y chromosome of organisms with male heterogamety is expected to show reduced levels of genetic diversity, because the effective population size is one-fourth that of autosomes. However, studies in mammals, flies, and plants show that Y chromosome diversity is lower than expected even when differences in effective population size are taken into account. This may be explained by skewed reproductive success among males, leading to low male effective population size, or by a strong role of selection in shaping levels of nucleotide diversity in nonrecombining chromosomes. We tested these hypotheses in a system with female heterogamety by estimating nucleotide diversity in the female-specific W chromosome of the domestic chicken by resequencing of 7,643 base pairs in 47 birds from 10 highly divergent breeds. The screening revealed only one single segregating site, which is in sharp contrast to our previous observation, using a similar panel of birds of, on average, one segregating site every 39 base pairs in autosomal sequence. When taking sex-specific mutation rates and differences in effective population size into account, the observed degree of W chromosome polymorphism is 28-fold lower than expected for the frequency of segregating sites and 13-fold lower than expected for estimates of nucleotide diversity (autosomes, 6.5×10^{-3} ; W, 7.0×10^{-5}). We note that selection is the only factor that can explain the reduced diversity in the sex-limited chromosome irrespective of mode of reproduction or whether there is male or female heterogamety. Reduced variability in female-specific W chromosomes is not easily explained by sexual selection.

Bill, C. A. and J. Summers (2004). "Genomic DNA double-strand breaks are targets for hepadnaviral DNA integration." PNAS **101**(30): 11135-11140.

<http://www.pnas.org/cgi/content/abstract/101/30/11135>

Integrated hepadnaviral DNA in livers and tumors of chronic hepatitis B patients has been reported for many years. In this study, we investigated whether hepatitis B virus DNA integration occurs preferentially at sites of cell DNA damage. A single I-SceI homing endonuclease recognition site was introduced into the DNA of the chicken hepatoma cell line LMH by stable DNA transfection, and double-strand breaks were induced by transient expression of I-SceI after transfection of an I-SceI expression vector. Alteration of the target cleavage site by imprecise nonhomologous end joining occurred at a frequency of $\sim 10^{-3}$ per transfected cell. When replication of an avian hepadnavirus, duck hepatitis B virus, occurred at the time of double-strand break repair, we observed integration of viral DNA at the site of the break with a frequency of $\sim 10^{-4}$ per transfected cell. Integration depended on the production of viral double-stranded linear DNA and the expression of I-SceI, and integrated DNA was stable through at least 17 cell divisions. Integration appeared to occur through nonhomologous end joining between the viral linear DNA ends and the I-SceI-induced break, because small deletions or insertions were observed at the sites of end joining. The results suggest that integration of hepadnaviral DNA in infected livers occurs at sites of DNA damage and may indicate the presence of more widespread genetic changes caused by viral DNA integration itself.

Bradshaw, A. D., D. C. Graves, et al. (2003). "SPARC-null mice exhibit increased adiposity without significant differences in overall body weight." PNAS **100**(10): 6045-6050.

<http://www.pnas.org/cgi/content/abstract/100/10/6045>

Secreted protein acidic and rich in cysteine/osteonectin/BM-40 (SPARC) is a matrix-associated protein that elicits changes in cell shape, inhibits cell-cycle progression, and influences the synthesis of extracellular matrix (ECM). The absence of SPARC in mice gives rise to aberrations in the structure and composition of the ECM that result in generation of cataracts, development of severe osteopenia, and accelerated closure of dermal wounds. In this report we show that SPARC-null mice have greater deposits of s.c. fat and larger epididymal fat pads in comparison with wild-type mice. Similar to earlier studies of SPARC-null dermis, we observed a reduction in collagen I in SPARC-null fat pads in comparison with wild-type. Although elevated levels of serum leptin were observed in SPARC-null mice, their overall body weights were not significantly different from those of wild-type counterparts. The diameters of adipocytes from SPARC-null versus wild-type epididymal fat pads were 252 ± 61 and 161 ± 33 μm (means $\pm$ SD), respectively, and there was an increase in adipocyte number within SPARC-null fat pads in comparison with wild-type pads. Thus the absence of SPARC appears to result in an increase in the size of individual adipocytes as well as an increase in the number of adipocytes per fat pad. In fat pads isolated from wild-type mice, SPARC mRNA was associated with both the stromal/vascular and adipocyte fractions. We propose that SPARC limits the accumulation of adipose tissue in mice in part through its demonstrated effects on the regulation of cell shape and production of ECM.

Busto, R., A. V. Schally, et al. (2002). "The expression of growth hormone-releasing hormone (GHRH) and splice variants of its receptor in human gastroenteropancreatic carcinomas." PNAS **99**(18): 11866-11871.

<http://www.pnas.org/cgi/content/abstract/99/18/11866>

Splice variants (SVs) of receptors for growth hormone-releasing hormone (GHRH) have been found in primary human prostate cancers and diverse human cancer cell lines. GHRH antagonists inhibit growth of various experimental human cancers, including pancreatic and colorectal, xenografted into nude mice or cultured in vitro, and their antiproliferative action could be mediated in part through SVs of GHRH receptors. In this study we examined the expression of mRNA for GHRH and for SVs of its receptors in tumors of human pancreatic, colorectal, and gastric cancer cell lines grown in nude mice. mRNA for both GHRH and SV1 isoform of GHRH receptors was expressed in tumors of pancreatic (SW1990, PANC-1, MIA PaCa-2, Capan-1, Capan-2, and CFPAC1), colonic (COLO 320DM and HT-29), and gastric (NCI-N87, HS746T, and AGS) cancer cell lines; mRNA for SV2 was also present in Capan-1, Capan-2, CFPAC1, HT-29, and NCI-N87 tumors. In proliferation studies in vitro, the growth of pancreatic, colonic, and gastric cancer cells was stimulated by GHRH(1-29)NH₂ and inhibited by GHRH antagonist JV-1-38. The stimulation of some gastroenteropancreatic cancer cells by GHRH was followed by an increase in cAMP production, and GHRH antagonist JV-1-38 competitively inhibited this effect. Our study indicates the presence of an autocrine/paracrine stimulatory loop based on GHRH and SV1 of GHRH receptors in human pancreatic, colorectal, and gastric cancers. The finding of SV1 receptor in human cancers provides an approach to an antitumor therapy based on the blockade of this receptor by specific GHRH antagonists.

Caramelli, D., C. Lalueza-Fox, et al. (2003). "Evidence for a genetic discontinuity between Neandertals and 24,000-year-old anatomically modern Europeans." PNAS **100**(11): 6593-6597.

<http://www.pnas.org/cgi/content/abstract/100/11/6593>

During the late Pleistocene, early anatomically modern humans coexisted in Europe with the anatomically archaic Neandertals for some thousand years. Under the recent variants of the multiregional model of human evolution, modern and archaic forms were different but related populations within a single evolving species, and both have contributed to the gene pool of current humans. Conversely, the Out-of-Africa model considers the transition between Neandertals and anatomically modern humans as the result of a demographic replacement, and hence it predicts a genetic discontinuity between them. Following the most stringent current standards for validation of ancient DNA sequences, we typed the mtDNA hypervariable region I of two anatomically modern *Homo sapiens sapiens* individuals of the Cro-Magnon type dated at about 23 and 25 thousand years ago. Here we show that the mtDNAs of these individuals fall well within the range of variation of today's humans, but differ sharply from the available sequences of the chronologically closer Neandertals. This discontinuity is difficult to reconcile with the hypothesis that both Neandertals and early anatomically modern humans contributed to the current European gene pool.

Carroll, J., M. Neitz, et al. (2004). "Functional photoreceptor loss revealed with adaptive optics: An alternate cause of color blindness." PNAS **101**(22): 8461-8466.

<http://www.pnas.org/cgi/content/abstract/101/22/8461>

There is enormous variation in the X-linked L/M (long/middle wavelength sensitive) gene array underlying "normal" color vision in humans. This variability has been shown to underlie individual variation in color matching behavior. Recently, red-green color blindness has also been shown to be associated with distinctly different genotypes. This has opened the possibility that there may be important phenotypic differences within classically defined groups of color blind individuals. Here, adaptive optics retinal imaging has revealed a mechanism for producing dichromatic color vision in which the expression of a mutant cone photopigment gene leads to the loss of the entire corresponding class of cone photoreceptor cells. Previously, the theory that common forms of inherited color blindness could be caused by the loss of photoreceptor cells had been discounted. We confirm that remarkably, this loss of one-third of the cones does not impair any aspect of vision other than color.

Castriconi, R., A. Dondero, et al. (2004). "Identification of 4Ig-B7-H3 as a neuroblastoma-associated molecule that exerts a protective role from an NK cell-mediated lysis." PNAS **101**(34): 12640-12645.

<http://www.pnas.org/cgi/content/abstract/101/34/12640>

In this study, in an attempt to identify neuroblastoma-associated surface antigens, we generated mAbs against the ACN neuroblastoma cell line. A mAb was selected (5B14) that reacted with all neuroblastoma cell lines analyzed and allowed detection of tumor cell infiltrates in bone marrow aspirates from neuroblastoma patients. In cytofluorimetric analysis, unlike anti-disialoganglioside mAb, 5B14 mAb did not display reactivity with normal bone marrow hematopoietic cell precursors, thus representing a highly specific marker for identifying neuroblastoma cells. Molecular analysis revealed that the 5B14 mAb-reactive surface glycoprotein corresponded to the recently identified 4Ig-B7-H3 molecule. Remarkably, mAb-mediated masking of the 4Ig-B7-H3

molecule on cell transfectants or on freshly isolated neuroblastoma cells resulted in enhancement of natural killer-mediated lysis of these target cells. These data suggest that 4Ig-B7-H3 molecules expressed at the tumor cell surface can exert a protective role from natural killer-mediated lysis by interacting with a still undefined inhibitory receptor expressed on natural killer cells.

Cong, B., J. Liu, et al. (2002). "Natural alleles at a tomato fruit size quantitative trait locus differ by heterochronic regulatory mutations." PNAS **99**(21): 13606-13611.

<http://www.pnas.org/cgi/content/abstract/99/21/13606>

fw2.2 is a major quantitative trait locus that accounts for as much as 30% of the difference in fruit size between wild and cultivated tomatoes. Evidence thus far indicates that fw2.2 alleles modulate fruit size through changes in gene regulation rather than in the FW2.2 protein itself. To investigate the nature of these regulatory changes and the manner in which they may affect fruit size, a pair of nearly isogenic lines has been subjected to detailed developmental, transcriptional, mitotic, and in situ hybridization studies. The results indicate that the large- and small-fruited alleles of fw2.2 differ in peak transcript levels by [approx]1 week. Moreover, this difference in timing of expression is associated with concomitant changes in mitotic activity in the early stage of fruit development. The changes in timing of gene expression (heterochronic allelic variation), combined with overall differences in total transcript levels, are sufficient to account for a large portion phenotypic differences in fruit weight associated with the two alleles.

Crooks, D. M., G. Pacheco-Rodriguez, et al. (2004). "Molecular and genetic analysis of disseminated neoplastic cells in lymphangioleiomyomatosis." PNAS **101**(50): 17462-17467.

<http://www.pnas.org/cgi/content/abstract/101/50/17462>

Lymphangioleiomyomatosis (LAM) is a multisystem disorder of women, characterized by cystic degeneration of the lungs, renal angiomyolipomas (AML), and lymphatic abnormalities. LAM lesions result from the proliferation of benign-appearing, smooth muscle-like LAM cells, which are characterized by loss of heterozygosity (LOH) of one of the tuberous sclerosis complex (TSC) genes. LAM cells are believed to migrate among the involved organs. Because of the apparently metastatic behavior of LAM, we tried to isolate LAM cells from body fluids. A cell fraction separated by density gradient centrifugation from blood had TSC2 LOH in 33 of 60 (55%) LAM patients. Cells with TSC2 LOH were also found in urine from 11 of 14 (79%) patients with AML and in chylous fluid from 1 of 3 (33%) patients. Identification of LAM cells with TSC2 LOH in body fluids was not correlated with severity of lung disease or extrapulmonary involvement and was found in one patient after double lung transplantation. These studies are compatible with a multisite origin for LAM cells. They establish the existence of disseminated, potentially metastatic LAM cells through a relatively simple, noninvasive procedure that should be valuable for molecular and genetic studies of somatic mutations in LAM and perhaps other metastatic diseases.

Dawson, S. C. and N. R. Pace (2002). "Novel kingdom-level eukaryotic diversity in anoxic environments." PNAS **99**(12): 8324-8329.

<http://www.pnas.org/cgi/content/abstract/99/12/8324>

Molecular evolutionary studies of eukaryotes have relied on a sparse collection of gene

sequences that do not represent the full range of eukaryotic diversity in nature. Anaerobic microbes, particularly, have had little representation in phylogenetic studies. Such organisms are the least known of eukaryotes and probably are the most phylogenetically diverse. To provide fresh perspective on the natural diversity of eukaryotes in anoxic environments and also to discover novel sequences for evolutionary studies, we conducted a cultivation-independent, molecular phylogenetic survey of three anoxic sediments, including both freshwater and marine samples. Many previously unrecognized eukaryotes were identified, including representatives of seven lineages that are not specifically related to any known organisms at the kingdom-level and branch below the eukaryotic "crown" radiation of animals, plants, fungi, stramenopiles, etc. The survey additionally identified new sequences characteristic of known ecologically important eukaryotic groups with anaerobic members. Phylogenetic analyses with the new sequences enhance our understanding of the diversity and pattern of eukaryotic evolution.

Doi, T., K. Kinoshita, et al. (2003). "Inaugural Article: De novo protein synthesis is required for the activation-induced cytidine deaminase function in class-switch recombination." PNAS **100**(5): 2634-2638.

<http://www.pnas.org/cgi/content/abstract/100/5/2634>

Activation-induced cytidine deaminase (AID) is required for class-switch recombination (CSR), somatic hypermutation, and gene conversion of Ig genes. Although AID has sequence similarity to an RNA-editing enzyme Apobec-1, how AID functions in CSR and somatic hypermutation is unknown. Because involvement of RNA-editing but not DNA-editing in CSR requires de novo protein synthesis after AID expression, we examined whether protein synthesis inhibitors could block CSR in the presence of the AID activity. For this purpose we constructed AID fused with the hormone-binding domain of the estrogen receptor (AID-ER), which was introduced into AID-deficient spleen B cells. When such transfectants were treated with an estrogen analogue, 4-hydroxytamoxifen (OHT), CSR was induced within 1 h. Cycloheximide or puromycin drastically suppressed OHT-induced CSR in AID-ER expressing AID^{-/-} B cells when added 1 h before OHT but not after OHT, suggesting that de novo protein synthesis is required for an event downstream to AID expression in CSR. The results lend the weight to RNA-editing hypothesis for the function of AID.

Dominski, Z., X.-c. Yang, et al. (2003). "Cloning and characterization of the Drosophila U7 small nuclear RNA." PNAS **100**(16): 9422-9427.

<http://www.pnas.org/cgi/content/abstract/100/16/9422>

Base pairing between the 5' end of U7 small nuclear RNA (snRNA) and the histone downstream element (HDE) in replication-dependent histone pre-mRNAs is the key event in 3'-end processing that leads to generation of mature histone mRNAs. We have cloned the Drosophila U7 snRNA and demonstrated that it is required for histone pre-mRNA 3'-end processing in a Drosophila nuclear extract. The 71-nt Drosophila U7 snRNA is encoded by a single gene that is embedded in the direct orientation in an intron of the Eip63E gene. The U7 snRNA gene contains conserved promoter elements typical of other Drosophila snRNA genes, and the coding sequence is followed by a 3' box indicating that the Drosophila U7 snRNA gene is an independent transcription unit. Drosophila U7 snRNA contains a trimethyl-guanosine cap at the 5' end and a putative Sm-binding site similar to the unique Sm-binding site found in other U7 snRNAs. Drosophila U7 snRNA is {approx}10 nt longer than mammalian U7 snRNAs because of an extended 5' sequence and has only a limited potential to form a stem-loop structure near the 3' end. The extended 5' end of Drosophila U7 snRNA can base pair with the HDE in all five Drosophila histone pre-mRNAs. Blocking the 5' end of the U7 snRNA with a complementary

oligonucleotide specifically blocks processing of a *Drosophila* histone pre-mRNA. Changes in the HDE that abolish or decrease processing efficiency result in a reduced ability to recruit U7 snRNA to the pre-mRNA.

Drummond, D. A., J. J. Silberg, et al. (2005). "On the conservative nature of intragenic recombination." PNAS **102**(15): 5380-5385.

<http://www.pnas.org/cgi/content/abstract/102/15/5380>

Intragenic recombination rapidly creates protein sequence diversity compared with random mutation, but little is known about the relative effects of recombination and mutation on protein function. Here, we compare recombination of the distantly related β -lactamases PSE-4 and TEM-1 to mutation of PSE-4. We show that, among β -lactamase variants containing the same number of amino acid substitutions, variants created by recombination retain function with a significantly higher probability than those generated by random mutagenesis. We present a simple model that accurately captures the differing effects of mutation and recombination in real and simulated proteins with only four parameters: (i) the amino acid sequence distance between parents, (ii) the number of substitutions, (iii) the average probability that random substitutions will preserve function, and (iv) the average probability that substitutions generated by recombination will preserve function. Our results expose a fundamental functional enrichment in regions of protein sequence space accessible by recombination and provide a framework for evaluating whether the relative rates of mutation and recombination observed in nature reflect the underlying imbalance in their effects on protein function.

Dvorak, M. M., A. Siddiqua, et al. (2004). "Physiological changes in extracellular calcium concentration directly control osteoblast function in the absence of calciotropic hormones." PNAS **101**(14): 5140-5145.

<http://www.pnas.org/cgi/content/abstract/101/14/5140>

We investigated the direct effects of changes in free ionized extracellular calcium concentrations ($[Ca^{2+}]_o$) on osteoblast function and the involvement of the calcium-sensing receptor (CaR) in mediating these responses. CaR mRNA and protein were detected in osteoblast models, freshly isolated fetal rat calvarial cells and murine clonal osteoblastic 2T3 cells, and in freshly frozen, undecalcified preparations of human mandible and rat femur. In fetal rat calvarial cells, elevating $[Ca^{2+}]_o$ and treatment with gadolinium, a nonpermeant CaR agonist, resulted in phosphorylation of the extracellular signal-regulated kinases 1 and 2, Akt, and glycogen synthase kinase 3 β , consistent with signals of cell survival and proliferation. In agreement, cell number was increased under these conditions. Expression of the osteoblast differentiation markers core binding factor $\alpha 1$, osteocalcin, osteopontin, and collagen I mRNAs was increased by high $[Ca^{2+}]_o$, as was mineralized nodule formation. Alkaline phosphatase activity was maximal for $[Ca^{2+}]_o$ between 1.2 and 1.8 mM. Inhibition of CaR by NPS 89636 blocked responses to the CaR agonists. In conclusion, we show that small deviations of $[Ca^{2+}]_o$ from physiological values have a profound impact on bone cell fate, by means of the CaR and independently of systemic calciotropic peptides.

Ericsson, T. A., Y. Takeuchi, et al. (2003). "Identification of receptors for pig endogenous retrovirus." PNAS **100**(11): 6759-6764.

<http://www.pnas.org/cgi/content/abstract/100/11/6759>

Xenotransplantation of porcine tissues has the potential to treat a wide variety of major health problems including organ failure and diabetes. Balanced against the potential benefits of xenotransplantation, however, is the risk of human infection with a porcine microorganism. In particular, the transmission of porcine endogenous retrovirus (PERV) is a major concern [Chapman, L. E. & Bloom, E. T. (2001) *J. Am. Med. Assoc.* 285, 2304-2306]. Here we report the identification of two, sequence-related, human proteins that act as receptors for PERV-A, encoded by genes located on chromosomes 8 and 17. We also describe homologs from baboon and porcine cells that also are active as receptors. Conversely, activity could not be demonstrated with a syntenic murine receptor homolog. Sequence analysis indicates that PERV-A receptors [human PERV-A receptor (HuPAR)-1, HuPAR-2, baboon PERV-A receptor 2, and porcine PERV-A receptor] are multiple membrane-spanning proteins similar to receptors for other gammaretroviruses. Expression is widespread in human tissues including peripheral blood mononuclear cells, but their biological functions are unknown. The identification of the PERV-A receptors opens avenues of research necessary for a more complete assessment of the retroviral risks of pig to human xenotransplantation.

Esteve, P.-O., H. G. Chin, et al. (2005). "Human maintenance DNA (cytosine-5)-methyltransferase and p53 modulate expression of p53-repressed promoters." *PNAS* **102**(4): 1000-1005.

<http://www.pnas.org/cgi/content/abstract/102/4/1000>

DNA (cytosine-5)-methyltransferase (DNMT) 1 participates in transcriptional repression of genes by methylation-dependent and -independent mechanisms. Here, DNMT1 is shown to bind p53 and colocalize in the nucleus. DNMT1-mediated methylation is stimulated by p53 in vitro. Upon p53 induction, a reporter construct containing the antiapoptotic gene survivin promoter, which contains a natural p53 binding site, was methylated in WT HCT116 cells but not in DNMT1 null or p53 null cells. Endogenous survivin gene repression involves cooperation between DNMT1 and p53 and is relieved by introduction of DNMT1- or p53-specific small inhibitory RNA. DNMT1 null cells did not exhibit a significant repressive effect for p53 responsive survivin and cdc25C gene expression compared with the parental cells. Normal human fibroblasts also exhibited similar DNMT1- and p53-mediated methylation of the survivin promoter, suggesting cooperation between p53 and DNMT1 in gene silencing.

Fearnhead, N. S., J. L. Wilding, et al. (2004). "Multiple rare variants in different genes account for multifactorial inherited susceptibility to colorectal adenomas." *PNAS* **101**(45): 15992-15997.

<http://www.pnas.org/cgi/content/abstract/101/45/15992>

Clear-cut inherited Mendelian traits, such as familial adenomatous polyposis or hereditary nonpolyposis colorectal cancer, account for <4% of colorectal cancers. Another 20% of all colorectal cancers are thought to occur in individuals with a significant inherited multifactorial susceptibility to colorectal cancer that is not obviously familial. Incompletely penetrant, comparatively rare missense variants in the adenomatous polyposis coli gene, which is responsible for familial adenomatous polyposis, have been described in patients with multiple colorectal adenomas. These variants represent a category of variation that has been suggested, quite generally, to account for a substantial fraction of such multifactorial inherited susceptibility. The aim of this study was to explore this rare variant hypothesis for multifactorial inheritance by using multiple colorectal adenomas as the model. Patients with multiple adenomas were screened for germ-line variants in a panel of candidate genes. Germ-line DNA was obtained from

124 patients with between 3 and 100 histologically proven synchronous or metachronous adenomatous polyps. All patients were tested for the adenomatous polyposis coli variants I1307K and E1317Q, and variants were also sought in AXIN1 (axin), CTNNB1 (β -catenin), and the mismatch repair genes hMLH1 and hMSH2. The control group consisted of 483 random controls. Thirty of 124 (24.9%) patients carried potentially pathogenic germ-line variants as compared with 55 (approx 12%) of the controls. This overall difference is highly significant, suggesting that many rare variants collectively contribute to the inherited susceptibility to colorectal adenomas.

Gaskin, J. F. and B. A. Schaal (2002). "Hybrid Tamarix widespread in U.S. invasion and undetected in native Asian range." PNAS **99**(17): 11256-11259.

<http://www.pnas.org/cgi/content/abstract/99/17/11256>

Biological invasions are drastically altering natural habitats and threatening biodiversity on both local and global levels. In one of the United States' worst invasions, Eurasian Tamarix plant species have spread rapidly to dominate over 600,000 riparian and wetland hectares. The largest Tamarix invasion consists of Tamarix chinensis and Tamarix ramosissima, two morphologically similar species. To clarify the identity, origins, and population structuring of this invasion, we analyzed DNA sequence data from an intron of a nuclear gene, phosphoenolpyruvate carboxylase (PepC). This intron proved to be highly variable at the population level, and the 269 native and invasive specimens yielded 58 haplotypes, from which we constructed a gene genealogy. Only four of these haplotypes were common to both the U.S. and Eurasia. Surprisingly, we found that the most common plant in this U.S. invasion is a hybrid combination of two species-specific genotypes that were geographically isolated in their native Eurasian range. Less extensive hybrids exist in the invasion, involving combinations of T. ramosissima and T. chinensis with Tamarix parviflora and Tamarix gallica. The presence of potentially novel hybrids in the U.S. illustrates how importation of exotics can alter population structures of species and contribute to invasions.

Goldammer, T., S. R. Kata, et al. (2002). "A comparative radiation hybrid map of bovine chromosome 18 and homologous chromosomes in human and mice." PNAS **99**(4): 2106-2111.

<http://www.pnas.org/cgi/content/abstract/99/4/2106>

A comprehensive radiation hybrid (RH) map and a high resolution comparative map of Bos taurus (BTA) chromosome 18 were constructed, composed of 103 markers and 76 markers, respectively, by using a cattle-hamster somatic hybrid cell panel and a 5,000 rad whole-genome radiation hybrid (WGRH) panel. These maps include 65 new assignments (56 genes, 3 expressed-sequence tags, 6 microsatellites) and integrate 38 markers from the first generation WGRH5,000 map of BTA18. Fifty-nine assignments of coding sequences were supported by somatic hybrid cell mapping to markers on BTA18. The total length of the comprehensive map was 1666 cR5,000. Break-point positions within the chromosome were refined and a new telomeric RH linkage group was established. Conserved synteny between cattle, human, and mouse was found for 76 genes of BTA18 and human chromosomes (HSA) 16 and 19 and for 34 cattle genes and mouse chromosomes (MMU) 7 and 8. The new RH map is potentially useful for the identification of candidate genes for economically important traits, contributes to the expansion of the existing BTA18 gene map, and provides new information about the chromosome evolution in cattle, humans, and mice.

Grundemann, D., S. Harlfinger, et al. (2005). "Discovery of the ergothioneine transporter." PNAS **102**(14): 5256-5261.

<http://www.pnas.org/cgi/content/abstract/102/14/5256>

Variants of the SLC22A4 gene are associated with susceptibility to rheumatoid arthritis and Crohn's disease. SLC22A4 codes for an integral membrane protein, OCTN1, that has been presumed to carry organic cations like tetraethylammonium across the plasma membrane. Here, we show that the key substrate of this transporter is in fact ergothioneine (ET). Human OCTN1 was expressed in 293 cells. A substrate lead, stachydrine (alias proline betaine), was identified by liquid chromatography MS difference shading, a new substrate search strategy. Analysis of transport efficiency of stachydrine-related solutes, affinity, and Na⁺ dependence indicates that the physiological substrate is ET. Efficiency of transport of ET was as high as 195 {micro}l per min per mg of protein. By contrast, the carnitine transporter OCTN2 from rat did not transport ET at all. Because ET is transported >100 times more efficiently than tetraethylammonium and carnitine, we propose the functional name ETT (ET transporter) instead of OCTN1. ET, all of which is absorbed from food, is an intracellular antioxidant with metal ion affinity. Its particular purpose is unresolved. Cells with expression of ETT accumulate ET to high levels and avidly retain it. By contrast, cells lacking ETT do not accumulate ET, because their plasma membrane is virtually impermeable for this compound. The real-time PCR expression profile of human ETT, with strong expression in CD71+ cells, is consistent with a pivotal function of ET in erythrocytes. Moreover, prominent expression of ETT in monocytes and SLC22A4 polymorphism associations suggest a protective role of ET in chronic inflammatory disorders.

Guo, L., J. Hu-Li, et al. (2002). "In TH2 cells the Il4 gene has a series of accessibility states associated with distinctive probabilities of IL-4 production." PNAS **99**(16): 10623-10628.

<http://www.pnas.org/cgi/content/abstract/99/16/10623>

TH2 clones may produce very variable amounts of IL-4. Among six TH2 clones prepared from homozygous or heterozygous mice in which Gfp replaced the first exon of Il4, a range of patterns of CpG methylation in the Il4/Il13 locus was observed correlating with the degree of expression of IL-4 or green fluorescence protein. Patterns of histone acetylation also showed differences between "high" and "low" TH2 clones. These results indicate that in TH2 cells the Il4 locus may display variable patterns of chromatin accessibility associated with distinct degrees of IL-4 expression. This finding suggests a regulation of IL-4 expression keyed to the function of this cytokine in cell/cell interactions and in the regulation of threshold responses.

Hamaguchi, M., J. L. Meth, et al. (2002). "DBC2, a candidate for a tumor suppressor gene involved in breast cancer." PNAS **99**(21): 13647-13652.

<http://www.pnas.org/cgi/content/abstract/99/21/13647>

A previously uncharacterized gene, DBC2 (deleted in breast cancer), was cloned from a homozygously deleted region at human chromosome 8p21. DBC2 contains a highly conserved RAS domain and two putative protein interacting domains. Our analyses indicate that DBC2 is the best candidate tumor suppressor gene from this region. It lies within the epicenter of the deletions and is homozygously deleted in 3.5% (7/200) of breast tumors. Mutation analysis of DBC2 led to discovery of two instances of somatic missense mutations in breast tumor specimens, whereas no missense mutations were found in other candidates from the region. Unlike other genes in the region, expression of DBC2 is often extinguished in breast cancer cells or tissues. Moreover, our

functional analysis revealed that DBC2 expression in breast cancer cells lacking DBC2 transcripts causes growth inhibition. By contrast, expression of a somatic mutant discovered in a breast cancer specimen does not suppress the growth of breast cancer cells.

Hanley, P. J., B. Musset, et al. (2004). "Extracellular ATP induces oscillations of intracellular Ca²⁺ and membrane potential and promotes transcription of IL-6 in macrophages." *PNAS* **101**(25): 9479-9484.

<http://www.pnas.org/cgi/content/abstract/101/25/9479>

The effects of low concentrations of extracellular ATP on cytosolic Ca²⁺, membrane potential, and transcription of IL-6 were studied in monocyte-derived human macrophages. During inflammation or infection many cells secrete ATP. We show here that application of 10 {micro}M ATP or 10 {micro}M UTP induces oscillations in cytosolic Ca²⁺ with a frequency of {approx}12 min⁻¹ and oscillations in membrane potential. RT-PCR analysis showed expression of P2Y1, P2Y2, P2Y11, P2X1, P2X4, and P2X7 receptors, large-conductance (KCNMA1 and KCNMB1-4), and intermediate-conductance (KCNN4) Ca²⁺-activated K⁺ channels. The Ca²⁺-oscillations were unchanged after removal of extracellular Ca²⁺, indicating that they were mainly due to movements of Ca²⁺ between intracellular compartments. Comparison of the effects of different nucleotides suggests that the Ca²⁺ oscillations were elicited by activation of P2Y2 receptors coupled to phospholipase C. Patch-clamp experiments showed that ATP induced a transient depolarization, probably mediated by activation of P2X4 receptors, followed by membrane potential oscillations due to opening of Ca²⁺-activated K⁺ channels. We also found that 10 {micro}M ATP{gamma}S increased transcription of IL-6 {approx}40-fold within 2 h. This effect was abolished by blockade of P2Y receptors with 100 {micro}M suramin. Our results suggest that ATP released from inflamed, damaged, or metabolically impaired cells represents a "danger signal" that plays a major role in activating the innate immune system.

Hearn, M. G., Y. Ren, et al. (2002). "A Drosophila dopamine 2-like receptor: Molecular characterization and identification of multiple alternatively spliced variants." *PNAS* **99**(22): 14554-14559.

<http://www.pnas.org/cgi/content/abstract/99/22/14554>

Dopamine is an important neurotransmitter in the central nervous system of both Drosophila and mammals. Despite the evolutionary distance, functional parallels exist between the fly and mammalian dopaminergic systems, with both playing roles in modulating locomotor activity, sexual function, and the response to drugs of abuse. In mammals, dopamine exerts its effects through either dopamine 1-like (D1-like) or D2-like G protein-coupled receptors. Although pharmacologic data suggest the presence of both receptor subtypes in insects, only cDNAs encoding D1-like proteins have been isolated previously. Here we report the cloning and characterization of a newly discovered Drosophila dopamine receptor. Sequence analysis reveals that this putative protein shares highest homology with known mammalian dopamine 2-like receptors. Eight isoforms of the Drosophila D2-like receptor (DD2R) transcript have been identified, each the result of alternative splicing. The encoded heptahelical receptors range in size from 461 to 606 aa, with variability in the length and sequence of the third intracellular loop. Pharmacologic assessment of three DD2R isoforms, DD2R-606, DD2R-506, and DD2R-461, revealed that among the endogenous biogenic amines, dopamine is most potent at each receptor. As established for mammalian D2-like receptors, stimulation of the Drosophila homologs with dopamine triggers pertussis toxin-sensitive Gi/o-mediated signaling. The D2-like receptor agonist, bromocriptine, has nanomolar potency at DD2R-606, -506, and -461, whereas multiple D2-like receptor antagonists (as established with mammalian receptors) have markedly reduced if any affinity when assessed at the fly receptor isoforms. The isolation of cDNAs

encoding *Drosophila* D2-like receptors extends the range of apparent parallels between the dopaminergic system in flies and mammals. Pharmacologic and genetic manipulation of the DD2Rs will provide the opportunity to better define the physiologic role of these proteins in vivo and further explore the utility of invertebrates as a model system for understanding dopaminergic function in higher organisms.

Hinman, V. F., A. T. Nguyen, et al. (2003). "Developmental gene regulatory network architecture across 500 million years of echinoderm evolution." PNAS **100**(23): 13356-13361.

<http://www.pnas.org/cgi/content/abstract/100/23/13356>

Evolutionary change in morphological features must depend on architectural reorganization of developmental gene regulatory networks (GRNs), just as true conservation of morphological features must imply retention of ancestral developmental GRN features. Key elements of the provisional GRN for embryonic endomesoderm development in the sea urchin are here compared with those operating in embryos of a distantly related echinoderm, a starfish. These animals diverged from their common ancestor 520-480 million years ago. Their endomesodermal fate maps are similar, except that sea urchins generate a skeletogenic cell lineage that produces a prominent skeleton lacking entirely in starfish larvae. A relevant set of regulatory genes was isolated from the starfish *Asterina miniata*, their expression patterns determined, and effects on the other genes of perturbing the expression of each were demonstrated. A three-gene feedback loop that is a fundamental feature of the sea urchin GRN for endoderm specification is found in almost identical form in the starfish: a detailed element of GRN architecture has been retained since the Cambrian Period in both echinoderm lineages. The significance of this retention is highlighted by the observation of numerous specific differences in the GRN connections as well. A regulatory gene used to drive skeletogenesis in the sea urchin is used entirely differently in the starfish, where it responds to endomesodermal inputs that do not affect it in the sea urchin embryo. Evolutionary changes in the GRNs since divergence are limited sharply to certain cis-regulatory elements, whereas others have persisted unaltered.

Hoffmann, E., K. Mahmood, et al. (2002). "Rescue of influenza B virus from eight plasmids." PNAS **99**(17): 11411-11416.

<http://www.pnas.org/cgi/content/abstract/99/17/11411>

Influenza B virus causes a significant amount of morbidity and mortality, yet the systems to produce high yield inactivated vaccines for these viruses have lagged behind the development of those for influenza A virus. We have established a plasmid-only reverse genetics system for the generation of recombinant influenza B virus that facilitates the generation of vaccine viruses without the need for time consuming coinfection and selection procedures currently required to produce reassortants. We cloned the eight viral cDNAs of influenza B/Yamanashi/166/98, which yields relatively high titers in embryonated chicken eggs, between RNA polymerase I and RNA polymerase II transcription units. Virus was detected as early as 3 days after transfection of cocultured COS7 and Madin-Darby canine kidney cells and achieved levels of 10⁶-10⁷ plaque-forming units per ml of cell supernatant 6 days after transfection. The full-length sequence of the recombinant virus after passage into embryonated chicken eggs was identical to that of the input plasmids. To improve the utility of the eight-plasmid system for generating 6 + 2 reassortants from recently circulating influenza B strains, we optimized the reverse transcriptase-PCR for cloning of the hemagglutinin (HA) and neuraminidase (NA) segments. The six internal genes of B/Yamanashi/166/98 were used as the backbone to generate 6 + 2 reassortants including the HA and NA gene segments from B/Victoria/504/2000, B/Hong Kong/330/2001, and B/Hawaii/10/2001. Our results demonstrate that the eight-plasmid system can be used for the

generation of high yields of influenza B virus vaccines expressing current HA and NA glycoproteins from either of the two lineages of influenza B virus.

Huang, X., S. M. Gollin, et al. (2002). "High-resolution mapping of the 11q13 amplicon and identification of a gene, TAOS1, that is amplified and overexpressed in oral cancer cells." PNAS **99**(17): 11369-11374.

<http://www.pnas.org/cgi/content/abstract/99/17/11369>

Amplification of chromosomal band 11q13 is a common event in human cancer. It has been reported in about 45% of head and neck carcinomas and in other cancers including esophageal, breast, liver, lung, and bladder cancer. To understand the mechanism of 11q13 amplification and to identify the potential oncogene(s) driving it, we have fine-mapped the structure of the amplicon in oral squamous cell carcinoma cell lines and localized the proximal and distal breakpoints. A 5-Mb physical map of the region has been prepared from which sequence is available. We quantified copy number of sequence-tagged site markers at 42-550 kb intervals along the length of the amplicon and defined the amplicon core and breakpoints by using TaqMan-based quantitative microsatellite analysis. The core of the amplicon maps to a 1.5-Mb region. The proximal breakpoint localizes to two intervals between sequence-tagged site markers, 550 kb and 160 kb in size, and the distal breakpoint maps to a 250 kb interval. The cyclin D1 gene maps to the amplicon core, as do two new expressed sequence tag clusters. We have analyzed one of these expressed sequence tag clusters and now report that it contains a previously uncharacterized gene, TAOS1 (tumor amplified and overexpressed sequence 1), which is both amplified and overexpressed in oral cancer cells. The data suggest that TAOS1 may be an amplification-dependent candidate oncogene with a role in the development and/or progression of human tumors, including oral squamous cell carcinomas. The approach described here should be useful for characterizing amplified genomic regions in a wide variety of tumors.

Hubert, N. and M. W. Hentze (2002). "Previously uncharacterized isoforms of divalent metal transporter (DMT)-1: Implications for regulation and cellular function." PNAS **99**(19): 12345-12350.

<http://www.pnas.org/cgi/content/abstract/99/19/12345>

Divalent metal transporter 1 (DMT1) mediates apical iron uptake into duodenal enterocytes and also transfers iron from the endosome into the cytosol after cellular uptake via the transferrin receptor. Hence, mutations in DMT1 cause systemic iron deficiency and anemia. DMT1 mRNA levels are increased in the duodenum of iron-deficient animals. This regulation has been observed for DMT1 mRNA harboring an iron-responsive element (IRE) in its 3' UTR, but not for a processing variant lacking a 3'UTR IRE, suggesting that the IRE regulates the expression of DMT1 mRNA in response to iron levels. Here, we show that iron regulation of DMT1 involves the expression of a previously unrecognized upstream 5' exon (exon 1A) of the human and murine DMT1 gene. The expression of this previously uncharacterized 5' exon is tissue-specific and particularly prevalent in the duodenum and kidney. It adds an in-frame AUG translation initiation codon extending the DMT1 ORF by a conserved sequence of 29-31 amino acids. In combination with the IRE- and non-IRE variants in the 3'UTR, our results reveal the existence of four DMT1 mRNA isoforms predicting the synthesis of four different DMT1 proteins. We show that two regulatory regions, the 5' promoter/exon 1A region and the IRE-containing terminal exon participate in iron regulation of DMT1 expression, which operate in a tissue-specific way. These results uncover an unexpected complexity of DMT1 expression and regulation, with implications for understanding the physiology, cell biology, and pathophysiology of mammalian iron metabolism.

Jiang, H., S. Curran, et al. (2003). "Regulatory CD8⁺ T cells fine-tune the myelin basic protein-reactive T cell receptor V β repertoire during experimental autoimmune encephalomyelitis." PNAS **100**(14): 8378-8383.

<http://www.pnas.org/cgi/content/abstract/100/14/8378>

A significant number of self-reactive T cell clones escape thymic negative selection and are released into the periphery, where some are potentially pathogenic. The clonal expansion of self-reactive T cells is known to be limited during initial antigen encounter by apoptotic or anergic mechanisms, regulatory CD4⁺ T cells, and cytokines. Here we report that superimposed on these mechanisms, during the evolution of autoimmunity in experimental autoimmune encephalomyelitis (EAE), CD8⁺ T cells are induced, which fine-tune the peripheral self-reactive T cell receptor (TCR) repertoire. We assayed the myelin basic protein-reactive TCR repertoire in naive, EAE-recovered mice as well as EAE-recovered mice depleted of CD8⁺ T cells by TCRV β surface expression, complementarity-determining region 3 length distribution, and complementarity-determining region 3 sequencing analysis. In EAE-recovered mice, certain myelin basic protein-reactive CD4⁺V β 8.2⁺ clones are significantly decreased and this decrease is not observed if CD8⁺ T cells were depleted from these mice. The clones that persist in CD8⁺ T cell-intact mice are highly diverse in contrast to the clones expanded in CD8⁺ T cell-depleted mice, which are dominated by the significant outgrowth of a few clones. Importantly, the T cell clones that expand in the absence of CD8⁺ T cell control are enriched in potentially pathogenic self-reactive T cell clones capable of inducing EAE in vivo.

Jonsson, K., B. P. Guo, et al. (2004). "Molecular cloning and characterization of two *Helicobacter pylori* genes coding for plasminogen-binding proteins." PNAS **101**(7): 1852-1857.

<http://www.pnas.org/cgi/content/abstract/101/7/1852>

Helicobacter pylori binds a number of host cell proteins, including the plasma protein plasminogen, which is the proenzyme of the serine protease plasmin. Two *H. pylori* plasminogen-binding proteins have been described; however, no genes were identified. Here we report the use of a phage display library to clone two genes from the *H. pylori* CCUG 17874 genome that mediate binding to plasminogen. DNA sequence analysis of one of these genes revealed 96.6% homology with *H. pylori* 26695 HP0508. A subsequent database search revealed that the amino acid sequence of a lysine-rich C-terminal segment of HP0508 is identical to the C terminus of HP0863. Recombinant proteins expressed from HP0508 and HP0863 bound plasminogen specifically and in a lysine-dependent manner. We designate these genes pgbA and pgbB, respectively. These proteins are expressed by a variety of *H. pylori* strains, have surface-exposed domains, and do not inhibit plasminogen activation. These results indicate that pgbA and pgbB may allow *H. pylori* to coat its exterior with plasminogen, which subsequently can be activated to plasmin. The surface acquisition of protease activity may enhance the virulence of *H. pylori*.

Jordan, B., A. Charest, et al. (2002). "Genome complexity reduction for SNP genotyping analysis." PNAS **99**(5): 2942-2947.

<http://www.pnas.org/cgi/content/abstract/99/5/2942>

Efficient single nucleotide polymorphism (SNP) genotyping methods are necessary to accomplish many current gene discovery goals. A crucial element in large-scale SNP genotyping is the

number of individual biochemical reactions that must be performed. An efficient method that can be used to simultaneously amplify a set of genetic loci across a genome with high reliability can provide a valuable tool for large-scale SNP genotyping studies. In this paper we describe and characterize a method that addresses this goal. We have developed a strategy for reducing genome complexity by using degenerate oligonucleotide primer (DOP)-PCR and applied this strategy to SNP genotyping in three complex eukaryotic genomes; human, mouse, and *Arabidopsis thaliana*. Using a single DOP-PCR primer, SNP loci spread throughout a genome can be amplified and accurately genotyped directly from a DOP-PCR product mixture. DOP-PCRs are extremely reproducible. The DOP-PCR method is transferable to many species of interest. Finally, we describe an *in silico* approach that can effectively predict the SNP loci amplified in a given DOP-PCR, permitting the design of an efficient set of reactions for large-scale, genome-wide SNP studies.

Kanashiro, C. A., A. V. Schally, et al. (2003). "Inhibition of mutant p53 expression and growth of DMS-153 small cell lung carcinoma by antagonists of growth hormone-releasing hormone and bombesin." *PNAS* **100**(26): 15836-15841.

<http://www.pnas.org/cgi/content/abstract/100/26/15836>

We investigated the effects of growth hormone-releasing hormone (GHRH) antagonists, JV-1-65 and JV-1-63, and bombesin/gastrin-releasing peptide (BN/GRP) antagonist RC-3940-II on DMS-153 human small cell lung carcinoma xenografted into nude mice. Treatment with 10 {micro}g/day JV-1-65 or RC-3940-II decreased tumor volume by 28% ($P < 0.05$) and 77% ($P < 0.01$), respectively, after 42 days compared with controls. Combination of JV-1-65 and RC-3940-II induced the greatest inhibition of tumor proliferation (95%; $P < 0.01$), suggesting a synergism. Western blotting showed that the antitumor effects of these antagonists were associated with inhibition of the expression of the mutant tumor suppressor protein p53 (Tp53). Mutation was detected by sequence analysis of the p53 gene at codon 155: ACC [Thr] [-&gt] CCC [Pro]. Combination of JV-1-65 and RC-3940-II decreased the levels of mutant p53 protein by 42% ($P < 0.01$) compared with controls. JV-1-65, JV-1-63, and RC-3940-II, given singly, reduced mutant p53 protein expression by 18-24% ($P < 0.05$). Serum insulin-like growth factor (IGF)-I levels were diminished in animals receiving GHRH antagonists. mRNA levels for IGF-II, IGF receptor-I, GRP receptor, and EGF receptor in tumors were significantly decreased by combined treatment with JV-1-65 and RC-3940-II. DMS-153 tumors expressed mRNAs for GHRH and GHRH receptor splice variants 1 and 2, suggesting that GHRH could be an autocrine growth factor. Proliferation of DMS-153 cells *in vitro* was stimulated by GRP and IGF-II and inhibited by JV-1-65. This study indicates that GHRH antagonists and BN/GRP antagonist inhibit the growth of DMS-153 small cell lung carcinoma concomitantly with the expression of mutant Tp53, which might uncouple the signal transduction pathways for cell growth stimulation.

Karanth, K. P., T. Delefosse, et al. (2005). "Ancient DNA from giant extinct lemurs confirms single origin of Malagasy primates." *PNAS* **102**(14): 5090-5095.

<http://www.pnas.org/cgi/content/abstract/102/14/5090>

The living Malagasy lemurs constitute a spectacular radiation of >50 species that are believed to have evolved from a common ancestor that colonized Madagascar in the early Tertiary period. Yet, at least 15 additional Malagasy primate species, some of which were relative giants, succumbed to extinction within the past 2,000 years. Their existence in Madagascar is recorded predominantly in its Holocene subfossil record. To rigorously test the hypothesis that all endemic Malagasy primates constitute a monophyletic group and to determine the evolutionary relationships among living and extinct taxa, we have conducted an ancient DNA analysis of

subfossil species. A total of nine subfossil individuals from the extinct genera *Palaeopropithecus* and *Megaladapis* yielded amplifiable DNA. Phylogenetic analysis of cytochrome b sequences derived from these subfossils corroborates the monophyly of endemic Malagasy primates. Our results support the close relationship of sloth lemurs to living indriids, as has been hypothesized on morphological grounds. In contrast, *Megaladapis* does not show a sister-group relationship with the living genus *Lepilemur*. Thus, the classification of the latter in the family Megaladapidae is misleading. By correlating the geographic location of subfossil specimens with relative amplification success, we reconfirm the global trend of increased success rates of ancient DNA recovery from nontropical localities.

Kiaris, H., A. V. Schally, et al. (2002). "Expression of a splice variant of the receptor for GHRH in 3T3 fibroblasts activates cell proliferation responses to GHRH analogs." *PNAS* **99**(1): 196-200.

<http://www.pnas.org/cgi/content/abstract/99/1/196>

The stimulatory effects of growth hormone-releasing hormone (GHRH) and the antiproliferative action of GHRH antagonists have been demonstrated in various cancers, but the receptors that mediate these responses are not clearly identified. Recently, we reported that human cancer cell lines express splice variants (SVs) of the receptors for GHRH. SV1 exhibits the greatest similarity to the pituitary GHRH receptor and is most likely to be functional. To ascertain whether SV1 mediates mitogenic effects on nonpituitary tissues, we expressed SV1 in 3T3 mouse fibroblasts and studied the properties of the transfected cells. Radioligand binding assays with ¹²⁵I-labeled GHRH antagonist JV-1-42 detected high affinity ($K_d = 0.58 \pm 0.17$ nM) binding sites for GHRH with a maximal binding capacity (B_{max}) of 103 ± 17.4 fmol/mg of membrane protein in 3T3 cells transfected with pcDNA3-SV1, whereas the control cells transfected with the empty vector did not show any GHRH binding. Cell proliferation studies showed that cells expressing SV1 are much more sensitive to GHRH analogs than the pcDNA3 controls. Thus, the expression of SV1 augments the stimulatory responses to GHRH(1-29)NH₂ or GHRH agonist JI-38 and inhibitory responses to GHRH antagonist JV-1-38 as compared with pcDNA3 controls. The stimulation of SV1-expressing cells by GHRH or JI-38 is followed by an increase in cAMP production, but no GH release occurs. Vasoactive intestinal peptide had no effect, and its antagonist JV-1-53 did not inhibit the proliferation of SV1-expressing cells stimulated by GHRH. Our results suggest that SV1 could mediate responses of nonpituitary cells and various tumors to GHRH and GHRH antagonists. The presence of SV1 in several human cancer cell lines provides a rationale for antitumor therapy based on the blockade of this receptor by specific GHRH antagonists.

Klebig, M. L., M. D. Wall, et al. (2003). "Mutations in the clathrin-assembly gene *Picalm* are responsible for the hematopoietic and iron metabolism abnormalities in *fit1* mice." *PNAS* **100**(14): 8360-8365.

<http://www.pnas.org/cgi/content/abstract/100/14/8360>

Recessive N-ethyl-N-nitrosourea (ENU)-induced mutations recovered at the fitness-1 (*fit1*) locus in mouse chromosome 7 cause hematopoietic abnormalities, growth retardation, and shortened life span, with varying severity of the defects in different alleles. Abnormal iron distribution and metabolism and frequent scoliosis have also been associated with an allele of intermediate severity (*fit14R*). We report that *fit14R*, as well as the most severe *fit15R* allele, are nonsense point mutations in the mouse ortholog of the human phosphatidylinositol-binding clathrin assembly protein (*PICALM*) gene, whose product is involved in clathrin-mediated endocytosis. A variety of leukemias and lymphomas have been associated with translocations that fuse human *PICALM* with the putative transcription factor gene *AF10*. The *Picalmfit1-5R* and *Picalmfit1-4R* mutations are splice-donor alterations resulting in transcripts that are less abundant than normal and missing exons 4 and 17, respectively. These exon deletions introduce premature termination

codons predicted to truncate the proteins near the N and C termini, respectively. No mutations in the genes encoding Picalm, clathrin, or components of the adaptor protein complex 2 (AP2) have been previously described in which the suite of disorders present in the Picalm^{fl}1 mutant mice is apparent. These mutants thus provide unique models for exploring how the endocytic function of mouse Picalm and the transport processes mediated by clathrin and the AP2 complex contribute to normal hematopoiesis, iron metabolism, and growth.

Koh, E. G. L., K. Lam, et al. (2003). "Hox gene clusters in the Indonesian coelacanth, *Latimeriamenadoensis*." PNAS **100**(3): 1084-1088.

<http://www.pnas.org/cgi/content/abstract/100/3/1084>

The Hox genes encode transcription factors that play a key role in specifying body plans of metazoans. They are organized into clusters that contain up to 13 paralogue group members. The complex morphology of vertebrates has been attributed to the duplication of Hox clusters during vertebrate evolution. In contrast to the single Hox cluster in the amphioxus (*Branchiostoma floridae*), an invertebrate-chordate, mammals have four clusters containing 39 Hox genes. Ray-finned fishes (*Actinopterygii*) such as zebrafish and fugu possess more than four Hox clusters. The coelacanth occupies a basal phylogenetic position among lobe-finned fishes (*Sarcopterygii*), which gave rise to the tetrapod lineage. The lobe fins of sarcopterygians are considered to be the evolutionary precursors of tetrapod limbs. Thus, the characterization of Hox genes in the coelacanth should provide insights into the origin of tetrapod limbs. We have cloned the complete second exon of 33 Hox genes from the Indonesian coelacanth, *Latimeria menadoensis*, by extensive PCR survey and genome walking. Phylogenetic analysis shows that 32 of these genes have orthologs in the four mammalian HOX clusters, including three genes (HoxA6, D1, and D8) that are absent in ray-finned fishes. The remaining coelacanth gene is an ortholog of *hoxc1* found in zebrafish but absent in mammals. Our results suggest that coelacanths have four Hox clusters bearing a gene complement more similar to mammals than to ray-finned fishes, but with an additional gene, HoxC1, which has been lost during the evolution of mammals from lobe-finned fishes.

Koliatsos, V. E., T. M. Dawson, et al. (2004). "Cortical interneurons become activated by deafferentation and instruct the apoptosis of pyramidal neurons." PNAS **101**(39): 14264-14269.

<http://www.pnas.org/cgi/content/abstract/101/39/14264>

Unlike peripheral nervous system neurons and certain groups of nerve cells in the CNS, cortical projection neurons are tolerant of axonal lesions. This resistance is incongruent with the massive death of pyramidal neurons in age-associated neurodegenerative diseases that proceed along corticocortical connections. Some insights have emerged from our previous work showing that pyramidal cells in piriform cortex undergo classical apoptosis within 24 h after bulbectomy via transsynaptic, but not retrograde, signaling. These findings allow the investigation of cellular and molecular changes that take place in the context of experimental cortical degeneration. In the present study, we show that the transsynaptic death of pyramidal neurons in piriform cortex is a nitric oxide-mediated event signaled by activated interneurons in layer I. Thus, we demonstrate that cortical interneurons play an essential role in transducing injury to apoptotic signaling that selectively targets pyramidal neurons. We propose that this mechanism may be generic to cortical degenerations and amenable to therapeutic interventions.

Kumar, S., G. K. Christophides, et al. (2003). "The role of reactive oxygen species on Plasmodium melanotic encapsulation in Anopheles gambiae." PNAS **100**(24): 14139-14144.

<http://www.pnas.org/cgi/content/abstract/100/24/14139>

Malaria transmission depends on the competence of some Anopheles mosquitoes to sustain Plasmodium development (susceptibility). A genetically selected refractory strain of Anopheles gambiae blocks Plasmodium development, melanizing, and encapsulating the parasite in a reaction that begins with tyrosine oxidation, and involves three quantitative trait loci. Morphological and microarray mRNA expression analysis suggest that the refractory and susceptible strains have broad physiological differences, which are related to the production and detoxification of reactive oxygen species. Physiological studies corroborate that the refractory strain is in a chronic state of oxidative stress, which is exacerbated by blood feeding, resulting in increased steady-state levels of reactive oxygen species, which favor melanization of parasites as well as Sephadex beads.

Kuwahara, T., A. Yamashita, et al. (2004). "Genomic analysis of Bacteroides fragilis reveals extensive DNA inversions regulating cell surface adaptation." PNAS **101**(41): 14919-14924.

<http://www.pnas.org/cgi/content/abstract/101/41/14919>

Bacteroides are predominant human colonic commensals, but the principal pathogenic species, Bacteroides fragilis (BF), lives closely associated with the mucosal surface, whereas a second major species, Bacteroides thetaiotaomicron (BT), concentrates within the colon. We find corresponding differences in their genomes, based on determination of the genome sequence of BF and comparative analysis with BT. Both species have acquired two mechanisms that contribute to their dominance among the colonic microbiota: an exceptional capability to use a wide range of dietary polysaccharides by gene amplification and the capacity to create variable surface antigenicities by multiple DNA inversion systems. However, the gene amplification for polysaccharide assimilation is more developed in BT, in keeping with its internal localization. In contrast, external antigenic structures can be changed more systematically in BF. Thereby, at the mucosal surface, where microbes encounter continuous attack by host defenses, BF evasion of the immune system is favored, and its colonization and infectious potential are increased.

Laub, M. T., S. L. Chen, et al. (2002). "Genes directly controlled by CtrA, a master regulator of the Caulobacter cell cycle." PNAS **99**(7): 4632-4637.

<http://www.pnas.org/cgi/content/abstract/99/7/4632>

Studies of the genetic network that controls the Caulobacter cell cycle have identified a response regulator, CtrA, that controls, directly or indirectly, one-quarter of the 553 cell cycle-regulated genes. We have performed in vivo genomic binding site analysis of the CtrA protein to identify which of these genes have regulatory regions bound directly by CtrA. By combining these data with previous global analysis of cell cycle transcription patterns and gene expression profiles of mutant ctrA strains, we have determined that CtrA directly regulates at least 95 genes. The total group of CtrA-regulated genes includes those involved in polar morphogenesis, DNA replication initiation, DNA methylation, cell division, and cell wall metabolism. Also among the genes in this notably large regulon are 14 that encode regulatory proteins, including 10 two-component signal transduction regulatory proteins. Identification of additional regulatory genes activated by CtrA will serve to directly connect new regulatory modules to the network controlling cell cycle progression.

Lavitrano, M., M. L. Bacci, et al. (2002). "Efficient production by sperm-mediated gene transfer of human decay accelerating factor (hDAF) transgenic pigs for xenotransplantation." PNAS **99**(22): 14230-14235.

<http://www.pnas.org/cgi/content/abstract/99/22/14230>

A large number of hDAF transgenic pigs to be used for xenotransplantation research were generated by using sperm-mediated gene transfer (SMGT). The efficiency of transgenesis obtained with SMGT was much greater than with any other method. In the experiments reported, up to 80% of pigs had the transgene integrated into the genome. Most of the pigs carrying the hDAF gene transcribed it in a stable manner (64%). The great majority of pigs that transcribed the gene expressed the protein (83%). The hDAF gene was transmitted to progeny. Expression was stable and found in caveolae as it is in human cells. The expressed gene was functional based on in vitro experiments performed on peripheral blood mononuclear cells. These results show that our SMGT approach to transgenesis provides an efficient procedure for studies involving large animal models.

Leabman, M. K., C. C. Huang, et al. (2003). "Natural variation in human membrane transporter genes reveals evolutionary and functional constraints." PNAS **100**(10): 5896-5901.

<http://www.pnas.org/cgi/content/abstract/100/10/5896>

Membrane transporters maintain cellular and organismal homeostasis by importing nutrients and exporting toxic compounds. Transporters also play a crucial role in drug response, serving as drug targets and setting drug levels. As part of a pharmacogenetics project, we screened exons and flanking intronic regions for variation in a set of 24 membrane transporter genes (96 kb; 57% coding) in 247 DNA samples from ethnically diverse populations. We identified 680 single nucleotide polymorphisms (SNPs), of which 175 were synonymous and 155 caused amino acid changes, and 29 small insertions and deletions. Amino acid diversity ([pi]NS) in transmembrane domains (TMDs) was significantly lower than in loop domains, suggesting that TMDs have special functional constraints. This difference was especially striking in the ATP-binding cassette superfamily and did not parallel evolutionary conservation: there was little variation in the TMDs, even in evolutionarily unconserved residues. We used allele frequency distribution to evaluate different scoring systems (Grantham, BLOSUM62, SIFT, and evolutionarily conserved/evolutionarily unconserved) for their ability to predict which SNPs affect function. Our underlying assumption was that alleles that are functionally deleterious will be selected against and thus under represented at high frequencies and over represented at low frequencies. We found that evolutionary conservation of orthologous sequences, as assessed by evolutionarily conserved/evolutionarily unconserved and SIFT, was the best predictor of allele frequency distribution and hence of function. European Americans had an excess of high frequency alleles in comparison to African Americans, consistent with a historic bottleneck. In addition, African Americans exhibited a much higher frequency of population specific medium-frequency alleles than did European Americans.

Lee, J. H., S. M. P. Yi, et al. (2004). "Propagation of infectious human papillomavirus type 16 by using an adenovirus and Cre/LoxP mechanism." PNAS **101**(7): 2094-2099.

<http://www.pnas.org/cgi/content/abstract/101/7/2094>

Human papillomavirus type 16 (HPV16) infection is a major risk factor for the development of squamous cell cancers of the cervix and of the head and neck. A major barrier to understanding the progression from initial infection to cancer has been the lack of in vitro models that allow infection, replication, and persistence of the viral genome as an episome in differentiated epithelial cells. To overcome this barrier, we designed an adenoviral delivery vector that contained a full HPV16 genome flanked by LoxP homologous recombination sites and a fluorescent reporter that was expressed only after the HPV genome was excised by Cre recombinase. This system delivered circular HPV16 genomes to cervical epithelial cells and well differentiated human airway epithelia. After delivery, the HPV16 genome replicated and persisted as an episome in cervical keratinocytes. These cells developed an immortalized phenotype and a dysplastic epithelial appearance. Moreover, induction of differentiation led to the expression of late genes and production of infectious HPV16 virions. This work provides a means of introducing biologically active HPV genomes into epithelial cells, which are normally difficult to transfect. These methods allow the study of HPV genome replication and gene expression in the earliest stages of HPV genome establishment, and they may provide a means to study nononcogenic HPV viral types.

Lepp, P. W., M. M. Brinig, et al. (2004). "Methanogenic Archaea and human periodontal disease." PNAS **101**(16): 6176-6181.

<http://www.pnas.org/cgi/content/abstract/101/16/6176>

Archaea have been isolated from the human colon, vagina, and oral cavity, but have not been established as causes of human disease. In this study, we reveal a relationship between the severity of periodontal disease and the relative abundance of archaeal small subunit ribosomal RNA genes (SSU rDNA) in the subgingival crevice by using quantitative PCR. Furthermore, the relative abundance of archaeal small subunit rDNA decreased at treated sites in association with clinical improvement. Archaea were harbored by 36% of periodontitis patients and were restricted to subgingival sites with periodontal disease. The presence of archaeal cells at these sites was confirmed by fluorescent in situ hybridization. The archaeal community at diseased sites was dominated by a *Methanobrevibacter oralis*-like phylotype and a distinct *Methanobrevibacter* subpopulation related to archaea that inhabit the gut of numerous animals. We hypothesize that methanogens participate in syntrophic relationships in the subgingival crevice that promote colonization by secondary fermenters during periodontitis. Because they are potential alternative syntrophic partners, our finding of larger *Treponema* populations sites without archaea provides further support for this hypothesis.

Liang, G., J. C. Y. Lin, et al. (2004). "Distinct localization of histone H3 acetylation and H3-K4 methylation to the transcription start sites in the human genome." PNAS **101**(19): 7357-7362.

<http://www.pnas.org/cgi/content/abstract/101/19/7357>

Almost 1-2% of the human genome is located within 500 bp of either side of a transcription initiation site, whereas a far larger proportion ($\approx 25\%$) is potentially transcribable by elongating RNA polymerases. This observation raises the question of how the genome is packaged into chromatin to allow start sites to be recognized by the regulatory machinery at the same time as transcription initiation, but not elongation, is blocked in the 25% of intragenic DNA. We developed a chromatin scanning technique called ChAP, coupling the chromatin immunoprecipitation assay with arbitrarily primed PCR, which allows for the rapid and unbiased comparison of histone modification patterns within the eukaryotic nucleus. Methylated lysine 4 (K4) and acetylated K9/14 of histone H3 were both highly localized to the 5' regions of transcriptionally active human genes but were greatly decreased downstream of the start sites.

Our results suggest that the large transcribed regions of human genes are maintained in a deacetylated conformation in regions read by elongating polymerase. Common models depicting widespread histone acetylation and K4 methylation throughout the transcribed unit do not therefore apply to the majority of human genes.

Liang, T., J. Spence, et al. (2003). "alpha -Synuclein maps to a quantitative trait locus for alcohol preference and is differentially expressed in alcohol-preferring and -nonpreferring rats." PNAS **100**(8): 4690-4695.

<http://www.pnas.org/cgi/content/abstract/100/8/4690>

Total gene expression analysis (TOGA) was used to identify genes that are differentially expressed in brain regions between the alcohol-naive, inbred alcohol-preferring (iP), and -nonpreferring (iNP) rats. [alpha]-Synuclein, expressed at >2-fold higher levels in the hippocampus of the iP than the iNP rat, was prioritized for further study. In situ hybridization was used to determine specific brain regions and cells expressing [alpha]-synuclein in the iP and iNP rats. Similar to [alpha]-synuclein mRNA levels, protein levels in the hippocampus were higher in iP rats than iNP rats. Higher protein levels were also observed in the caudate putamen of iP rats compared with iNP rats. Sequence analysis identified two single nucleotide polymorphisms in the 3' UTR of the cDNA. The polymorphism was used to map the gene, by using recombination-based methods, to chromosome 4, within a quantitative trait locus for alcohol consumption that was identified in the iP and iNP rats. A nucleotide exchange in the iNP 3' UTR reduced expression of the luciferase reporter gene in SK-N-SH neuroblastoma cells. These results suggest that differential expression of the [alpha]-synuclein gene may contribute to alcohol preference in the iP rats.

Licht, T., M. Haskins, et al. (2002). "Drug selection with paclitaxel restores expression of linked IL-2 receptor gamma -chain and multidrug resistance (MDR1) transgenes in canine bone marrow." PNAS **99**(5): 3123-3128.

<http://www.pnas.org/cgi/content/abstract/99/5/3123>

Unstable expression of transferred genes is a major obstacle to successful gene therapy of hematopoietic diseases. We have investigated in a canine large-animal model whether expression of transduced genes can be recovered in vivo. Mixed-breed dogs had undergone autologous bone marrow transplantation (BMT) with stem cell factor and granulocyte-colony-stimulating factor-mobilized retrovirally marked hematopoietic cells. The bicistronic retroviral vector construct allowed for coexpression of MDR1 and human IL-2 receptor common [gamma]-chain cDNAs. The latter gene is deficient in X-linked severe combined immunodeficiency. After initial high-level expression, P-glycoprotein and the [gamma]-chain were undetectable in blood and bone marrow 17 months post-BMT. Six months later, one dog was treated i.v. with 125 mg/m² paclitaxel. Three administrations restored expression of the two linked genes to high levels in blood and bone marrow. Two dogs treated with higher paclitaxel doses died from myelosuppression after the first administration. As determined by flow cytometry, both genes were expressed in granulocytes, monocytes, and lymphocytes of the surviving animal. PCR analysis of DNA from peripheral blood confirmed that the retroviral cDNA was increased after paclitaxel treatment, suggesting enrichment of transduced cells. P-glycoprotein was detectable for more than 1 year after cessation of paclitaxel. Repeated analyses of blood and bone marrow aspirates gave no indication of hematopoietic disturbance after BMT with transduced cells and paclitaxel treatment. In summary, we have shown that with the use of a drug-selectable marker gene, chemotherapy can select for cells that express an otherwise nonselected therapeutic gene in blood and bone marrow.

Liu, H., G. R. Abecasis, et al. (2002). "Genetic variation in the 22q11 locus and susceptibility to schizophrenia." PNAS **99**(26): 16859-16864.

<http://www.pnas.org/cgi/content/abstract/99/26/16859>

An increased prevalence of microdeletions at the 22q11 locus has been reported in samples of patients with schizophrenia. 22q11 microdeletions represent the highest known genetic risk factor for the development of schizophrenia, second only to that of the monozygotic cotwin of an affected individual or the offspring of two schizophrenic parents. It is therefore clear that a schizophrenia susceptibility locus maps to chromosome 22q11. In light of evidence for suggestive linkage for schizophrenia in this region, we hypothesized that, whereas deletions of chromosome 22q11 may account for only a small proportion of schizophrenia cases in the general population (up to {approx}2%), nondeletion variants of individual genes within the 22q11 region may make a larger contribution to susceptibility to schizophrenia in the wider population. By studying a dense collection of markers (average one single nucleotide polymorphism/20 kb over 1.5 Mb) in the vicinity of the 22q11 locus, in both family- and population-based samples, we present here results consistent with this assumption. Moreover, our results are consistent with contribution from more than one gene to the strikingly increased disease risk associated with this locus. Finer-scale haplotype mapping has identified two subregions within the 1.5-Mb locus that are likely to harbor candidate schizophrenia susceptibility genes.

Liu, H., S. C. Heath, et al. (2002). "Genetic variation at the 22q11 PRODH2/DGCR6 locus presents an unusual pattern and increases susceptibility to schizophrenia." PNAS **99**(6): 3717-3722.

<http://www.pnas.org/cgi/content/abstract/99/6/3717>

The location of a schizophrenia susceptibility locus at chromosome 22q11 has been suggested by genome-wide linkage studies. Additional support was provided by the observation of a higher-than-expected frequency of 22q11 microdeletions in patients with schizophrenia and the demonstration that [approx]20-30% of individuals with 22q11 microdeletions develop schizophrenia or schizoaffective disorder in adolescence and adulthood. Analysis of the extent of these microdeletions by using polymorphic markers afforded further refinement of this locus to a region of [approx]1.5 Mb. Recently, a high rate of 22q11 microdeletions was also reported for a cohort of 47 patients with Childhood Onset Schizophrenia, a rare and severe form of schizophrenia with onset by age 13. It is therefore likely that this 1.5-Mb region contains one or more genes that predispose to schizophrenia. In three independent samples, we provide evidence for a contribution of the PRODH2/DGCR6 locus in 22q11-associated schizophrenia. We also uncover an unusual pattern of PRODH2 gene variation that mimics the sequence of a linked pseudogene. Several of the pseudogene-like variants we identified result in missense changes at conserved residues and may prevent synthesis of a fully functional enzyme. Our results have implications for understanding the genetic basis of the 22q11-associated psychiatric phenotypes and provide further insights into the genomic instability of this region.

Lossos, I. S., A. A. Alizadeh, et al. (2002). "Transformation of follicular lymphoma to diffuse large-cell lymphoma: Alternative patterns with increased or decreased expression of c-myc and its regulated genes." PNAS **99**(13): 8886-8891.

<http://www.pnas.org/cgi/content/abstract/99/13/8886>

The natural history of follicular lymphoma (FL) is frequently characterized by transformation to a more aggressive diffuse large B cell lymphoma (DLBCL). We compared the gene-expression profiles between transformed DLBCL and their antecedent FL. No genes were observed to increase or decrease their expression in all of the cases of histological transformation. However, two different gene-expression profiles associated with the transformation process were defined, one in which c-myc and genes regulated by c-myc showed increased expression and one in which these same genes showed decreased expression. Further, there was a striking difference in gene-expression profiles between transformed DLBCL and de novo DLBCL, because the gene-expression profile of transformed DLBCL was more similar to their antecedent FL than to de novo DLBCL. This study demonstrates that transformation from FL to DLBCL can occur by alternative pathways and that transformed DLBCL and de novo DLBCL have very different gene-expression profiles that may underlie the different clinical behaviors of these two types of morphologically similar lymphomas.

Mai, W., M. F. Janier, et al. (2004). "Thyroid hormone receptor {alpha} is a molecular switch of cardiac function between fetal and postnatal life." PNAS **101**(28): 10332-10337.

<http://www.pnas.org/cgi/content/abstract/101/28/10332>

Thyroid hormones are involved in the regulation of many physiological processes and regulate gene transcription by binding to their nuclear receptors TR{alpha} and TR{beta}. In the absence of triiodothyronine (T3), the unliganded receptors (aporeceptors) do bind DNA and repress the transcription of target genes. The role of thyroid hormone aporeceptors as repressors was observed in hypothyroid adult mice, but its physiological relevance in nonpathological hypothyroid conditions remained to be determined. Here we show that, in the normal mouse fetus, TR{alpha} aporeceptors repress heart rate as well as the expression of TR{beta} and several genes encoding ion channels involved in cardiac contractile activity. Right after birth, when T3 concentration sharply increases, liganded TR{alpha} (holoreceptors) turn on the expression of some of these same genes concomitantly with heart rate increase. These data describe a physiological situation under which conversion of TR{alpha} from apo-receptors into holo-receptors, upon changes in T3 availability, plays a determinant role in a developmental process.

Marijt, W. A. E., M. H. M. Heemskerk, et al. (2003). "Hematopoiesis-restricted minor histocompatibility antigens HA-1- or HA-2-specific T cells can induce complete remissions of relapsed leukemia." PNAS **100**(5): 2742-2747.

<http://www.pnas.org/cgi/content/abstract/100/5/2742>

Donor lymphocyte infusion (DLI) into patients with a relapse of their leukemia or multiple myeloma after allogeneic stem cell transplantation (alloSCT) has been shown to be a successful treatment approach. The hematopoiesis-restricted minor histocompatibility antigens (mHAg) HA-1 or HA-2 expressed on malignant cells of the recipient may serve as target antigens for alloreactive donor T cells. Recently we treated three mHAg HA-1- and/or HA-2-positive patients with a relapse of their disease after alloSCT with DLI from their mHAg HA-1- and/or HA-2-negative donors. Using HLA-A2/HA-1 and HA-2 peptide tetrameric complexes we showed the emergence of HA-1- and HA-2-specific CD8+ T cells in the blood of the recipients 5-7 weeks after DLI. The appearance of these tetramer-positive cells was followed immediately by a complete remission of the disease and restoration of 100% donor chimerism in each of the patients. Furthermore, cloned tetramer-positive T cells isolated during the clinical response specifically recognized HA-1 and HA-2 expressing malignant progenitor cells of the recipient and inhibited the growth of leukemic precursor cells in vitro. Thus, HA-1- and HA-2-specific cytotoxic T lymphocytes emerging in the blood of patients after DLI demonstrate graft-versus-leukemia or

myeloma reactivity resulting in a durable remission. This finding implies that in vitro generated HA-1- and HA-2-specific cytotoxic T lymphocytes could be used as adoptive immunotherapy to treat hematological malignances relapsing after alloSCT.

Martin, E. S., R. Cesari, et al. (2003). "The BCSC-1 locus at chromosome 11q23-q24 is a candidate tumor suppressor gene." PNAS **100**(20): 11517-11522.

<http://www.pnas.org/cgi/content/abstract/100/20/11517>

Frequent allelic loss at human chromosome 11q23-q24 occurs in a wide variety of cancers, suggesting that this region may harbor a tumor suppressor gene. By constructing a physical map of the LOH11CR2 minimal region of loss on 11q23-q24 associated with lung and breast carcinomas, we were able to clone a hereditary translocation, t(11;12)(q23;q24), in a patient with early-onset breast cancer and family history of cancer. The breakpoint was found within 6 kb of the BCSC-1 candidate tumor suppressor gene located in the LOH11CR2 region whereas additional loss of heterozygosity (LOH) analysis in breast and ovarian tumors, including that of the patient with the t(11;12)(q23;q24), implicated the BCSC-1 locus as the primary target of deletion. Northern analysis of the BCSC-1 mRNA revealed a lack of expression in 33 of 41 (80%) tumor cell lines, and its ectopic expression led to the suppression of colony formation in vitro and tumorigenicity in vivo. These data suggest that BCSC-1 may exert a tumor suppressor activity and is a likely target of the LOH observed on 11q23-q24 in cancer.

Matisoo-Smith, E. and J. H. Robins (2004). "From The Cover: Origins and dispersals of Pacific peoples: Evidence from mtDNA phylogenies of the Pacific rat." PNAS **101**(24): 9167-9172.

<http://www.pnas.org/cgi/content/abstract/101/24/9167>

The human settlement of the Pacific in general, and the origin of the Polynesians in particular, have been topics of debate for over two centuries. Polynesian origins are most immediately traced to people who arrived in the Fiji, Tonga, and Samoa region {approx}3,000 B.P. and are clearly associated with the Lapita Cultural Complex. Although this scenario of the immediate origins of the Polynesians is generally accepted, the debate on the ultimate origin of the Polynesians and the Lapita cultural complex continues. Our previous research has shown that analyses of mtDNA variation in the Pacific rat (*Rattus exulans*), often transported as a food item in the colonizing canoes, are valuable for tracing prehistoric human migration within Polynesia. Here we present mtDNA phylogenies based on {approx}240 base pairs of the D-loop from both archaeological and modern samples collected from Island Southeast Asia and the Pacific. We identify three major haplogroups, two of which occur in the Pacific. Comparing our results with Lapita models of Oceanic settlement, we are able to reject two often cited but simplistic models, finding support instead for multifaceted models incorporating a more complex view of the Lapita intrusion. This study is unique and valuable in that *R. exulans* is the only organism associated with the Lapita dispersal for which there are sufficient ancient and extant populations available for genetic analysis. By tracking population changes through time, we can understand more fully the settlement process and population interactions in both Near and Remote Oceania.

Maxfield, L. F., C. D. Fraize, et al. (2005). "From The Cover: Relationship between retroviral DNA-integration-site selection and host cell transcription." PNAS **102**(5): 1436-1441.

<http://www.pnas.org/cgi/content/abstract/102/5/1436>

Retroviral DNA integration occurs throughout the genome; however, local "hot spots" exist where a strong preference for certain sites over others are seen, and more global preferences associated with genes have been reported. Previous data from our laboratory suggested that there are fewer integration events into a DNA template when it is undergoing active transcription than when it is not. Because these data were generated by using a stably transfected foreign gene that was only weakly inducible, we have extended this observation by comparing integration events into a highly inducible endogenous gene under both induced and uninduced transcriptional states. To examine the influence of transcription on site selection directly, we analyzed the frequency and distribution of integration of avian retrovirus DNA into the metallothionein gene, before and after its induction to a highly sustained level of expression by addition of ZnSO₄. We found a 6-fold reduction in integration events after 100-fold induction of transcription. This result implies that, despite an apparent preference for integration of retroviral DNA into transcribed regions of host DNA, high-level transcription can be inhibitory to the integration process. Several possible models for our observation are as follows. First, when a DNA template is undergoing active transcription, integration might be blocked by the RNA polymerase II complex because of steric hindrance. Alternatively, the integrase complex may require DNA to be in a double-stranded conformation, which would not be the case during active transcription. Last, transcription might lead to remodeling of chromatin into a structure that is less favorable for integration.

Miller, C. R. and L. P. Waits (2003). "The history of effective population size and genetic diversity in the Yellowstone grizzly (Ursus arctos): Implications for conservation." *PNAS* **100**(7): 4334-4339.

<http://www.pnas.org/cgi/content/abstract/100/7/4334>

Protein, mtDNA, and nuclear microsatellite DNA analyses have demonstrated that the Yellowstone grizzly bear has low levels of genetic variability compared with other *Ursus arctos* populations. Researchers have attributed this difference to inbreeding during a century of anthropogenic isolation and population size reduction. We test this hypothesis and assess the seriousness of genetic threats by generating microsatellite data for 110 museum specimens collected between 1912 and 1981. A loss of variability is detected, but it is much less severe than hypothesized. Variance in allele frequencies over time is used to estimate an effective population size of [approx]80 across the 20th century and >100 currently. The viability of the population is unlikely to be substantially reduced by genetic factors in the next several generations. However, gene flow from outside populations will be beneficial in avoiding inbreeding and the erosion of genetic diversity in the future.

Min, B., G. Foucras, et al. (2004). "Spontaneous proliferation, a response of naive CD4 T cells determined by the diversity of the memory cell repertoire." *PNAS* **101**(11): 3874-3879.

<http://www.pnas.org/cgi/content/abstract/101/11/3874>

T cell numbers are maintained within narrow ranges in vivo. Introduction of naive cells into lymphopenic environments results in proliferation and differentiation driven by the recognition of peptide/MHC complexes and by cytokine signaling. This process, often described as homeostatic proliferation, is here referred to as spontaneous proliferation. We show that, although the presence of memory CD4 T cells of broad repertoire efficiently inhibits proliferation/differentiation of naive CD4 T cells, a memory population of similar size comprised of cells with a repertoire of limited diversity fails to do so, implying that cells of a given specificity prevent responses of cells of the same or related specificity. This finding suggests that the immune system has evolved mechanisms to attain a memory cell repertoire of great diversity independently of foreign antigens.

Min, B., B. T. Weinert, et al. (2004). "Interplay between Drosophila Bloom's syndrome helicase and Ku autoantigen during nonhomologous end joining repair of P element-induced DNA breaks." PNAS **101**(24): 8906-8911.

<http://www.pnas.org/cgi/content/abstract/101/24/8906>

P transposable elements in Drosophila are mobilized via a cut-and-paste mechanism. The broken DNA ends generated during transposition can be repaired via the homology-directed synthesis-dependent strand annealing or by nonhomologous end joining (NHEJ). Genetic studies have demonstrated an interaction between the gene (*mus309*, for mutagen-sensitive) encoding the Drosophila Bloom's syndrome helicase homolog (DmBLM) and the Ku70 gene, which is involved in NHEJ. We have used RNA interference (RNAi) to knock down expression of DmBLM and one or both of the Drosophila Ku subunits, DmKu70 or DmKu80. Our results show that upon reduction of DmKu, an increase in small deletions (1-49 bp) and large deletions (≥ 50 bp) flanking the site of P element-induced breaks is observed, and a reduction in large deletions at these sites is found upon reduction of DmBLM. Moreover, double RNAi of DmKu and DmBLM results in an increase in small deletions characteristic of the DmKu RNAi and also partially suppresses the reduction in repair efficiency observed with DmKu RNAi. These results suggest that there are DNA double-strand break recognition and/or processing events involving DmKu and DmBLM that, when eliminated by RNAi, lead to deletions. Finally, these results raise the possibility that, unlike the situation in mammals, where BLM appears to function exclusively in the homologous repair pathway, in Drosophila, DmBLM may be directly involved in, or at least influence the double-strand break recognition that leads to the NHEJ repair pathway.

Mitra, R. D., V. L. Butty, et al. (2003). "Digital genotyping and haplotyping with polymerase colonies." PNAS **100**(10): 5926-5931.

<http://www.pnas.org/cgi/content/abstract/100/10/5926>

Polymerase colony (polony) technology amplifies multiple individual DNA molecules within a thin acrylamide gel attached to a microscope slide. Each DNA molecule included in the reaction produces an immobilized colony of double-stranded DNA. We genotype these colonies by performing single base extensions with dye-labeled nucleotides, and we demonstrate the accurate quantitation of two allelic variants. We also show that polony technology can determine the phase, or haplotype, of two single-nucleotide polymorphisms (SNPs) by coamplifying distally located targets on a single chromosomal fragment. We correctly determine the genotype and phase of three different pairs of SNPs. In one case, the distance between the two SNPs is 45 kb, the largest distance achieved to date without separating the chromosomes by cloning or somatic cell fusion. The results indicate that polony genotyping and haplotyping may play an important role in understanding the structure of genetic variation.

Moore, J. B., R. K. Blanchard, et al. (2003). "Dietary zinc modulates gene expression in murine thymus: Results from a comprehensive differential display screening." PNAS **100**(7): 3883-3888.

<http://www.pnas.org/cgi/content/abstract/100/7/3883>

Differential mRNA display was used to comprehensively screen the murine thymic transcriptome for genes modulated in vivo by dietary zinc. A moderate feeding protocol rendered young adult, outbred mice zinc-deficient and zinc-supplemented without alterations in feeding behavior or

growth. However, these levels of deficiency and supplementation altered specific mRNA abundances in a manner detectable by differential display. In total, 240 primer-pair combinations were used to generate >48,000 interpretable cDNA bands derived from thymic total RNA, of which only 265 or 0.55% were identified as zinc-modulated under these moderate dietary conditions. The most strongly zinc-modulated cDNAs identified by display were reamplified and sequenced. No cDNAs encoding zinc-metalloenzymes or zinc-finger transcription factors were identified as zinc-modulated in this global screening. Those zinc-regulated genes independently confirmed by quantitative PCR included: heat shock proteins 40 and 60; heat shock cognate 70; histocompatibility 2, class II antigen A, [alpha]; and the T cell cytokine receptor. In addition, a variety of transcription- and translation-related factors (such as ribosomal proteins L3, L5, and L28; nuclear matrix protein 84; matrin cyclophilin; the H3 histone family 3A protein; [beta]2 microglobulin; and a cleavage and polyadenylation factor) were identified as zinc-modulated. These profiling data show that differential expression of genes in the thymus in response to the dietary zinc supply precedes many of the phenotypic effects on thymic function associated with severe zinc restriction or supplementation. Several genes involved in T cell development were identified as regulated by zinc and will be targets to evaluate the effects of zinc on immune function.

Mori, H., S. M. Colman, et al. (2002). "Chromosome translocations and covert leukemic clones are generated during normal fetal development." PNAS **99**(12): 8242-8247.

<http://www.pnas.org/cgi/content/abstract/99/12/8242>

Studies on monozygotic twins with concordant leukemia and retrospective scrutiny of neonatal blood spots of patients with leukemia indicate that chromosomal translocations characteristic of pediatric leukemia often arise prenatally, probably as initiating events. The modest concordance rate for leukemia in identical twins ([approx]5%), protracted latency, and transgenic modeling all suggest that additional postnatal exposure and/or genetic events are required for clinically overt leukemia development. This notion leads to the prediction that chromosome translocations, functional fusion genes, and preleukemic clones should be present in the blood of healthy newborns at a rate that is significantly greater than the cumulative risk of the corresponding leukemia. Using parallel reverse transcriptase-PCR and real-time PCR (Taqman) screening, we find that the common leukemia fusion genes, TEL-AML1 or AML1-ETO, are present in cord bloods at a frequency that is 100-fold greater than the risk of the corresponding leukemia. Single-cell analysis by cell enrichment and immunophenotype/fluorescence in situ hybridization multicolor staining confirmed the presence of translocations in restricted cell types corresponding to the B lymphoid or myeloid lineage of the leukemias that normally harbor these fusion genes. The frequency of positive cells (10⁻⁴ to 10⁻³) indicates substantial clonal expansion of a progenitor population. These data have significant implications for the pathogenesis, natural history, and etiology of childhood leukemia.

Nagaraju, J., M. Kathirvel, et al. (2002). "Genetic analysis of traditional and evolved Basmati and non-Basmati rice varieties by using fluorescence-based ISSR-PCR and SSR markers." PNAS **99**(9): 5836-5841.

<http://www.pnas.org/cgi/content/abstract/99/9/5836>

The objective of the present study was to make use of efficient molecular marker systems to reveal genetic relationships in traditional and evolved Basmati (EB) and semidwarf non-Basmati (NB) rice varieties. A subset of three rice groups was analyzed by using 19 simple sequence repeat (SSR) loci and 12 inter-SSR-PCR primers. A total of 70 SSR alleles and 481 inter-SSR-PCR markers were revealed in 24 varieties from the three groups. The lowest genetic diversity

was observed among the traditional Basmati varieties, whereas the EB varieties showed the highest genetic diversity by both the marker assays. The results indicated that the subset of aromatic rice varieties analyzed in the present study is probably derived from a single land race. The traditional Basmati (TB) and semidwarf NB rice varieties used in the present study were clearly delineated by both marker assays. A number of markers, which could unambiguously distinguish the TB varieties used in the present study from the evolved and NB rice varieties, were identified. The potential use of these markers in Basmati rice-breeding programs and authentication of TB varieties used in the present study are envisaged.

Neff, M. W., K. R. Robertson, et al. (2004). "From the Cover: Breed distribution and history of canine *mdr1-1*{Delta}, a pharmacogenetic mutation that marks the emergence of breeds from the collie lineage." *PNAS* **101**(32): 11725-11730.

<http://www.pnas.org/cgi/content/abstract/101/32/11725>

A mutation in the canine multidrug resistance gene, *MDR1*, has previously been associated with drug sensitivities in two breeds from the collie lineage. We exploited breed phylogeny and reports of drug sensitivity to survey other purebred populations that might be genetically at risk. We found that the same allele, *mdr1-1*{Delta}, segregated in seven additional breeds, including two sighthounds that were not expected to share collie ancestry. A mutant haplotype that was conserved among affected breeds indicated that the allele was identical by descent. Based on breed histories and the extent of linkage disequilibrium, we conclude that all dogs carrying *mdr1-1*{Delta} are descendants of a dog that lived in Great Britain before the genetic isolation of breeds by registry (ca. 1873). The breed distribution and frequency of *mdr1-1*{Delta} have applications in veterinary medicine and selective breeding, whereas the allele's history recounts the emergence of formally recognized breeds from an admixed population of working sheepdogs.

Ng, E. K. O., N. B. Y. Tsui, et al. (2003). "From the Cover: mRNA of placental origin is readily detectable in maternal plasma." *PNAS* **100**(8): 4748-4753.

<http://www.pnas.org/cgi/content/abstract/100/8/4748>

The discovery of circulating fetal nucleic acid in maternal plasma has opened up new possibilities for noninvasive prenatal diagnosis. Thus far, a gender- and polymorphism-independent fetal-specific target that can be used for prenatal screening and monitoring in all pregnant women has not been reported. In addition, the origin of such circulating nucleic acid has remained unclear. Here we provide direct evidence that the placenta is an important source of fetal nucleic acid release into maternal plasma by demonstrating that mRNA transcripts from placenta-expressed genes are readily detectable in maternal plasma. The surprising stability of such placental mRNA species in maternal plasma and their rapid clearance after delivery demonstrate that such circulating mRNA molecules are practical markers for clinical use. The measurement of such plasma mRNA markers has provided a gender-independent approach for noninvasive prenatal gene expression profiling and has opened up numerous research and diagnostic possibilities.

Pao, W., V. Miller, et al. (2004). "EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib." *PNAS* **101**(36): 13306-13311.

<http://www.pnas.org/cgi/content/abstract/101/36/13306>

Somatic mutations in the tyrosine kinase (TK) domain of the epidermal growth factor receptor (EGFR) gene are reportedly associated with sensitivity of lung cancers to gefitinib (Iressa), kinase inhibitor. In-frame deletions occur in exon 19, whereas point mutations occur frequently in codon 858 (exon 21). We found from sequencing the EGFR TK domain that 7 of 10 gefitinib-sensitive tumors had similar types of alterations; no mutations were found in eight gefitinib-refractory tumors ($P = 0.004$). Five of seven tumors sensitive to erlotinib (Tarceva), a related kinase inhibitor for which the clinically relevant target is undocumented, had analogous somatic mutations, as opposed to none of 10 erlotinib-refractory tumors ($P = 0.003$). Because most mutation-positive tumors were adenocarcinomas from patients who smoked <100 cigarettes in a lifetime ("never smokers"), we screened EGFR exons 2-28 in 15 adenocarcinomas resected from untreated never smokers. Seven tumors had TK domain mutations, in contrast to 4 of 81 non-small cell lung cancers resected from untreated former or current smokers ($P = 0.0001$). Immunoblotting of lysates from cells transiently transfected with various EGFR constructs demonstrated that, compared to wild-type protein, an exon 19 deletion mutant induced diminished levels of phosphotyrosine, whereas the phosphorylation at tyrosine 1092 of an exon 21 point mutant was inhibited at 10-fold lower concentrations of drug. Collectively, these data show that adenocarcinomas from never smokers comprise a distinct subset of lung cancers, frequently containing mutations within the TK domain of EGFR that are associated with gefitinib and erlotinib sensitivity.

Pendaries, C., B. Darblade, et al. (2002). "The AF-1 activation-function of ERalpha may be dispensable to mediate the effect of estradiol on endothelial NO production in mice." *PNAS* **99**(4): 2205-2210.

<http://www.pnas.org/cgi/content/abstract/99/4/2205>

Two isoforms of estrogen receptor (ER) have been described: ER[alpha] and ER[beta]. The initial gene targeting of ER[alpha], consisting in the introduction of a Neo cassette in exon 1 [[alpha]ERKO, hereafter called ER[alpha]-Neo KO (knockout)], was reported in 1993. More recently, another mouse deficient in ER[alpha] because of the deletion of exon 2 (ER[alpha]KO, hereafter called ER[alpha]-[Delta]2 KO) was generated. In ovariectomized ER[alpha]-wild-type mice, estradiol (E2) increases uterine weight and basal production of endothelial nitric oxide (NO). Both of these effects are abolished in ER[alpha]-[Delta]2 KO mice. In contrast, we show here that both of these effects of E2 are partially (uterine weight) or totally (endothelial NO production) preserved in ER[alpha]-Neo KO. We also confirm the presence of two ER[alpha] mRNA splice variants in uterus and aorta from ER[alpha]-Neo KO mice. One of them encodes a chimeric ER[alpha] protein (ER[alpha]55), partially deleted in the A/B domain, that was detected in both uterus and aorta by Western blot analysis. The other ER[alpha] mRNA splice variant codes for an isoform deleted for the A/B domain (ER[alpha]46), which was detected in uterus of ER[alpha]-Neo KO, and wild-type mice. This protein isoform was not detected in aorta. The identification of these two N-terminal modified isoforms in uterus, and at least one of them in aorta, probably explains the persistence of the E2 effects in ER[alpha]-Neo KO mice. Furthermore, ER[alpha]-Neo KO mice may help in the elucidation of the specific functions of full-length ER[alpha] (ER[alpha]66) and ER[alpha]46, both shown to be physiologically generated in vivo.

Peters, J., E. Fowler, et al. (2002). "High diversity and rapid changeover of expressed var genes during the acute phase of Plasmodium falciparum infections in human volunteers." *PNAS* **99**(16): 10689-10694.

<http://www.pnas.org/cgi/content/abstract/99/16/10689>

Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) proteins expressed on the

surface of *P. falciparum*-infected erythrocytes undergo antigenic variation by switching the gene expressed within a repertoire of approximately 50 var genes per haploid genome. The switching of PfEMP1 plays an important role in the survival and pathogenesis of the parasite. To understand how a parasite switches its var gene expression in human infections, we investigated the composition and change of var gene transcripts during the acute phase of well-defined laboratory-induced *P. falciparum* infections in naive human hosts. Multiple var transcripts, with the same dominant transcript, were identified in samples collected after three to four asexual-parasite cycles in two volunteers infected with cloned 3D7 *P. falciparum* via mosquito bites. A major change in composition and frequency of var gene transcripts was observed between the culture used to infect the mosquitoes and the parasites recovered from the infected volunteers. A further change was seen when infected blood from a mosquito-infected volunteer was either passaged to other volunteers or cultured in vitro. The diversity of var transcripts did not increase with time. The results suggest that the switch of var gene expression is reinitiated after mosquito transmission and that var genes may rapidly switch from the first gene expressed after liver stage, but subsequent switching occurs at a much lower rate.

Pinkoski, M. J., N. M. Droin, et al. (2002). "Nonlymphoid Fas ligand in peptide-induced peripheral lymphocyte deletion." PNAS **99**(25): 16174-16179.

<http://www.pnas.org/cgi/content/abstract/99/25/16174>

Peripheral lymphocyte deletion is required for reduction of lymphocyte numbers after expansion in response to antigen. Peripheral deletion is mediated in part by the activation of apoptosis by engagement of the death receptor, Fas (CD95), by its ligand, Fas ligand (FasL; CD95L), among other mechanisms. Here we used T cell receptor (TCR) transgenic animals to examine the role of inducible expression of nonlymphoid FasL in response to peptide antigen. Antigenic challenge of TCR transgenic mice resulted in increased expression of FasL in a number of nonlymphoid tissues including the epithelium of the small intestine. Similar results were obtained in an adoptive transfer system in which TCR transgenic T cells were transferred into recipient animals. The functional relevance of nonlymphoid FasL in peripheral deletion is supported by the observation that FasL-deficient *gld* animals showed a significantly reduced rate of clearance of transferred antigen-specific lymphocytes, although the lymphocytes themselves were wild type for FasL. These observations were supported further by studies in a transgenic mouse model where *lacZ* was expressed under the control of the proximal promoter of the FasL gene. Using these transgenic mice, we observed induced activity of the FasL promoter in intestinal epithelial cells throughout the crypts and villi, where we also observed infiltration of activated T cells. These data demonstrate that nonlymphoid FasL is expressed in response to peripheral T cell activation and participates in the regulation of T cells that infiltrate peripheral tissues.

Prak, E. T. L., A. W. Dodson, et al. (2003). "Tracking an embryonic L1 retrotransposition event." PNAS **100**(4): 1832-1837.

<http://www.pnas.org/cgi/content/abstract/100/4/1832>

Long interspersed nuclear elements 1 (L1) are active retrotransposons that reside in many species, including humans and rodents. L1 elements produce an RNA intermediate that is reverse transcribed to DNA and inserted in a new genomic location. We have tagged an active human L1 element (L1RP) with a gene encoding enhanced GFP (EGFP). Expression of GFP occurs only if L1-EGFP has undergone a cycle of transcription, reverse transcription, and integration into a transcriptionally permissive genomic region. We show here that L1-EGFP can undergo retrotransposition in vivo and produce fluorescence in mouse testis. The retrotransposition event characterized here has occurred at a very early stage in the development

of an L1-EGFP transgenic founder mouse.

Puffenberger, E. G., D. Hu-Lince, et al. (2004). "Mapping of sudden infant death with dysgenesis of the testes syndrome (SIDDT) by a SNP genome scan and identification of TSPYL loss of function." PNAS **101**(32): 11689-11694.

<http://www.pnas.org/cgi/content/abstract/101/32/11689>

We have identified a lethal phenotype characterized by sudden infant death (from cardiac and respiratory arrest) with dysgenesis of the testes in males [Online Mendelian Inheritance in Man (OMIM) accession no. 608800]. Twenty-one affected individuals with this autosomal recessive syndrome were ascertained in nine separate sibships among the Old Order Amish. High-density single-nucleotide polymorphism (SNP) genotyping arrays containing 11,555 single-nucleotide polymorphisms evenly distributed across the human genome were used to map the disease locus. A genome-wide autozygosity scan localized the disease gene to a 3.6-Mb interval on chromosome 6q22.1-q22.31. This interval contained 27 genes, including two testis-specific Y-like genes (TSPYL and TSPYL4) of unknown function. Sequence analysis of the TSPYL gene in affected individuals identified a homozygous frameshift mutation (457_458insG) at codon 153, resulting in truncation of translation at codon 169. Truncation leads to loss of a peptide domain with strong homology to the nucleosome assembly protein family. GFP-fusion expression constructs were constructed and illustrated loss of nuclear localization of truncated TSPYL, suggesting loss of a nuclear localization patch in addition to loss of the nucleosome assembly domain. These results shed light on the pathogenesis of a disorder of sexual differentiation and brainstem-mediated sudden death, as well as give insight into a mechanism of transcriptional regulation.

Qin, M., J. Kang, et al. (2002). "Increased rates of cerebral glucose metabolism in a mouse model of fragile X mental retardation." PNAS **99**(24): 15758-15763.

<http://www.pnas.org/cgi/content/abstract/99/24/15758>

In humans, failure to express the fragile X mental retardation protein (FMRP) gives rise to fragile X syndrome, the most common form of inherited mental retardation. A fragile X knockout (fmr1 KO) mouse has been described that has some of the characteristics of patients with fragile X syndrome, including immature dendritic spines and subtle behavioral deficits. In our behavioral studies, fmr1 KO mice exhibited hyperactivity and a higher rate of entrance into the center of an open field compared with controls, suggesting decreased levels of anxiety. Our finding of impaired performance of fmr1 KO mice on a passive avoidance task is suggestive of a deficit in learning and memory. In an effort to understand what brain regions are involved in the behavioral abnormalities, we applied the [¹⁴C]deoxyglucose method for the determination of cerebral metabolic rates for glucose (CMR_{glc}). We measured CMR_{glc} in 38 regions in adult male fmr1 KO and WT littermates. We found CMR_{glc} was higher in all 38 regions in fmr1 KO mice, and in 26 of the regions, differences were statistically significant. Differences in CMR_{glc} ranged from 12% to 46%, and the greatest differences occurred in regions of the limbic system and primary sensory and posterior parietal cortical areas. Regions most affected are consistent with behavioral deficiencies and regions in which FMRP expression is highest. Higher CMR_{glc} in fragile X mice may be a function of abnormalities found in dendritic spines.

Reeves, M. B., P. A. MacAry, et al. (2005). "Latency, chromatin remodeling, and reactivation of human

cytomegalovirus in the dendritic cells of healthy carriers." PNAS **102**(11): 4140-4145.

<http://www.pnas.org/cgi/content/abstract/102/11/4140>

Human cytomegalovirus (HCMV) persists as a subclinical, lifelong infection in the normal human host, but reactivation from latency in immunocompromised subjects results in serious disease. Latency and reactivation are defining characteristics of the herpesviruses and are key to understanding their biology; however, the precise cellular sites in which HCMV is carried and the mechanisms regulating its latency and reactivation during natural infection remain poorly understood. Here we present evidence, based entirely on direct analysis of material isolated from healthy virus carriers, to show that myeloid dendritic cell (DC) progenitors are sites of HCMV latency and that their ex vivo differentiation to a mature DC phenotype is linked with reactivation of infectious virus resulting from differentiation-dependent chromatin remodeling of the viral major immediate-early promoter. Thus, myeloid DC progenitors are a site of HCMV latency during natural persistence, and there is a critical linkage between their differentiation to DC and transcriptional reactivation of latent virus, which is likely to play an important role in the pathogenesis of HCMV infection.

Rothenburg, S., N. Deigendesch, et al. (2005). "A PKR-like eukaryotic initiation factor 2{alpha} kinase from zebrafish contains Z-DNA binding domains instead of dsRNA binding domains." PNAS **102**(5): 1602-1607.

<http://www.pnas.org/cgi/content/abstract/102/5/1602>

The double-stranded RNA (dsRNA)-dependent protein kinase (PKR) is induced as part of the IFN response in mammals and acts to shut down protein synthesis by the phosphorylation of eukaryotic initiation factor 2{alpha} (eIF2{alpha}). In fish, a PKR-like kinase activity has been detected, but the enzyme responsible has eluded characterization. Here, we describe a PKR-like kinase from zebrafish. Phylogenetic analysis shows that the C-terminal kinase domain is more closely related to the kinase domain of PKR than to any of the other three known eIF2{alpha} kinases. Surprisingly, instead of the two dsRNA binding domains found at the N terminus of PKR, there are two Z{alpha} domains. Z{alpha} domains specifically bind dsDNA and RNA in the left-handed Z conformation, often with high affinity. They have been found previously in two other IFN-inducible proteins, the dsRNA editing enzyme, ADAR1, and Z-DNA binding protein 1 (ZBP1), as well as in the poxvirus virulence factor, E3L. This previously undescribed kinase, designated PKZ (protein kinase containing Z-DNA binding domains), is transcribed constitutively at low levels and is highly induced after injection of poly(inosinic)-poly(cytidylic) acid, which simulates viral infection. Binding of Z-DNA by the Z{alpha} domain of PKZ was demonstrated by circular dichroism. PKZ inhibits translation in transfected cells; site-directed mutagenesis indicates that this inhibition depends on its catalytic activity. Identification of a gene combining Z{alpha} domains with a PKR-like kinase domain strengthens the hypothesis that the ability to bind left-handed nucleic acid plays a role in the host response to viruses.

Saez, A. G., I. Probert, et al. (2003). "Pseudo-cryptic speciation in coccolithophores." PNAS **100**(12): 7163-7168.

<http://www.pnas.org/cgi/content/abstract/100/12/7163>

Coccolithophores are a group of calcifying unicellular algae that constitute a major fraction of oceanic primary productivity, play an important role in the global carbon cycle, and are key biostratigraphic marker fossils. Their taxonomy is primarily based on the morphology of the

minute calcite plates, or coccoliths, covering the cell. These are diverse and include widespread fine scale variation, of which the biological/taxonomic significance is unknown. Do they represent phenotypic plasticity, genetic polymorphisms, or species-specific characters? Our research on five commonly occurring coccolithophores supports the hypothesis that such variation represents pseudocryptic speciation events, occurring between 0.3 and 12.9 million years ago from a molecular clock estimation. This finding suggests strong stabilizing selection acting on coccolithophorid phenotypes. Our results also provide strong support for the use of fine scale morphological characters of coccoliths in the fossil record to improve biostratigraphic resolution and paleoceanographic data retrieval.

Sawada, H., N. Sakai, et al. (2002). "Extracellular ubiquitination and proteasome-mediated degradation of the ascidian sperm receptor." PNAS **99**(3): 1223-1228.

<http://www.pnas.org/cgi/content/abstract/99/3/1223>

The ubiquitin-proteasome system is essential for intracellular protein degradation, but an extracellular role of this system has not been known until now. We have previously reported that the proteasome is secreted into the surrounding seawater from sperm of the ascidian (Urochordata) *Halocynthia roretzi* on sperm activation, and that the sperm proteasome plays a key role in fertilization. Here, we show that a 70-kDa component (HrVC70) of the vitelline coat is the physiological substrate for the ubiquitin-proteasome system during fertilization of *H. roretzi*. A cDNA clone encoding the HrVC70 precursor (HrVC120) was isolated, and a homology search revealed that HrVC120 contains 13 epidermal growth factor-like repeats and a mammalian zona pellucida glycoprotein-homologous domain. HrVC70 functions as a sperm receptor. We demonstrate that HrVC70 is ubiquitinated both in vitro and in vivo. The immunocytochemical localization of multiubiquitin chains in the vitelline coat and the inhibitory effect of monoclonal antibodies against the multiubiquitin chains on fertilization strongly support the role of the ubiquitin-proteasome system in ascidian fertilization. Taken together, these results indicate that the ubiquitin-proteasome system is responsible for extracellular degradation of the sperm receptor HrVC70 and, consequently, for sperm penetration of the vitelline coat during fertilization.

Shi, L., Y.-J. Jung, et al. (2003). "Expression of Th1-mediated immunity in mouse lungs induces a *Mycobacterium tuberculosis* transcription pattern characteristic of nonreplicating persistence." PNAS **100**(1): 241-246.

<http://www.pnas.org/cgi/content/abstract/100/1/241>

The lung is the primary target of infection with *Mycobacterium tuberculosis*. It is well established that, in mouse lung, expression of adaptive, Th1-mediated host immunity inhibits further multiplication of *M. tuberculosis*. Here, real-time RT-PCR was used to define the pattern of expression against time of lung infection of key genes involved in Th1-mediated immunity and of selected genes of *M. tuberculosis*. Inhibition of bacterial multiplication was preceded by increased mRNA synthesis for IFN- γ and inducible NO synthase (NOS2) and by NOS2 protein synthesis in infected macrophages. Concurrently, the pattern of transcription of bacterial genes underwent dramatic changes. mRNA synthesis increased for α -crystallin (acr), rv2626c, and rv2623 and decreased for superoxide dismutase C (sodC), sodA, and fibronectin-binding protein B (fbpB). This pattern of *M. tuberculosis* transcription is characteristic of the nonreplicating persistence [Wayne, L. G. & Sohaskey, C. D. (2001) *Annu. Rev. Microbiol.* 55, 139-163] associated with adaptation of tubercle bacilli to hypoxia in vitro. Based on this similarity, we infer that host immunity induces bacterial growth arrest. In IFN- γ gene-deleted mice, bacterial growth was not controlled; NOS2 protein was not detected in macrophages; sodC, sodA, and fbpB transcription showed no decrease; and acr, rv2626c, and rv2623 transcription increased

only at the terminal stages of lung pathology. These findings define the transcription signature of *M. tuberculosis* as it transitions from growth to persistence in the mouse lung. The bacterial transcription changes measured at onset of Th1-mediated immunity are likely induced, directly or indirectly, by nitric oxide generated by infected macrophages.

Silic-Benussi, M., I. Cavallari, et al. (2004). "Suppression of tumor growth and cell proliferation by p13II, a mitochondrial protein of human T cell leukemia virus type 1." *PNAS* **101**(17): 6629-6634.

<http://www.pnas.org/cgi/content/abstract/101/17/6629>

Human T cell leukemia virus type 1 encodes an "accessory" protein named p13II that is targeted to mitochondria and triggers a rapid flux of K⁺ and Ca²⁺ across the inner membrane. In this study, we investigated the effects of p13II on tumorigenicity in vivo and on cell growth in vitro. Results showed that p13II significantly reduced the incidence and growth rate of tumors arising from c-myc and Ha-ras-cotransfected rat embryo fibroblasts. Consistent with these findings, HeLa-derived cell lines stably expressing p13II exhibited markedly reduced tumorigenicity, as well as reduced proliferation at high density in vitro. Mixed culture assays revealed that the phenotype of the p13II cell lines was dominant over that of control lines and was mediated by a heat-labile soluble factor. The p13II cell lines exhibited an enhanced response to Ca²⁺-mediated stimuli, as measured by increased sensitivity to C2-ceramide-induced apoptosis and by cAMP-responsive element-binding protein (CREB) phosphorylation in response to histamine. p13II-expressing Jurkat T cells also exhibited reduced proliferation, suggesting that the protein might exert similar effects in T cells, the primary target of HTLV-1 infection. These findings provide clues into the function of p13II as a negative regulator of cell growth and underscore a link between mitochondria, Ca²⁺ signaling, and tumorigenicity.

Sleat, D. E., J. A. Wiseman, et al. (2004). "Genetic evidence for nonredundant functional cooperativity between NPC1 and NPC2 in lipid transport." *PNAS* **101**(16): 5886-5891.

<http://www.pnas.org/cgi/content/abstract/101/16/5886>

Niemann-Pick C (NPC) disease is a fatal neurodegenerative disorder characterized by a lysosomal accumulation of cholesterol and other lipids within the cells of patients. Clinically identical forms of NPC disease are caused by defects in either of two different proteins: NPC1, a lysosomal-endosomal transmembrane protein and NPC2, a soluble lysosomal protein with cholesterol binding properties. Although it is clear that NPC1 and NPC2 are required for the egress of lipids from the lysosome, the precise roles of these proteins in this process is unknown. To gain insight into the normal function of NPC2 and to investigate its interactions, if any, with NPC1, we have generated a murine NPC2 hypomorph that expresses 0-4% residual protein in different tissues and have examined its phenotype in the presence and absence of NPC1. The phenotypes of NPC1 and NPC2 single mutants and an NPC1;NPC2 double mutant are similar or identical in terms of disease onset and progression, pathology, neuronal storage, and biochemistry of lipid accumulation. These findings provide genetic evidence that the NPC1 and NPC2 proteins function in concert to facilitate the intracellular transport of lipids from the lysosome to other cellular sites.

Sobol, R. W., D. E. Watson, et al. (2002). "Mutations associated with base excision repair deficiency and methylation-induced genotoxic stress." *PNAS* **99**(10): 6860-6865.

<http://www.pnas.org/cgi/content/abstract/99/10/6860>

The long-term effect of exposure to DNA alkylating agents is entwined with the cell's genetic capacity for DNA repair and appropriate DNA damage responses. A unique combination of environmental exposure and deficiency in these responses can lead to genomic instability; this "gene-environment interaction" paradigm is a theme for research on chronic disease etiology. In the present study, we used mouse embryonic fibroblasts with a gene deletion in the base excision repair (BER) enzymes DNA [beta]-polymerase ([beta]-pol) and alkyladenine DNA glycosylase (AAG), along with exposure to methyl methanesulfonate (MMS) to study mutagenesis as a function of a particular gene-environment interaction. The [beta]-pol null cells, defective in BER, exhibit a modest increase in spontaneous mutagenesis compared with wild-type cells. MMS exposure increases mutant frequency in [beta]-pol null cells, but not in isogenic wild-type cells; UV light exposure or N-methyl-N'-nitro-N-nitrosoguanidine exposure increases mutant frequency similarly in both cell lines. The MMS-induced increase in mutant frequency in [beta]-pol null cells appears to be caused by DNA lesions that are AAG substrates, because overexpression of AAG in [beta]-pol null cells eliminates the effect. In contrast, [beta]-pol/AAG double null cells are slightly more mutable than the [beta]-pol null cells after MMS exposure. These results illustrate that BER plays a role in protecting mouse embryonic fibroblast cells against methylation-induced mutations and characterize the effect of a particular combination of BER gene defect and environmental exposure.

Song, X., B. Wang, et al. (2002). "Retroviral-mediated transmission of a mouse VL30 RNA to human melanoma cells promotes metastasis in an immunodeficient mouse model." *PNAS* **99**(9): 6269-6273.

<http://www.pnas.org/cgi/content/abstract/99/9/6269>

Infection of a human melanoma cell line by a retroviral vector resulted in transmission of a mouse VL30 (mVL30-1) retroelement RNA to some of the cells infected by the retrovirus, followed by synthesis, integration, and expression of the mVL30-1 cDNA. One vector carried a tissue factor (TF) transgene that generated high TF melanoma clones, and another vector was a control without the TF transgene that generated low TF clones. Some high TF melanoma clones contained the mVL30-1 retroelement and others did not, and some low TF melanoma clones contained the mVL30-1 retroelement and others did not. Each type of melanoma clone was tested for its metastatic potential in severe combined immunodeficient (SCID) mice, by i.v. injection of the cells to generate lung tumors. None of the low TF clones that either contained or lacked the mVL30-1 retroelement generated lung tumors, consistent with earlier results showing that high TF expression promoted metastasis. The high TF clones containing the mVL30-1 retroelement were strongly metastatic, in contrast to the high TF clones lacking the mVL30-1 retroelement, which were weakly metastatic. Southern hybridization analyses showed that the mVL30-1 cDNA integrated into different genomic sites in different melanoma clones, suggesting that the effect of the mVL30-1 retroelement on metastasis depends not on integration per se but instead on expression of the mVL30-1 RNA. A role for the mVL30-1 RNA in metastasis and possibly other cell functions is an unexpected finding, because the RNA appears to lack significant coding potential for a functional protein. The metastatic effect might be mediated directly by a noncoding mVL30-1 RNA or by a peptide or small protein encoded by one of the short ORFs in the mVL30-1 RNA.

Stanton, T., S. Boxall, et al. (2003). "A high-frequency polymorphism in exon 6 of the CD45 tyrosine phosphatase gene (PTPRC) resulting in altered isoform expression." *PNAS* **100**(10): 5997-6002.

<http://www.pnas.org/cgi/content/abstract/100/10/5997>

CD45 (leukocyte common) antigen is a hemopoietic cell-specific tyrosine phosphatase essential for antigen receptor-mediated signaling in lymphocytes. The molecule undergoes complex alternative splicing in the extracellular domain, and different patterns of CD45 splicing are associated with distinct functions. Lack of CD45 leads to severe combined immunodeficiency, and alterations of CD45 splicing, because of a polymorphism in exon 4, have been associated with altered immune function. Here we describe a polymorphism in exon 6 (A138G) of the gene encoding CD45 that interferes with alternative splicing. The polymorphism results in an amino acid substitution of Thr-47 to Ala in exon 6, a potential O- and N-linked glycosylation site. This exon 6 A138G variant is present at a frequency of 23.7% in the Japanese population but is absent in Caucasoids. Peripheral blood T cells from individuals carrying the A138G variant show a significant decrease in the proportion of cells expressing the A, B, and C CD45 isoforms and a high frequency of CD45R0+ cells. These phenotypic alterations in the A138G carriers may lead to changes in ligand binding, homodimerization of CD45, and altered immune responses, suggesting the involvement of natural selection in controlling the A138G carrier frequency.

Summers, J., A. R. Jilbert, et al. (2003). "Inaugural Article: Hepatocyte turnover during resolution of a transient hepatitis B infection." PNAS **100**(20): 11652-11659.

<http://www.pnas.org/cgi/content/abstract/100/20/11652>

We estimated the amount of hepatocyte turnover in the livers of three woodchucks undergoing clearance of a transient woodchuck hepatitis infection by determining the fate of integrated viral DNA as a genetic marker of the infected cell population. Integrated viral DNA was found to persist in liver tissue from recovered animals at essentially undiminished levels of 1 viral genome per 1,000-3,000 liver cells, suggesting that the hepatocytes in the recovered liver were derived primarily from the infected cell population. We determined the single and multicopy distribution of distinct viral cell junctions isolated from small pieces of liver after clearance of the infection to determine the cumulative amount of hepatocyte proliferation that had occurred during recovery. We estimated that proliferation was equivalent to a minimum of 0.7-1 complete random turnovers of the hepatocyte population of the liver. Our results indicated that during resolution of the transient infections a large fraction of the infected hepatocyte population was killed and replaced by hepatocyte cell division.

Sun, L. V., L. Chen, et al. (2003). "Protein-DNA interaction mapping using genomic tiling path microarrays in *Drosophila*." PNAS **100**(16): 9428-9433.

<http://www.pnas.org/cgi/content/abstract/100/16/9428>

We demonstrate the use of a chromosomal walk (or "tiling path") printed as DNA microarrays for mapping protein-DNA interactions across large regions of contiguous genomic DNA in *Drosophila melanogaster*. Microarrays were constructed with genomic DNA fragments 430-920 bp in length, covering 2.9 million base pairs of the *Adh-cactus* region of chromosome 2 and 85,000 base pairs of the 82F region of chromosome 3. We performed DNA localization mapping for the heterochromatin protein HP1 and for the sequence-specific GAGA transcription factor, producing a comprehensive, high-resolution map of in vivo protein-DNA interactions throughout these regions of the *Drosophila* genome.

Taddeo, B., A. Esclatine, et al. (2002). "The patterns of accumulation of cellular RNAs in cells infected with a wild-type and a mutant herpes simplex virus 1 lacking the virion host shutoff gene." PNAS

99(26): 17031-17036.

<http://www.pnas.org/cgi/content/abstract/99/26/17031>

Cellular RNA extracted from quiescent human foreskin fibroblasts harvested at 1, 3, 7, or 12 h after infection was profiled on Affymetrix HG-U95Av2 arrays designed to detect 12,626 unique human transcripts. We also profiled RNA extracted from cells harvested at 1 and 7 h after infection with a mutant lacking the gene (Δ UL41) encoding a protein (vhs) brought into cells by the virus and responsible for nonselective degradation of RNA early in infection. We report the following: (i) of the 12 tested genes, up-regulated at least 3-fold relative to the values of mock infected cells, 9 were confirmed by real-time PCR. The microchip assays analyses indicate that there were 475 genes up-regulated >3 -fold. The up-regulated genes were clustered into 15 groups with respect to temporal pattern of transcript accumulation, and classified into 20 groups on the basis of their function. The preponderance of cellular genes up-regulated early in infection play a predominant role in transcription, whereas those up-regulated at later times respond to intracellular stress or concern themselves with the cell cycle and apoptosis. (ii) The number of genes up-regulated early in infection was higher in cells infected with the Δ UL41 mutant. Conversely, more genes were down-regulated late in infection with wild-type virus than with mutant viruses. Both observations are compatible with the known function of the UL41 gene product early in infection and with degradation of cellular RNAs in the absence of replenishment by de novo transcription of cellular genes.

Tauschek, M., R. J. Gorrell, et al. (2002). "Identification of a protein secretory pathway for the secretion of heat-labile enterotoxin by an enterotoxigenic strain of *Escherichia coli*." *PNAS* 99(10): 7066-7071.

<http://www.pnas.org/cgi/content/abstract/99/10/7066>

Enterotoxigenic *Escherichia coli* (ETEC) is an enteric pathogen that causes cholera-like diarrhea in humans and animals. ETEC secretes a heat-labile enterotoxin (LT), which resembles cholera toxin, but the actual mechanism of LT secretion is presently unknown. We have identified a previously unrecognized type II protein secretion pathway in the prototypic human ETEC strain, H10407 (serotype O78:H11). The genes for this pathway are absent from *E. coli* K-12, although examination of the K-12 genome suggests that it probably once possessed them. The secretory pathway bears significant homology at the amino acid level to the type II protein secretory pathway required by *Vibrio cholerae* for the secretion of cholera toxin. With this in mind, we determined whether the homologous pathway of *E. coli* H10407 played a role in the secretion of LT. To this end, we inactivated the pathway by inserting a kanamycin-resistance gene into one of the genes (*gspD*) of the type II secretion pathway by homologous recombination. LT secretion by *E. coli* H10407 and the *gspD* mutant was assayed by enzyme immunoassay, and its biological activity was assessed by using Y-1 adrenal cells. This investigation showed that the protein secretory pathway is functional and necessary for the secretion of LT by ETEC. Our findings have revealed the mechanism for the secretion of LT by ETEC, which previously was unknown, and provide further evidence of close biological similarities of the LT and cholera toxin.

Timm, J., F. A. Post, et al. (2003). "Differential expression of iron-, carbon-, and oxygen-responsive mycobacterial genes in the lungs of chronically infected mice and tuberculosis patients." *PNAS* 100(24): 14321-14326.

<http://www.pnas.org/cgi/content/abstract/100/24/14321>

Pathogenetic processes that facilitate the entry, replication, and persistence of *Mycobacterium*

tuberculosis (MTB) in the mammalian host likely include the regulated expression of specific sets of genes at different stages of infection. Identification of genes that are differentially expressed in vivo would provide insights into host-pathogen interactions in tuberculosis (TB); this approach might be particularly valuable for the study of human TB, where experimental opportunities are limited. In this study, the levels of selected MTB mRNAs were quantified in vitro in axenic culture, in vivo in the lungs of mice, and in lung specimens obtained from TB patients with active disease. We report the differential expression of MTB mRNAs associated with iron limitation, alternative carbon metabolism, and cellular hypoxia, conditions that are thought to exist within the granulomatous lesions of TB, in the lungs of wild-type C57BL/6 mice as compared with bacteria grown in vitro. Analysis of the same set of mRNAs in lung specimens obtained from TB patients revealed differences in MTB gene expression in humans as compared with mice.

Tsai, K. Y., D. MacPherson, et al. (2002). "ARF mutation accelerates pituitary tumor development in Rb+/- mice." PNAS **99**(26): 16865-16870.

<http://www.pnas.org/cgi/content/abstract/99/26/16865>

Mice heterozygous for the retinoblastoma (Rb) tumor suppressor gene develop pituitary and thyroid tumors with high penetrance. We demonstrate here that loss of the ARF tumor suppressor strongly accelerates intermediate lobe pituitary tumorigenesis in Rb heterozygous mice. These effects in the pituitary are greater than those conferred by p53 loss in that Rb+/-;ARF-/- mice display significantly more early atypical lesions than Rb+/-; p53-/- mice. Also, Rb+/-;ARF-/- compound mutants do not develop many of the novel tumors or precancerous lesions seen in Rb+/-;p53-/- compound mutants. Although complete loss of ARF expression is not obligatory for pituitary tumorigenesis in Rb+/- mice, alterations of the ARF locus are observed in tumors from Rb+/-;ARF+/- mice, consistent with a selective advantage of ARF inactivation in this context. We conclude that inactivation of ARF acts more broadly than that of p53 in connecting abrogation of the Rb pathway to tumorigenesis.

Twigg, S. R. F., R. Kan, et al. (2004). "Mutations of ephrin-B1 (EFNB1), a marker of tissue boundary formation, cause craniofrontonasal syndrome." PNAS **101**(23): 8652-8657.

<http://www.pnas.org/cgi/content/abstract/101/23/8652>

Craniofrontonasal syndrome (CFNS) is an X-linked developmental disorder that shows paradoxically greater severity in heterozygous females than in hemizygous males. Females have frontonasal dysplasia and coronal craniosynostosis (fusion of the coronal sutures); in males, hypertelorism is the only typical manifestation. Here, we show that the classical female CFNS phenotype is caused by heterozygous loss-of-function mutations in EFNB1, which encodes a member of the ephrin family of transmembrane ligands for Eph receptor tyrosine kinases. In mice, the orthologous Efnb1 gene is expressed in the frontonasal neural crest and demarcates the position of the future coronal suture. Although EFNB1 is X-inactivated, we did not observe markedly skewed X-inactivation in either blood or cranial periosteum from females with CFNS, indicating that lack of ephrin-B1 does not compromise cell viability in these tissues. We propose that in heterozygous females, patchwork loss of ephrin-B1 disturbs tissue boundary formation at the developing coronal suture, whereas in males deficient in ephrin-B1, an alternative mechanism maintains the normal boundary. This is the only known mutation in the ephrin/Eph receptor signaling system in humans and provides clues to the biogenesis of craniosynostosis.

Valentin, A., M. Rosati, et al. (2002). "Persistent HIV-1 infection of natural killer cells in patients receiving highly active antiretroviral therapy." PNAS **99**(10): 7015-7020.

<http://www.pnas.org/cgi/content/abstract/99/10/7015>

We have identified a subset of CD56+CD3[-] human natural killer (NK) cells that express CD4 and the HIV coreceptors CCR5 and CXCR4. These cells can be productively infected in vitro by both CCR5- and CXCR4-using molecular clones of HIV-1 in a CD4-dependent manner. Analysis of HIV-infected persons showed that viral DNA is present in purified NK cells, and virus could be rescued from these cells after in vitro cultivation. Longitudinal analysis of the HIV-1 DNA levels in NK cells from patients after 1-2 years of highly active antiretroviral therapy indicated that NK cells remain persistently infected and account for a substantial amount of the viral DNA in peripheral blood mononuclear cells. These results demonstrate that a subset of non-T cells with NK markers are persistently infected and suggest that HIV infection of NK cells is important for virus persistence. The properties of the virus reservoir in these cells should be considered in attempts to further optimize antiretroviral therapies.

von Wintzingerode, F., S. Bocker, et al. (2002). "Base-specific fragmentation of amplified 16S rRNA genes analyzed by mass spectrometry: A tool for rapid bacterial identification." PNAS **99**(10): 7039-7044.

<http://www.pnas.org/cgi/content/abstract/99/10/7039>

A rapid approach to the 16S rRNA gene (16S rDNA)-based bacterial identification has been developed that combines uracil-DNA-glycosylase (UDG)-mediated base-specific fragmentation of PCR products with matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS). 16S rDNA signature sequences were PCR-amplified from both cultured and as-yet-uncultured bacteria in the presence of dUTP instead of dTTP. These PCR products then were immobilized onto a streptavidin-coated solid support to selectively generate either sense or antisense templates. Single-stranded amplicons were subsequently treated with uracil-DNA-glycosylase to generate T-specific abasic sites and fragmented by alkaline treatment. The resulting fragment patterns were analyzed by MALDI-TOF MS. Mass signals of 16S rDNA fragments were compared with patterns calculated from published 16S rDNA sequences. MS of base-specific fragments of amplified 16S rDNA allows reliable discrimination of sequences differing by only one nucleotide. This approach is fast and has the potential for high-throughput identification as required in clinical, pharmaceutical, or environmental microbiology. In contrast to identification by MS of intact whole bacterial cells, this technique allows for the characterization of both cultured and as-yet-uncultured bacteria.

Vranova, E., S. Atichartpongkul, et al. (2002). "Comprehensive analysis of gene expression in *Nicotiana tabacum* leaves acclimated to oxidative stress." PNAS **99**(16): 10870-10875.

<http://www.pnas.org/cgi/content/abstract/99/16/10870>

The molecular mechanisms by which plants acclimate to oxidative stress are poorly understood. To identify the processes involved in acclimation, we performed a comprehensive analysis of gene expression in *Nicotiana tabacum* leaves acclimated to oxidative stress. Combining mRNA differential display and cDNA array analysis, we estimated that at least 95 genes alter their expression in tobacco leaves acclimated to oxidative stress, of which 83% are induced and 17% repressed. Sequence analysis of 53 sequence tags revealed that, in addition to antioxidant genes, genes implicated in abiotic and biotic stress defenses, cellular protection and

detoxification, energy and carbohydrate metabolism, de novo protein synthesis, and signal transduction showed altered expression. Expression of most of the genes was enhanced, except for genes associated with photosynthesis and light-regulated processes that were repressed. During acclimation, two distinct groups of coregulated genes ("early-" and "late-response" gene regulons) were observed, indicating the presence of at least two different gene induction pathways. These two gene regulons also showed differential expression patterns on an oxidative stress challenge. Expression of "late-response" genes was augmented in the acclimated leaf tissues, whereas expression of "early-response" genes was not. Together, our data suggest that acclimation to oxidative stress is a highly complex process associated with broad gene expression adjustments. Moreover, our data indicate that in addition to defense gene induction, sensitization of plants for potentiated gene expression might be an important factor in oxidative stress acclimation.

White, S. N., K. H. Taylor, et al. (2003). "Haplotype variation in bovine Toll-like receptor 4 and computational prediction of a positively selected ligand-binding domain." *PNAS* **100**(18): 10364-10369.

<http://www.pnas.org/cgi/content/abstract/100/18/10364>

Toll-like receptor 4 (TLR4) is a cell-surface receptor that activates innate and adaptive immune responses. Because it recognizes a broad class of pathogen-associated molecular patterns presented by lipopolysaccharides and lipoteichoic acid, TLR4 is a candidate gene for resistance to a large number of diseases. In particular, mouse models suggest TLR4 as a candidate gene for resistance to major agents in bovine respiratory disease and Johne's disease. The coding sequence of bovine TLR4 is divided into three exons, with intron/exon boundaries and intron sizes similar to those of human TLR4 transcript variant 1. We amplified each exon in 40 individuals from 11 breeds and screened the sequence for single-nucleotide polymorphisms (SNPs). We identified 32 SNPs, 28 of which are in the coding sequence, for an average of one SNP per 90 bp of coding sequence. Eight SNPs were nonsynonymous and potentially alter specificity of pathogen recognition or efficiency of signaling. To evaluate the functional importance of these SNPs, we used codon-substitution models to detect diversifying selection in an extracellular region that may physically interact with ligands. One nonsynonymous SNP is located within this region, and other substitutions are in adjacent regions that may interact with coreceptor molecules. The 32 SNPs were found in 20 haplotypes that can be assigned to geographic ranges of origin. Haplotype-tagging SNP analysis indicated that 12 SNPs need to be genotyped to distinguish these 20 haplotypes. These data provide a basic understanding of bovine TLR4 sequence variation and supply haplotype markers for disease association studies.

Wolfe, J. L., T. Kawate, et al. (2002). "A genotyping strategy based on incorporation and cleavage of chemically modified nucleotides." *PNAS* **99**(17): 11073-11078.

<http://www.pnas.org/cgi/content/abstract/99/17/11073>

Aiming to facilitate the analysis of human genetic variations in the context of disease susceptibility and varied drug response, we have developed a genotyping method that entails incorporation of a chemically labile nucleotide by PCR followed by specific chemical cleavage of the resulting amplicon at the modified bases. The identity of the cleaved fragments determines the genotype of the DNA. This method, termed Incorporation and Complete Chemical Cleavage, utilizes modified nucleotides 7-deaza-7-nitro-dATP, 7-deaza-7-nitro-dGTP, 5-hydroxy-dCTP, and 5-hydroxy-dUTP, which have increased chemical reactivity but are able to form standard Watson-Crick base pairs. Thus one analog is substituted for the corresponding nucleotide during PCR, generating amplicons that contain nucleotide analogs at each occurrence of the selected base throughout

the target DNA except for the primer sequences. Subsequent treatment with an oxidant followed by an organic base results in chemical cleavage at each site of modification, which produces fragments of different lengths and/or molecular weights that reflect the genotype of the original DNA sample at the site of interest. This incorporation and cleavage chemistry are widely applicable to many existing nucleic acid analysis platforms, including gel electrophoresis and mass spectrometry. By combining DNA amplification and analog incorporation into one step, this strategy eliminates preamplification, DNA-strand separation, primer extension, and purification procedures associated with traditional chain-termination chemistry and therefore presents significant advantages in terms of speed, cost, and simplicity of genotyping.

Wong, N. A. C. S., M. P. Britton, et al. (2004). "Loss of CDX1 expression in colorectal carcinoma: Promoter methylation, mutation, and loss of heterozygosity analyses of 37 cell lines." PNAS **101**(2): 574-579.

<http://www.pnas.org/cgi/content/abstract/101/2/574>

Expression of the homeobox protein CDX1 is lost or reduced in a significant proportion of colorectal carcinomas (CRCs) but the underlying mechanism for this is unclear. We have demonstrated absence of CDX1 mRNA expression in 7 of 37 CRC cell lines and shown that all 7 cell lines have a methylated CDX1 promoter. Twenty-five cell lines showed both CDX1 mRNA expression and an unmethylated CDX1 promoter. The five remaining cell lines had a partially methylated CDX1 promoter and all expressed CDX1 mRNA; when treated with the demethylating agent, 5-aza-2'-deoxycytidine, these five cell lines all showed increased CDX1 expression. No mutations were found in the promoter and coding regions of CDX1. One polymorphism was demonstrated in each of the promoter, 5' UTR, and coding region of exon 1 of CDX1, but there were no associations between CDX1 mRNA expression and different polymorphic genotypes. Similarly, there was no association between CDX1 mRNA expression and loss of heterozygosity at the CDX1 locus. In conclusion, absence or reduction of CDX1 expression in CRC seems to be primarily regulated by promoter methylation and is probably selected for because of its impact on the differentiation of colonocytes.

Yamanishi, Y., D. L. Boyle, et al. (2002). "Regional analysis of p53 mutations in rheumatoid arthritis synovium." PNAS **99**(15): 10025-10030.

<http://www.pnas.org/cgi/content/abstract/99/15/10025>

The p53 tumor suppressor protein plays a central role in cell cycle regulation, DNA repair, and apoptosis. Recent studies indicate that DNA damage and somatic mutations in the p53 gene can occur because of genotoxic stress in many tissues, including the skin, colon, and synovium. Although somatic mutations in the p53 gene have been demonstrated in rheumatoid arthritis (RA) synovial tissue and synoviocytes, no information is available on the location or extent of p53 mutations. Using microdissected RA synovial tissue sections, we observed abundant p53 transition mutations, which are characteristic DNA damage caused by oxidative stress. p53 mutations, as well as p53 mRNA expression, were located mainly in the synovial intimal lining rather than the sublining ($P < 0.01$). Clusters of p53 mutant subclones were observed in some microdissected regions, suggesting oligoclonal expansion. Because IL-6 gene expression is regulated by wild-type p53, IL-6 mRNA expression in microdissected tissues was quantified by using real-time PCR. The regions with high rates of p53 mutations contained significantly greater amounts of IL-6 mRNA compared with the low mutation samples ($P < 0.02$). The microdissection findings suggest that p53 mutations are induced in RA synovial tissues by inflammatory oxidative stress. This process, as in sun-exposed skin and inflamed colonic epithelium, provides some of the mutant clones with a selective growth advantage. A relatively low percentage of cells

containing p53 mutations can potentially affect neighboring cells and enhance inflammation through the elaboration of proinflammatory cytokines.

Yoo, S.-H., S. Yamazaki, et al. (2004). "Inaugural Article: PERIOD2:LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues." PNAS **101**(15): 5339-5346.

<http://www.pnas.org/cgi/content/abstract/101/15/5339>

Mammalian circadian rhythms are regulated by the suprachiasmatic nucleus (SCN), and current dogma holds that the SCN is required for the expression of circadian rhythms in peripheral tissues. Using a PERIOD2:LUCIFERASE fusion protein as a real-time reporter of circadian dynamics in mice, we report that, contrary to previous work, peripheral tissues are capable of self-sustained circadian oscillations for >20 cycles in isolation. In addition, peripheral organs expressed tissue-specific differences in circadian period and phase. Surprisingly, lesions of the SCN in mPer2Luciferase knockin mice did not abolish circadian rhythms in peripheral tissues, but instead caused phase desynchrony among the tissues of individual animals and from animal to animal. These results demonstrate that peripheral tissues express self-sustained, rather than damped, circadian oscillations and suggest the existence of organ-specific synchronizers of circadian rhythms at the cell and tissue level.

Zhong, X.-b., R. Reynolds, et al. (2003). "Single-nucleotide polymorphism genotyping on optical thin-film biosensor chips." PNAS **100**(20): 11559-11564.

<http://www.pnas.org/cgi/content/abstract/100/20/11559>

Single-nucleotide polymorphisms (SNPs) constitute the bulk of human genetic variation and provide excellent markers to identify genetic factors contributing to complex disease susceptibility. A rapid, sensitive, and inexpensive assay is important for large-scale SNP scoring. Here we report the development of a multiplex SNP detection system using silicon chips coated to create a thin-film optical biosensor. Allele-discriminating, aldehyde-labeled oligonucleotides are arrayed and covalently attached to a hydrazinederivatized chip surface. Target sequences (e.g., PCR amplicons) then are hybridized in the presence of a mixture of biotinylated detector probes, one for each SNP, and a thermostable DNA ligase. After a stringent wash (0.01 M NaOH), ligation of biotinylated detector probes to perfectly matched capture oligomers is visualized as a color change on the chip surface (gold to blue/purple) after brief incubations with an anti-biotin IgG-horseradish peroxidase conjugate and a precipitable horseradish peroxidase substrate. Testing of PCR fragments is completed in 30-40 min. Up to several hundred SNPs can be assayed on a 36-mm² chip, and SNP scoring can be done by eye or with a simple digital-camera system. This assay is extremely robust, exhibits high sensitivity and specificity, and is format-flexible and economical. In studies of mutations associated with risk for venous thrombosis and genotyping/haplotyping of African-American samples, we document high-fidelity analysis with 0 misassignments in 500 assays performed in duplicate.

Malhi, H., B. Atac, et al. (2004). "Warfarin and celecoxib interaction in the setting of cytochrome P450 (CYP2C9) polymorphism with bleeding complication." Postgrad. Med. J. **80**(940): 107-109.

<http://pmj.bmjournals.com/cgi/content/abstract/80/940/107>

Drug metabolism may be perturbed by genetically determined differences in the metabolic activity of cytochrome P450 enzymes. The authors encountered extensive bleeding in a patient receiving warfarin for anticoagulation after the introduction of celecoxib, an anti-inflammatory drug. As the CYP2C9 enzyme metabolises these drugs, it was determined whether variant alleles were responsible for altering warfarin handling. Genetic analysis established that the patient was a compound heterozygote with CYP2C9*2 and *3 variant alleles, which exhibit lower drug metabolising capacity and enhance susceptibility to drug toxicity.

Postharvest Biology and Technology (3)

Boysen, M. E., S. Bjorneholm, et al. (2000). "Effect of the biocontrol yeast *Pichia anomala* on interactions between *Penicillium roqueforti*, *Penicillium carneum*, and *Penicillium paneum* in moist grain under restricted air supply." Postharvest Biology and Technology **19**(2): 173.

<http://www.sciencedirect.com/science/article/B6TBJ-407GJ3B-8/2/8498a668b9436fd414c336501776274c>

Penicillium roqueforti was recently reclassified into the three species *P. roqueforti*, *Penicillium carneum*, and *Penicillium paneum* based on differences in ribosomal DNA sequences and secondary metabolites, e.g. mycotoxins. This is the first report on interaction between these closely related mould species under stress conditions. The yeast *Pichia anomala* (J121) inhibits growth of *P. roqueforti* in grain stored in malfunctioning airtight storage systems. The ability of *P. anomala* to inhibit all three species of the *P. roqueforti* group was examined in separate experiments as well as the competition between the three mould species when co-cultured with or without the yeast in non-sterile wheat grain (aw 0.95) under restricted air supply. Mould growth was analysed by dilution plating after 14 days and the individual colonies identified by random amplified polymorphic DNA (RAPD) fingerprinting. When co-culturing the *P. roqueforti* group in wheat without *P. anomala* all three species were able to grow to the same extent. Also, when co-culturing all species of the *P. roqueforti* group together with *P. anomala*, the growth response of the three species was very similar. At yeast levels of 104 CFU g⁻¹ grain a pronounced inhibition was observed and at 105 CFU g⁻¹ grain a fungicidal effect was detected, indicating a potentiated effect of *P. anomala* when co-culturing the three mould species.

Narumi, T., Y. Kanno, et al. (2005). "Cloning of a cDNA encoding an ethylene receptor (DG-ERS1) from chrysanthemum and comparison of its mRNA level in ethylene-sensitive and -insensitive cultivars." Postharvest Biology and Technology **36**(1): 21.

<http://www.sciencedirect.com/science/article/B6TBJ-4FG899S-1/2/b3d3f06e934f3144d24284b14c539946>

A cDNA encoding a putative ethylene receptor (DG-ERS1) was isolated from chrysanthemum [*Dendranthema grandiflorum* (Ramat.) Kitamura] using a combination of reverse transcription

PCR (RT-PCR), cDNA library screening and 5'-RACE techniques. The cDNA (2427 bp) contained an open reading frame of 1920 bp coding for 640 amino acids. The predicted DG-ERS1 protein has an amino-terminal ethylene sensor domain and a histidine kinase domain, but lacks a receiver domain. The DG-ERS1 protein has 72, 70 and 69% similarity to Arabidopsis ERS1, tomato Never ripe (NR) and carnation DC-ERS2, respectively. Real time PCR analysis revealed that DG-ERS1 mRNA was present in a large amount in ligulate corollas (hereafter, petals for short) and mature leaves of an ethylene-sensitive cultivar 'Seiko-no-makoto' at the full-opening stage of the flower, and the amount decreased with time or in response to a 12-h ethylene treatment. In an ethylene-insensitive cultivar 'Iwa-no-hakusen', the amount of DG-ERS1 mRNA in petals was one-fourth and that in mature leaves was only one-twentieth of the amount in 'Seiko-no-makoto' at the full-opening stage, and its amount in both tissues scarcely changed with time or in response to a 12-h ethylene treatment. These findings suggest the involvement of DG-ERS1 in the perception of ethylene in cut chrysanthemum plants, especially in those of 'Seiko-no-makoto' cultivar.

Nishikawa, F., M. Kato, et al. (2003). "Two ascorbate peroxidases from broccoli: identification, expression and characterization of their recombinant proteins." Postharvest Biology and Technology **27**(2): 147.

<http://www.sciencedirect.com/science/article/B6TBJ-46Y5881-2/2/6725fa548dc14d77ba35bfe949858af0>

Two distinct clones having high nucleotide identity to the sequences encoding ascorbate peroxidase (APX) were isolated from broccoli (*Brassica oleracea* L. var. *italica*). Deduced amino acid sequences of both cDNAs, BO-APX 1 (accession number AB078599) and BO-APX 2 (accession number AB078600), shared identity of 92.8% and there was more than 80% identity between BO-APXs and other plant cytosolic APXs at the protein level. Gene expression and protein levels of BO-APX 1 and BO-APX 2 were investigated in various parts of broccoli after harvest. Transcript levels of BO-APX 2 gradually increased in florets, while those of BO-APX 1 decreased in florets after harvest. BO-APX 1 and BO-APX 2 were expressed in *Escherichia coli* as a fusion protein with glutathione S-transferase (GST) and purified to homogeneity by glutathione sepharose 4B column chromatography. Both proteins of BO-APX 1 and BO-APX 2 appeared as a single major band on SDS-PAGE corresponding to a mass of 25 kDa and reacted with polyclonal antibodies raised against recombinant BO-APX 1. Both enzymes showed high specificities for ascorbate and hydrogen peroxide. The K_m values of recombinant BO-APX 1 and BO-APX 2 for ascorbate were 395 and 526 μM and those for hydrogen peroxide were 15 and 7 μM , respectively. The role of APX was discussed in relation to ascorbate breakdown in broccoli florets during senescence.

Preventive Veterinary Medicine (1)

Milian-Suazo, F., V. Banda-Ruiz, et al. (2002). "Genotyping of *Mycobacterium bovis* by geographic location within Mexico." Preventive Veterinary Medicine **55**(4): 255.

<http://www.sciencedirect.com/science/article/B6TBK-45S9CHT-1/2/f32a690a77e9483b44dc84637601db16>

The spacer oligonucleotide typing (spoligotyping) method was used to differentiate 62 *Mycobacterium bovis* isolates obtained from tissues with macroscopic lesions typical of tuberculosis in dairy cattle from different regions of Mexico. Our purpose was to see if a strain from one region was genetically different from those of other regions (with the long-term aim of doing molecular trace back of isolates obtained in the laboratory). Results from the genetic analysis indicate that *M. bovis* isolates cannot be grouped by geographic location due to a wide range of genetic types involved in dairy cattle infections. Isolates even from the same herd showed different spoligotypes but some isolates from different region had similar genetic patterns. Genetic typing without epidemiologic information does not seem to be a plausible method to trace back animals to source of origin to detect and eliminate sources of infection.

Process Biochemistry (1)

Lin, Y.-S., M.-L. Chao, et al. (2004). "Cloning and expression of the transglutaminase gene from *Streptovorticillium ladakanum* in *Streptomyces lividans*." Process Biochemistry **39**(5): 591.

<http://www.sciencedirect.com/science/article/B6THB-49PRGPT-1/2/877fa90cfb9125ab47a7d47e99aa553>

A gene, tgB1, encoding transglutaminase (TGase) in *Streptovorticillium ladakanum* B1 was cloned and expressed in *Streptomyces lividans*. The tgB1 gene consisted of an open reading frame of 1230 nucleotides encoding a protein of 410 amino acids with a calculated molecular weight of 45780 Da. The deduced amino acid sequence is highly homologous to TGases from *Streptovorticillium* spp. but exhibits little homology with TGases of *Bacillus subtilis* and mammalian origins. The putative active site, YGCVG, conserved in *Streptovorticillium* TGases is also present in TgB1. No -10 and -35 regions of the putative promoter could be identified. Two A+T-rich regions, characteristics of a promoter sequence, were found at bp 238-269 and bp 631-681. The tgB1 gene was expressed in *S. lividans* JT46 under the control of its endogenous promoter. Immunoblotting of SDS-PAGE revealed that, in addition to protein bands with sizes corresponding to those of the unprocessed and mature TgB1, several bands with sizes in between reacting with anti-TgB1 IgG were present in the culture supernatant of the recombinant strain. This suggests that the recombinant TgB1 was not correctly processed during secretion in the transformed *S. lividans* JT46.

Progress in Neuro-Psychopharmacology and Biological Psychiatry (3)

Fehr, C., B. L. S. Rademacher, et al. (2003). "Evaluation of the glutamate decarboxylase genes Gad1 and Gad2 as candidate genes for acute ethanol withdrawal severity in mice." Progress in Neuro-Psychopharmacology and Biological Psychiatry **27**(3): 467.

<http://www.sciencedirect.com/science/article/B6TBR-480CSPJ-3/2/c9854359f74ce9fa0edf4ac819445cb8>

Previous studies in crosses between the C57BL/6J (B6) and the DBA/2J (D2) mice have implicated a role of the genes encoding for the 67- and 65-kDa isoforms of the glutamate decarboxylase (Gad1 and Gad2) in the manifestation and severity of multiple ethanol-related traits such as acute ethanol withdrawal severity [Buck, K.J., Metten, P., Belknap, J.K., Crabbe, J.C., 1997. Quantitative trait loci involved in genetic predisposition to acute alcohol withdrawal in mice. *J. Neurosci.* 17, 3946-3955], ethanol preference [Phillips, T.J., Belknap, J.K., Buck, K.J., Cunningham, C.L., 1998. Genes on mouse chromosomes 2 and 9 determine variation in ethanol consumption. *Mamm. Genome* 9, 936-941] and ethanol-induced locomotion [Demarest, K., McCaughan Jr., J., Mahjubi, E., Cipp, L., Hitzemann, R., 1999. Identification of an acute ethanol response quantitative trait locus on mouse chromosome 2. *J. Neurosci.* 19, 549-561]. Strain-specific sequencing experiments as well as gene expression studies in drug-naive and ethanol-treated D2 and B6 mice were carried out. The Gad1 sequence was similar, the Gad2 cDNA carried only a silent polymorphism (1017 G>C) between both strains. In addition, no significant GAD65 or GAD67 expression differences were detected in either drug-naive or acute ethanol withdrawn animals by Western blot experiments. Therefore, these results do not support the hypothesis of an involvement of Gad1 or Gad2 in the pathophysiology of acute ethanol withdrawal severity and the other ethanol related traits.

Fehr, C., A. Schleicher, et al. (2001). "Serotonergic polymorphisms in patients suffering from alcoholism, anxiety disorders and narcolepsy." Progress in Neuro-Psychopharmacology and Biological Psychiatry **25**(5): 965.

<http://www.sciencedirect.com/science/article/B6TBR-43S179D-1/2/a7702e7b3755f3805b44ba7575ac8cfc>

1. Alterations in the serotonergic neurotransmission have been frequently described for patients suffering from alcoholism, anxiety disorders and narcolepsy. 2. The authors tested for association of the 5-HT_{2A} receptor polymorphism (T102C) and the intron 7 tryptophan hydroxylase (TPH) polymorphism (A218C) among 176 alcohol dependent patients, 35 patients with panic disorder, 50 patients with generalized anxiety disorder, 55 patients with narcolepsy and 87 healthy controls. 3. Allele and genotype frequencies of the 5-HT_{2A} receptor polymorphism (T102C), the intron 7 TPH polymorphism (A218C) were almost similar between the patients suffering from alcohol dependence, panic disorder, generalized anxiety disorder and narcolepsy. 4. There was no association between the 5-HT_{2A} receptor polymorphism (T102C), the intron 7 TPH (A218C) polymorphisms and alcohol dependence, panic disorder, generalized anxiety disorder and narcolepsy in our subsets of German patients.

Murayama, O., M. Murayama, et al. (1999). "Twenty-nine missense mutations linked with familial Alzheimer's disease alter the processing of presenilin 1." Progress in Neuro-Psychopharmacology and Biological Psychiatry **23**(5): 905.

<http://www.sciencedirect.com/science/article/B6TBR-3XP0K3F-C/2/c84f91b9a59f34154ff3885895ee8fd7>

1. 1. Full-length form of human presenilin I (PS1) is processed and an N-terminal fragment (28 KD) and C-terminal fragment (19 KD) are generated. To elucidate the possible role of presenilin mutations in Alzheimer's disease (AD), the authors analyze the effects of AD-linked mutations on PS1 processing in cultured cells. 2. 2. Complementary DNAs encoding genes for human PS1 harboring twenty-nine missense mutations linked with familial Alzheimer's disease (FAD) were introduced into PC12 cells. Human PS1 exogenously expressed in the cells was detected by immunoblotting using a monoclonal antibody that recognized the N-terminal region of human PS1. The amounts of full-length form (48 KD) and N-terminal fragment (28 KD) of PS1 was

quantified by densitometrical analysis.3. 3. The ratio of the N-terminal fragment to total PS1 was reduced by twenty-nine mutations. The specific effects on PS1 processing varied according to mutation.4. 4. These results suggest that AD-linked missense mutations of PS1 are involved in neurodegeneration via inhibition of PS1 processing.

Progress in Neurobiology (1)

Ojika, K., S. Mitake, et al. (2000). "Hippocampal cholinergic neurostimulating peptides (HCNP)." Progress in Neurobiology **60**(1): 37.

<http://www.sciencedirect.com/science/article/B6T0R-3Y56KDG-3/2/a4bfd0eafea59e39863e37e91edff553>

Neuronal development and differentiation require a variety of cell interactions. Diffusible molecules from target neurons play an important part in mediating such interactions. Our early studies used explant culture technique to examine the factors that enhance the differentiation of septo-hippocampal cholinergic neurons, and they revealed that several components resident in the hippocampus are involved in the differentiation of presynaptic cholinergic neurons in the medial septal nucleus. One of these components, originally purified from young rat hippocampus, is a novel undecapeptide (hippocampal cholinergic neurostimulating peptide; HCNP); this enhances the production of ChAT, but not of AchE. Later experiments revealed that: (1) a specific receptor appears to mediate this effect; (2) NGF and HCNP act cooperatively to regulate cholinergic phenotype development in the medial septal nucleus in culture; and (3) these two molecules differ both in their mechanism of release from the hippocampus and their mechanism of action on cholinergic neurons. The amino acid sequence deduced from base sequence analysis of cloned HCNP-precursor protein cDNA shows that HCNP is located at the N-terminal domain of its precursor protein. The 21 kDa HCNP precursor protein shows homology with other proteins, and it functions not only as an HCNP precursor, but also as a binding protein for ATP, opioids and phosphatidylethanolamine. The distribution and localization of HCNP-related components and the expression of their mRNAs support the notion that the precursor protein is multifunctional. In keeping with its multiple functions, the multiple enhancers and promoters found in the genomic DNA for HCNP precursor protein may be involved in the regulation of its gene in a variety of cells and at different stages of development. Furthermore, several lines of evidence obtained from studies of humans and animal models suggest that certain types of memory and learning disorders are associated with abnormal accumulation and expression of HCNP analogue peptide and/or its precursor protein mRNA in the hippocampus.

Prostaglandines and Medicine(1)

Young, B. K., J. Noumoff, et al. (1981). "Effects of prostagiandin F2[alpha] or oxytocin on serum estriol and its conjugates during induced labor." Prostaglandines and Medicine **6**(3): 257.

<http://www.sciencedirect.com/science/article/B7GHB-4BTXW56->

1/2/8487491a2a9345060e91468a7b52ed20

Twelve patients underwent induction of labor at term, seven by prostaglandin F2[alpha] (PGF) and five by oxytocin infusion. Serum samples were drawn before induction and at two hour intervals after infusion was started, throughout labor. The samples were analyzed for estriol (E3) and its principal conjugates, estriol-3-sulfate (E3-3S), estriol-16-glucosiduronate (E3-16G), estriol-3-glucosiduronate (E3-3G), and estriol-3-sulfate-16-glucosiduronate (E3-SG). No significant changes occurred before eight hours of infusion. Two pre-eclamptic patients showed a 100[deg] increase in serum E3 while receiving PGF. The increases correlated best with rising levels of E3-SG. None of the five pre-eclamptic patients given oxytocin showed this striking change. These observations raise the possibility that PGF may alter the metabolism of estriol in pre-eclamptic patients, during labor.

Prostaglandins (2)

Cortese, J. F., E. W. Spannake, et al. (1995). "The 5-lipoxygenase pathway in cultured human intestinal epithelial cells." Prostaglandins **49**(3): 155.

<http://www.sciencedirect.com/science/article/B7CRH-4F5T6BT-3/2/517257ef4b7f1c387ee97b67a9a117e8>

Leukotrienes (LTs), the 5-lipoxygenase (5-LOX) metabolites of arachidonic acid, have roles in many biological processes relevant to the gastrointestinal tract, including intestinal inflammation. We screened two well-known human intestinal epithelial cell lines, HT29 and Caco-2, for evidence of LT-associated enzyme transcripts and LT synthesis. Northern blot analysis of total RNA from both intestinal lines demonstrated high levels of transcripts for LTA4 hydrolase, a multisubstrate enzyme that converts the 5-LOX metabolite, LTA4, to LTB4. With total RNA, the 5-LOX transcript was detected only in HT29. Caco-2 failed to show 5-LOX message even with poly A-containing RNA, although the transcript could be amplified with the polymerase chain reaction. Messenger RNA for FLAP, the 5-lipoxygenase-activating protein, was detectable in both cell lines, but only with poly A-containing RNA. In a sonicated cell preparation, HT29, but not Caco-2, revealed detectable levels of 5-HETE and LTB4. These results suggest that certain intestinal epithelial cells possess a limited capacity to synthesize LTs.

Ponton, A., J.-P. Thirion, et al. (1997). "Repression of the 5-lipoxygenase gene by c-myc overexpression in differentiated HL-60 cells." Prostaglandins **53**(1): 49.

<http://www.sciencedirect.com/science/article/B7CRH-4F66YRD-R/2/df271feeab83aadfc43dab3e557f48a5>

This paper reports on the involvement of c-MYC in the regulation of 5-lipoxygenase gene expression during differentiation of human HL-60 cells. We demonstrate that c-MYC binds, the 5-lipoxygenase promoter in undifferentiated cells but not in DMSQ-differentiated cells. Also, we show that overexpression of c-myc cDNA in differentiated HL-60 cells represses the 5-lipoxygenase gene expression.

Lyons, C., K. Beharry, et al. (2003). "In vitro response of prostaglandin E2 receptor (EP3) in the term pregnant rat uterus and cervix to misoprostol." Prostaglandins & Other Lipid Mediators **70**(3-4): 317.

<http://www.sciencedirect.com/science/article/B6T3H-47GYJNS-1/2/61ea76529c5791eb548057645fda81f6>

We examined and compared the in vitro effects of misoprostol (synthetic prostaglandin E1 (PGE1) analogue) on prostaglandin E2 (PGE2) secretion and EP3 receptor mRNA expression in the pregnant rat myometrium and cervix at 19 days gestation. Myometrial and cervical tissue samples were exposed to media with or without misoprostol (50 or 100 pg/ml) and incubated for 15 and 30 min, and 1, 3, 6, 12, and 24 h. Media and tissue samples were collected for quantification of PGE2 and mRNA expression of rEP3[alpha] and rEP3[beta] receptor, respectively. PGE2 secretion increased (P2 secretion increased following exposure to the 100 pg/ml dose only. In the myometrium, 50 and 100 pg/ml misoprostol induced elevations in rEP3[alpha] and rEP3[beta] receptor mRNA expression. rEP3[alpha] and rEP3[beta] receptor mRNA expression in the cervix was not different from controls. These data demonstrate that the EP3 receptor is differentially expressed in the myometrium and cervix in response to misoprostol. This may account for the ability of misoprostol to stimulate the myometrium when administered for cervical ripening.

Narayansingh, R. M., M. Senchyna, et al. (2002). "Treatment with prostaglandin F2[alpha] increases expression of prostaglandin synthase-2 in the rat corpus luteum." Prostaglandins & Other Lipid Mediators **70**(1-2): 145.

<http://www.sciencedirect.com/science/article/B6T3H-46RM318-1/2/a1265e80638cf96663fc58a722553d7a>

Recent studies indicate that the corpus luteum (CL) may be a source of prostaglandin F2[alpha] (PGF2[alpha]) for regression. We investigated expression of mRNA and protein for prostaglandin G/H synthase (PGHS) in the CL of immature superovulated rats following administration of PGF2[alpha]. We observed an increase in mRNA for PGHS-2, the induced isoform, at 1 h and protein at 8 and 24 h after treatment. One hour after PGF2[alpha], there was also a progressive decrease in plasma progesterone concentration. There were no changes, however, in expression of PGHS-1, the constitutive isoform, over the 24 h sampling period. These results indicate that PGHS-2 increases following PGF2[alpha] treatment and that expression of this enzyme in the rat CL may contribute to the luteolytic mechanism.

Tumbaga, P., K. Beharry, et al. (1999). "Biochemical changes in prostanoids and cerebral expression of cyclooxygenase (COX)-1 and COX-2 during morphine sulfate infusion in the newborn piglet*." Prostaglandins & Other Lipid Mediators **58**(5-6): 273.

<http://www.sciencedirect.com/science/article/B6T3H-3Y0J1NJ-6/2/ac7beb5fd85a6016577658a143d35341>

To examine the biochemical regulation of morphine sulfate (MS) on prostanoid synthesis, conscious newborn piglets received a bolus dose of 100 [mu]g/kg followed by a continuous infusion dose of 100 [mu]g/kg/h. The control group received equivalent volume bolus and continuous infusion of 5% dextrose. Blood samples were drawn from the femoral artery and sagittal sinus vein before, during and after infusion for measurement of prostanoids. The expression of mRNAs encoding cyclooxygenases (COX)-1 and -2 in the brainstem, thalamus, cortex, and cerebellum of the newborn piglets were also examined. Systemic PGE2 levels declined substantially during and post MS infusion (p 2 levels increased following the bolus dose (p p 1[alpha] levels, however, in the cerebral circulation 6-ketoPGF1[alpha] levels increased 146% (p 2 levels increased transiently at 4 h (p 2 increased at 0.5 and 1 h (p p p < 0.001) increased expression of COX-1 mRNA in the MS-infused brain samples. In contrast, no differences in COX-2 mRNA were detected between the groups. These data imply that MS may have significant effects on prostanoid synthesis in the newborn. The data further show that the MS-induced prostanoid responses appear to be mediated via COX-1.

Wu, Y.-L. and M. C. Wiltbank (2001). "Differential regulation of prostaglandin endoperoxide synthase-2 transcription in ovine granulosa and large luteal cells." Prostaglandins & Other Lipid Mediators **65**(2-3): 103.

<http://www.sciencedirect.com/science/article/B6T3H-433NRKR-3/2/7ce4a187680dca48629c3903cf5f2223>

The objective of the present study was to evaluate second messenger regulation of prostaglandin synthase-2 (PGS-2) mRNA and PGS-2 promoter in ovine granulosa cells and large luteal cells. In granulosa cells, PGS-2 mRNA was induced by forskolin (PKA activator) but not by phorbol didecanoate (PDD; PKC activator) with maximal stimulation at 24 h. In contrast, PDD was the more potent inducer in large luteal cells with the most dramatic effect by 4 h. Similarly, forskolin but not PDD increased media PGF2[alpha] in granulosa cells at 24 h; whereas, PDD but not forskolin increased PGF2[alpha] at 4 h and 24 h in large luteal cells. To evaluate if these effects were due to activation of transcription, a plasmid was constructed containing 1500 bp of PGS-2 promoter linked to a luciferase gene. Forskolin stimulated transcription from this construct in granulosa cells (5-fold); whereas, PDD but not forskolin stimulated transcription in large luteal cells (40-fold). Taking these findings together, we propose that transcriptional regulation of the PGS-2 gene changes from PKA-dependence in granulosa cells to PKC-dependence in large luteal cells after luteinization.

Prostaglandins & Other Lipid Mediators (1)

Stark, K., J. Bylund, et al. (2005). "On the mechanism of biosynthesis of 19-hydroxyprostaglandins of human seminal fluid and expression of cyclooxygenase-2, PGH 19-hydroxylase (CYP4F8) and microsomal PGE synthase-1 in seminal vesicles and vas deferens." Prostaglandins & Other Lipid Mediators **75**(1-4): 47.

<http://www.sciencedirect.com/science/article/B6T3H-4DVW1XX-2/2/7aa6b94a89af70d65e038657b00773bb>

The predominating prostaglandins of human seminal fluid are 19R-hydroxyprostaglandins E1 and

E2, conceivably formed sequentially by prostaglandin H (PGH) synthase-2, PGH 19-hydroxylase (CYP4F8), and microsomal PGE synthase-1 of seminal vesicles. Our aim was to study this enzyme system. Quantification by real-time PCR suggested that the transcripts of PGH synthase-2, CYP4F8, and microsomal PGE synthase-1 were abundant and correlated in seminal vesicles of seven patients (p n-2 hydroxylation of PGH1 and PGH2 and n-3 hydroxylation of arachidonic acid). Arachidonic acid was oxidized to 18-hydroxyarachidonic acid and to PGE2 and by microsomes of seminal vesicles in the presence of NADPH and GSH, and to relatively small amounts of 19-hydroxy-PGE2. We conclude that PGH synthase-2, CYP4F8, and PGE synthase-1 likely forms 19-hydroxy-PGE compounds in seminal vesicles and vas deferens, but the catalytic properties of CYP4F8 suggest additional biological functions. Recombinant CYP4F8 was also found to catalyze n-2 hydroxylation of PGI2 and carbaprostacyclin (Km [not, vert, similar] 40 [μM]), and n-2 and n-3 hydroxylation of carbocyclic TXA2.

Protein Eng. (1)

Kusharyoto, W., J. Pleiss, et al. (2002). "Mapping of a hapten-binding site: molecular modeling and site-directed mutagenesis study of an anti-atrazine antibody." Protein Eng. **15**(3): 233-241.

<http://peds.oupjournals.org/cgi/content/abstract/15/3/233>

A three-dimensional model of the variable domain of the atrazine-specific Fab fragment K411B was constructed by molecular modeling using known structures of highly homologous immunoglobulins as templates. Molecular dynamic simulations and cross-reactivity data were used to predict residues responsible for the binding of the hapten 4-chloro-6-(isopropylamino)-1,3,5-triazine-2-(6-aminohexanecarboxylic acid) (iPr/Cl/C6) instead of atrazine. Specific binding pockets could be defined for the chlorine, the isopropylamino group and the C6-spacer of the hapten. The influence of various amino acids on hapten binding was investigated by site-directed mutagenesis, and the effect of these mutations was analyzed by capture ELISA using the hapten iPr/Cl/C6 and 4-amino-6-chloro-1,3,5-triazine-2-(6-aminohexanecarboxylic acid) (H/Cl/C6). GlyH100a seems to be important in determining the conformation of the heavy-chain complementarity determining region H3; replacing it with any other residue prevented the binding of the hapten. Altering residues responsible for the binding of the chlorine atom (TrpH33, GluH50 and TyrL96) decreased the affinity significantly. Hapten-spacer recognition can be attributed to the interaction with PheL32; replacing PheL32 by leucine reduced the affinity towards iPr/Cl/C6. A triple mutant Fab fragment (GlnL89Glu, ValH37Ile and GluL3Val) showed an affinity 5-fold greater towards iPr/Cl/C6 compared to the wild-type K411B, as a result of better recognition of the isopropylamino group of iPr/Cl/C6.

Protein Eng. Des. Sel. (2)

Sacchi, S., E. Rosini, et al. (2004). "Modulating D-amino acid oxidase substrate specificity: production of an enzyme for analytical determination of all D-amino acids by directed evolution." Protein Eng. Des. Sel. **17**(6): 517-525.

<http://peds.oupjournals.org/cgi/content/abstract/17/6/517>

Recent research on the flavoenzyme D-amino acid oxidase from *Rhodotorula gracilis* (RgDAAO) has revealed new, intriguing properties of this catalyst and offers novel biotechnological applications. Among them, the reaction of RgDAAO has been exploited in the analytical determination of the D-amino acid content in biological samples. However, because the enzyme does not oxidize acidic D-amino acids, it cannot be used to detect the total amount of D-amino acids. We now present the results obtained using a random mutagenesis approach to produce RgDAAO mutants with a broader substrate specificity. The libraries of RgDAAO mutants were generated by error-prone PCR, expressed in BL21(DE3)pLysS *Escherichia coli* cells and screened for their ability to oxidize different substrates by means of an activity assay. Five random mutants that have a modified substrate specificity, more useful for the analytical determination of the entire content of D-amino acids than wild-type RgDAAO, have been isolated. With the only exception of Y223 and G199, none of the effective amino acid substitutions lie in segments predicted to interact directly with the bound substrate. The substitutions appear to cluster on the protein surface: it would not have been possible to predict that these substitutions would enhance DAAO activity. We can only conclude that these substitutions synergistically generate small structural changes that affect the dynamics and/or stability of the protein in a way that enhances substrate binding or subsequently catalytic turnover.

Xiang, J. and N. S. Sampson (2004). "Library screening studies to investigate substrate specificity in the reaction catalyzed by cholesterol oxidase." *Protein Eng. Des. Sel.* **17**(4): 341-348.

<http://peds.oupjournals.org/cgi/content/abstract/17/4/341>

We tested whether it is possible to alter the substrate specificity of cholesterol oxidase for similarly sized sterols, i.e. cholesterol, β -sitosterol and stigmasterol. Using existing X-ray crystal structures, we made a model of the predicted Michaelis complex of cholesterol and cholesterol oxidase. Based on this model, we identified five residues that are in direct contact with the steroid tail, Met58, Leu82, Val85, Met365 and Phe433. We prepared seven mutant libraries that contained the codon NYS (N = A, C, G, T; Y = C, Y; S = C, G) at one, two or three of the targeted positions by cassette mutagenesis. The libraries were screened for catalytic activity against three different sterols under k_{cat}/K_m conditions with 25 mol% sterol/DOPC unilamellar vesicles. The results of our screens suggest that specific packing interactions are not realized in the transition state of binding and that loss of active site water may be the predominant source of binding energy.

Protein Expression and Purification (8)

Dieckelmann, M., L. F. Roddam, et al. (2003). "Purification of post-translationally modified proteins from bacteria: homologous expression and purification of histidine-tagged pilin from *Neisseria meningitidis*." *Protein Expression and Purification* **30**(1): 69.

<http://www.sciencedirect.com/science/article/B6WPJ-48H87K9-1/2/9864d2d5389c9c899c4efd1ff5f0a8c8>

Until recently, glycosylation of proteins in prokaryotes was regarded as uncommon and thought to

be limited to special cases such as S-layer proteins and some archeal outer membrane proteins. Now, there are an increasing number of reports of bacterial proteins that are glycosylated. Pilin of pathogenic *Neisseria* is one of the best characterised post-translationally modified bacterial proteins, with four different types of modifications reported, including a novel glycosylation. Pilin monomers assemble to form pilus fibres, which are long protein filaments that protrude from the surface of bacterial cells and are key virulence factors. To aid in the investigation of these modifications, pure pilin is required. A number of pilin purification methods have been published, but none are appropriate for the routine purification of pilin from many different isolates. This study describes a novel, rapid, and simple method of pilin purification from *Neisseria meningitidis* C311#3, which facilitates the production of consistent quantities of pure, native pilin. A 6 x histidine tag was fused to the C-terminus of the pilin subunit structural gene, pilE, via homologous recombination placing the 6 x histidine-tagged allele in the chromosome of *N. meningitidis* C311#3. Pilin was purified under non-denaturing conditions via a two-step process using immobilised metal affinity chromatography (IMAC), followed by dye affinity chromatography. Analysis of the purified pilin confirmed that it retained both of the post-translational modifications examined. This novel approach may prove to be a generally applicable method for purification and analysis of post-translationally modified proteins in bacteria.

Dunn, J. J., B. N. Lade, et al. (1990). "Outer surface protein a (OspA) from the lyme disease spirochete, *Borrelia burgdorferi*: High level expression and purification of a soluble recombinant form of OspA." Protein Expression and Purification 1(2): 159.

<http://www.sciencedirect.com/science/article/B6WPJ-4DXK9WF-C/2/f121889f8448bc6f8d3e224f9840af40>

The ospA gene of *Borrelia burgdorferi* encodes an outer membrane protein which is a major antigen of the Lyme disease agent. Two sequence-specific sets of oligonucleotide primers were used to specify the amplification of the ospA coding sequence by the polymerase chain reaction. One set allowed the entire ospA sequence to be amplified, while the other primed amplification of a truncated form of ospA lacking the first 17 codons specified by the wild-type ospA structural gene, residues believed to constitute a signal sequence which normally would direct localization of the ospA protein to the *Borrelia* cell's outer membrane. Each set of primers also contained sequences near their 5' ends which facilitated cloning of the amplified DNA directly into a high level expression system based on bacteriophage T7 genetic elements. We showed that the fulllength OspA protein is synthesized poorly in *Escherichia coli* and it is associated with the insoluble membrane fraction. In contrast, the truncated form can be expressed to very high levels and it is soluble. The truncated protein was purified to homogeneity and partially characterized. Its N-terminal sequence and molecular weight derived from sodium dodecyl sulfate-polyacrylamide gel electrophoresis agree with those deduced from the DNA sequence. It is a monomer with a native molecular weight of 28,000 and it is very resistant to digestion by trypsin even though it is rather rich in lysine residues (16 mol%). Recombinant OspA protein synthesized in *E. coli* is recognized by antibodies in sera of Lyme patients, which suggests that the protein may be useful in immunoassays and as a possible immunogen to protect against Lyme borreliosis.

Gilbert, H. F., M. L. Kruzel, et al. (1991). "Expression and purification of recombinant rat protein disulfide isomerase from *Escherichia coli*." Protein Expression and Purification 2(2-3): 194.

<http://www.sciencedirect.com/science/article/B6WPJ-4DXK9WF-30/2/14a890b8d70c4404e1c3153521eff833>

Rat liver protein disulfide isomerase (PDI) catalyzes the oxidative folding of proteins containing

disulfide bonds. We have developed an efficient method for its overproduction in *Escherichia coli*. Using a T7 RNA polymerase expression system, isolated yields of 15-30 mg/liter of recombinant rat PDI are readily obtained. Convenient purification of the enzyme from *E. coli* lysates involves ion-exchange (DEAE) chromatography combined with zinc chelate chromatography. The recombinant PDI shows catalytic activity identical to that of PDI isolated from bovine liver in both the reduction of insulin and the oxidative folding of ribonuclease A. The enzyme is expressed in *E. coli* as a soluble, cytoplasmic protein. After complete reduction and denaturation in 6 guanidinium hydrochloride, PDI regains complete activity within 3 min after removal of the denaturant, implying that disulfide bonds are not essential for the maintenance of PDI tertiary structure. Both the protein isolated from *E. coli* and the protein isolated from liver contained free cysteine residues (1.8 ± 0.2 and 1.4 ± 0.3 SH/monomer, respectively).

Goodin, J. L., R. W. Raab, et al. (2005). "Yersinia pestis outer membrane type III secretion protein YscC: expression, purification, characterization, and induction of specific antiserum." Protein Expression and Purification **40**(1): 152.

<http://www.sciencedirect.com/science/article/B6WPJ-4DW8PPM-1/2/76ad2abb28f4b11bc311a7610a0a11e3>

The type III secretion system (YscC) protein of *Yersinia pestis* plays an essential role in the translocation of *Yersinia* outer proteins (Yops) into eukaryotic target cells through a type III secretion mechanism. To assess the immunogenicity and potential protective efficacy of YscC against lethal plague challenge, we cloned, overexpressed, and purified YscC using two different bacterial expression and purification systems. The resulting expression plasmids for YscC, pETBlue-2-YscC and pTYB11-YscC, were regulated by robust T7 promoters that were induced with isopropyl-[beta]-d-thiogalactopyranoside. The intein-fusion pTYB11-YscC system and the six-histidine-tagging pETBlue-2-YscC system were both successful for producing and purifying YscC. The intein-mediated purification system produced about 1 mg of soluble YscC per liter of bacterial culture while the YscC-His6-tag method resulted in 16 mg of insoluble YscC per liter of bacterial culture. Protein identity for purified YscC-His6 was confirmed by ion trap mass spectrometry. Antisera were produced against both YscC and YscC-His6. The specific immune response generated in YscC-vaccinated mice was relative to the particular purified protein, YscC or YscC-His6, which was used for vaccination as determined by Western blot analysis and ELISA. Regardless of the purification method, either form of the YscC protein failed to elicit a protective immune response against lethal plague challenge with either F1 capsule forming *Y. pestis* CO92 or the isogenic F1- *Y. pestis* C12.

Kunes, Y. Z., M.-C. Sanz, et al. (2002). "Expression and characterization of a synthetic protein C activator in *Pichia pastoris*." Protein Expression and Purification **26**(3): 406.

<http://www.sciencedirect.com/science/article/B6WPJ-47731DH-5/2/dfd306c22eb5ff0bf43054e6e08b5a4a>

Li, Z., R. Gambino, et al. (1992). "Expression and purification of kringle 4-type 2 of human apolipoprotein (a) in *Escherichia coli*." Protein Expression and Purification **3**(3): 212.

<http://www.sciencedirect.com/science/article/B6WPJ-4DXK9WF-T/2/59cedf2018b50bd02a7113e7c836827a>

The most frequently occurring kringle 4 domain of human apolipoprotein (a), Kringle 4-subtype 2 (K42), was expressed as a fusion protein with the maltose binding protein in *Escherichia coli* using the "tac" promoter. Although the fusion protein was expressed without a signal sequence, 25% was secreted into the periplasmic space; the remainder was found associated with the soluble cytosolic fraction. The fusion protein was readily isolated from whole cell lysate by amylose agarose affinity chromatography. Although a factor Xa cleavage site was engineered into the fusion protein, it was found that release of the K42 protein was most conveniently achieved by proteolysis with subtilisin A. The cleavage product produced in this way was shown to be intact K42 with only the first three amino acid residues of the leading flanking peptide missing, as judged by N-terminal sequence analysis. K42 was isolated from the hydrolysate by FPLC on a Mono-Q column with a yield of 170 +/- 30 [mu]g/g wet cells. The resulting protein was monomeric in phosphate-buffered saline as judged by size-exclusion chromatography and appeared to be folded as shown by spectroscopic and immunological assays. The recombinant K42 did not bind to either lysine- or proline-Sepharose, suggesting that the ligand binding activities of lipoprotein (a) may reside in the other kringle domains of apolipoprotein (a).

Lindler, L. E., J. C. Anders, et al. (1991). "Mutation in the *Escherichia coli* htpR locus results in stabilization of recombinant expression products that are susceptible to proteolytic degradation." Protein Expression and Purification **2**(5-6): 321.

<http://www.sciencedirect.com/science/article/B6WPJ-4DYVPKY-3/2/0b72c645055e6569c7d20995097eb311>

We compared the expression and degradation of three cloned malarial proteins in a pair of isogenic strains of *Escherichia coli* that differed at the htpR locus. The htpR locus encodes an alternate [sigma] factor necessary for the transcription of heat shock promoters. Plasmodium sequences were cloned from polymerase chain reaction-amplified DNA initiated by oligonucleotide primers that were specific for the gene coding regions to be expressed. The amplified DNA was cloned and expressed in a vector that encodes a strong T7 promoter and translation-initiation signal. The total cell yield of two of the expressed proteins was found to be increased when synthesis occurred in a *E. coli* htpR mutant. Pulse-chase experiments showed that the increased protein yield correlated with a decrease in the degradation of the protein in the htpR strain. A two- to seven-fold increase in the half-life of the malaria proteins was observed in the *E. coli* htpR- background as compared to htpR+. We found no difference in survival of the *E. coli* K165 htpR mutant and isogenic parent during thermal induction. Since the synthesis of the heat shock [sigma] factor did not significantly influence survival of *E. coli* and htpR expression results in increased degradation of foreign proteins, the *E. coli* htpR mutant was a valuable host strain for production of foreign proteins.

Nielsen, B. R., J. Lehmbeck, et al. (2002). "Cloning, heterologous expression, and enzymatic characterization of a thermostable glucoamylase from *Talaromyces emersonii*." Protein Expression and Purification **26**(1): 1.

<http://www.sciencedirect.com/science/article/B6WPJ-46SW7S6-4/2/eab1d24211c7e1d0b1d312a9160abe5e>

Glowacki, G., R. Braren, et al. (2002). "The family of toxin-related ecto-ADP-ribosyltransferases in humans and the mouse." Protein Sci. **11**(7): 1657-1670.

<http://www.proteinscience.org/cgi/content/abstract/11/7/1657>

ADP-ribosyltransferases including toxins secreted by *Vibrio cholera*, *Pseudomonas aeruginosa*, and other pathogenic bacteria inactivate the function of human target proteins by attaching ADP-ribose onto a critical amino acid residue. Cross-species polymerase chain reaction (PCR) and database mining identified the orthologs of these ADP-ribosylating toxins in humans and the mouse. The human genome contains four functional toxin-related ADP-ribosyltransferase genes (ARTs) and two related intron-containing pseudogenes; the mouse has six functional orthologs. The human and mouse ART genes map to chromosomal regions with conserved linkage synteny. The individual ART genes reveal highly restricted expression patterns, which are largely conserved in humans and the mouse. We confirmed the predicted extracellular location of the ART proteins by expressing recombinant ARTs in insect cells. Two human and four mouse ARTs contain the active site motif (R-S-EXE) typical of arginine-specific ADP-ribosyltransferases and exhibit the predicted enzyme activities. Two other human ARTs and their murine orthologues deviate in the active site motif and lack detectable enzyme activity. Conceivably, these ARTs may have acquired a new specificity or function. The position-sensitive iterative database search program PSI-BLAST connected the mammalian ARTs with most known bacterial ADP-ribosylating toxins. In contrast, no related open reading frames occur in the four completed genomes of lower eucaryotes (yeast, worm, fly, and mustard weed). Interestingly, these organisms also lack genes for ADP-ribosylhydrolases, the enzymes that reverse protein ADP-ribosylation. This suggests that the two enzyme families that catalyze reversible mono-ADP-ribosylation either were lost from the genomes of these nonchordata eucaryotes or were subject to horizontal gene transfer between kingdoms.

Kenig, M., S. Berbic, et al. (2004). "Differences in aggregation properties of three site-specific mutants of recombinant human stefin B." Protein Sci. **13**(1): 63-70.

<http://www.proteinscience.org/cgi/content/abstract/13/1/63>

We describe expression, purification, and characterization of three site-specific mutants of recombinant human stefin B: H75W, P36G, and P79S. The far- and near-UV CD spectra have shown that they have similar secondary and tertiary structures to the parent protein. The elution on gel-filtration suggests that recombinant human stefin B and the P36G variant are predominantly monomers, whereas the P79S variant is a dimer. ANS dye binding, reflecting exposed hydrophobic patches, is highest for the P36G variant, both at pH 5 and 3. ANS dye binding also is increased for stefin B and the other two variants at pH 3. Under the chosen conditions the highest tendency to form amyloid fibrils has been shown for the recombinant human stefin B. The P79S variant demonstrates a longer lag phase and a lower rate of fibril formation, while the P36G variant is most prone to amorphous aggregation. This was demonstrated by ThT fluorescence as a function of time and by transmission electron microscopy.

B. Stein, M., M. J. Chartier, et al. (1998). "Genetic linkage to the serotonin transporter protein and 5HT2A receptor genes excluded in generalized social phobia." Psychiatry Research **81**(3): 283.

<http://www.sciencedirect.com/science/article/B6TBV-3VFF65B-1/2/4b559500e71385bf53122813187d2ef6>

Social phobia, particularly the generalized form, is strongly familial and frequently comorbid with major depression, panic disorder, and obsessive-compulsive disorder. It has also recently been shown to be responsive to selective serotonin reuptake inhibitors. We conducted a study to determine if generalized social phobia is genetically linked to either of two candidate genes: the serotonin transporter protein (5HTT) gene, or the 5HT2A receptor (5HT2AR) gene. Rates of social phobia (using several phenotype definitions) were ascertained and blood samples obtained from consenting first-degree family members of generalized social phobic probands. 5HT2AR and 5HTT genotyping was performed using the polymerase chain reaction (PCR). Linkage was tested using LINKAGE and GENEHUNTER software. No evidence of linkage was found; power analysis indicated that failure to find linkage was unlikely due to inadequate statistical power. These findings reasonably exclude linkage between generalized social phobia and the 5HTT or 5HT2AR genes in these samples, although modifier effects cannot be ruled out. Other 5HT receptor subtypes or indirect modulatory effects of 5HT on other neurotransmitter systems may be involved.

Fehr, C., N. Gritschuk, et al. (2000). "The HTR1B 861G>C receptor polymorphism among patients suffering from alcoholism, major depression, anxiety disorders and narcolepsy." Psychiatry Research **97**(1): 1.

<http://www.sciencedirect.com/science/article/B6TBV-41TN3YJ-1/2/b39283f1ce713efc432eb07b4d61d976>

The HTR1B receptor gene has been linked to antisocial alcoholism in a Finnish population and an American Indian tribe [Lappalainen et al., Arch. Gen. Psychiatry, 55 (1998) 989]. Using a candidate gene approach, we genotyped 209 patients with alcoholism, 108 patients with major depression, 32 patients with panic disorder, 50 patients with generalized anxiety disorder, 58 patients with narcolepsy and 74 healthy volunteers for the HTR1B 861G>C polymorphism. There was a higher frequency of the HTR1B 861G alleles among the alcohol-dependent patients as compared to the control subjects ($\chi^2=4.02$, d.f.=2, $P=0.04$). However, the association resulted from higher frequencies of the opposite alleles (HTR1B 861G), as originally reported by Lappalainen et al. (1998). Although the association in our study might be due to a type I error, the higher degree of HTR1B allele sharing within both populations could also argue for another alcoholism-relevant gene within the proximity of the HTR1B gene on human chromosome 6.

Gourion, D., S. Leroy, et al. (2004). "Cerebellum development and schizophrenia: an association study of the human homeogene Engrailed 2." Psychiatry Research **126**(2): 93.

<http://www.sciencedirect.com/science/article/B6TBV-4CC1WY5-1/2/0155f8382293dc03c374d42db34c0dc9>

Epidemiological data and family studies in schizophrenia show that genetic factors contribute to the vulnerability to this disorder. The homeogene Engrailed 2 (EN2) is specifically involved in patterning the region that gives rise to the cerebellum and controls the plasticity of midbrain

dopaminergic neurons. We carried out an association study for a CA repeat polymorphism located in the 3' region of the homeogene EN2. The subjects consisted of 165 patients with schizophrenia and 97 controls matched for age and ethnicity from a French Caucasian population. We found no significant association of schizophrenia with this bi-nucleotide repeat polymorphism of the EN2 gene.

Iwase, T., N. Kajimura, et al. (2002). "Mutation screening of the human Clock gene in circadian rhythm sleep disorders." Psychiatry Research **109**(2): 121.

<http://www.sciencedirect.com/science/article/B6TBV-45DVXT1-1/2/7d871ec0aa9fb4172ca29136fe8914a3>

We tested whether the human Clock (hClock) gene, one of the essential components of the circadian oscillator, is implicated in the vulnerability to delayed sleep phase syndrome (DSPS) and non-24-hour sleep-wake syndrome (N-24). Screening in the entire coding region of the hClock gene with PCR amplification revealed three polymorphisms, of which two predicted the amino acid substitutions R533Q and H542R. The frequencies of the R533Q and H542R alleles in patients with DSPS or N-24 were very low and not significantly different from those in control subjects. A T3111C polymorphism in the 3'-untranslated region of hClock, which had been reportedly associated with morning or evening preference for activity, was also investigated; the results showed that the 3111C allele frequency decreased in DSPS. Polymorphisms in the coding region of the hClock gene are unlikely to play an important role in the development of DSPS or N-24. The possible contribution of the T3111C polymorphism to DSPS susceptibility should be studied further.

Rietschel, M., M. M. Nothen, et al. (1993). "A serine to glycine substitution at position 9 in the extracellular N-terminal part of the dopamine D3 receptor protein: No role in the genetic predisposition to bipolar affective disorder." Psychiatry Research **46**(3): 253.

<http://www.sciencedirect.com/science/article/B6TBV-46041G4-9F/2/c62461c9863714131a988cf233a5c416>

Association studies offer a promising tool to investigate the potential role of DNA sequence variation affecting the expression or sequence of proteins in susceptibility to common diseases. We determined the frequency of a DNA polymorphism resulting in a glycine to serine substitution at position 9 in the extracellular N-terminal part of the dopamine D3 receptor protein in a sample of 83 patients suffering from bipolar affective disorder and 100 control subjects. No significant differences between the groups were found. Thus, this substitution, which is the first sequence variation identified in the dopamine D3 receptor gene altering the amino acid sequence of the protein, can be regarded as a protein variant with no major effect on the susceptibility to bipolar affective disorder.

Pulmonary Pharmacology & Therapeutics (1)

Oshiro, T., Y. Asato, et al. (2005). "Differential effects of vitamin A on fetal lung growth and diaphragmatic

formation in nitrofen-induced rat model." Pulmonary Pharmacology & Therapeutics **18**(3): 155.

<http://www.sciencedirect.com/science/article/B6WPM-4F49595-1/2/9aebaa5316f6f657920e2e113db500ef>

Congenital diaphragmatic hernia (CDH) is associated with high neonatal mortality and morbidity due to pulmonary hypoplasia and pulmonary hypertension. Antenatal interventions have been developed in an attempt to reduce the unacceptable mortality rate of CDH. The pathogenesis of pulmonary hypoplasia is not fully understood. It is not clear whether the increase of lung growth would be necessary for diaphragmatic closure. Vitamin A is important for various aspects of lung development. Therefore, the aim of this study was to examine whether antenatal treatment with vitamin A can increase lung growth and reduce the incidence of CDH in a nitrofen-treated rat model. The animals were randomly assigned to four groups: control, vitamin A, nitrofen, and nitrofen/vitamin A (NIP/Vit A). The incidence of CDH in the NIP/Vit A group (54%) was markedly lower than that in the nitrofen-treated group (85%). Although lung weight was decreased in the nitrofen-treated and NIP/vitamin A groups, the fetal lung weight-to-body weight ratio was slightly increased in the NIP/vitamin A group, compared to the nitrofen-treated group. The mRNA levels of lung surfactant proteins were decreased in the NIP/vitamin A group. We conclude that antenatal treatment with vitamin A reduced the incidence of CDH without lung maturation in the nitrofen-induced rat model.

Quaternary Research (1)

Hofreiter, M., J. L. Betancourt, et al. (2003). "Phylogeny, diet, and habitat of an extinct ground sloth from Cuchillo Cura, Neuquen Province, southwest Argentina." Quaternary Research **59**(3): 364.

<http://www.sciencedirect.com/science/article/B6WPN-48F5JSY-1/2/60b5ba20bc574f5504bcb1ac9318eee>

Advancements in ancient DNA analyses now permit comparative molecular and morphological studies of extinct animal dung commonly preserved in caves of semiarid regions. These new techniques are showcased using a unique dung deposit preserved in a late glacial vizcacha (*Lagidium* sp.) midden from a limestone cave in southwestern Argentina (38.5[deg] S). Phylogenetic analyses of the mitochondrial DNA show that the dung originated from a small ground sloth species not yet represented by skeletal material in the region, and not closely related to any of the four previously sequenced extinct and extant sloth species. Analyses of pollen and plant cuticles, as well as analyses of the chloroplast DNA, show that the Cuchillo Cura ground sloth browsed on many of the same herb, grass, and shrub genera common at the site today, and that its habitat was treeless Patagonian scrub-steppe. We envision a day when molecular analyses are used routinely to supplement morphological identifications and possibly to provide a time-lapse view of molecular diversification.

Radiotherapy and Oncology (6)

Alsner, J., S. B. Sorensen, et al. (2001). "TP53 mutation is related to poor prognosis after radiotherapy, but not surgery, in squamous cell carcinoma of the head and neck." Radiotherapy and Oncology **59**(2): 179.

<http://www.sciencedirect.com/science/article/B6TBY-42VV7K4-9/2/e379808f2277dca1e95e84549e50c674>

Background and Purpose: TP53 gene-mutation and expression of p53 have been described to influence the radiosensitivity of tumour cells from head and neck carcinomas. The present study was performed to evaluate whether TP53 mutation may influence the clinical outcome of head and neck cancer patients treated with radiotherapy or surgery. **Materials and methods:** DNA was extracted from formalin-fixed paraffin-embedded tissue sections from primary biopsies taken before radiotherapy. Gene mutations (in exons 5-9) were identified using denaturing gradient gel electrophoresis (DGGE) as the initial scanning procedure and characterized by sequencing. Patients were treated with primary radiotherapy or surgery alone. Treatment was given according to the DAHANCA schedules with 5 or 6 weekly fractions (2 Gy) of radiotherapy (66-68 Gy). Most patients were also treated with the hypoxic radiosensitizer Nimorazole. The results are reported as 5-year actuarial values, and differences estimated by log-rank analysis. **Results:** The present analysis is based on 114 patients with squamous cell carcinoma of the larynx, pharynx and oral cavity diagnosed between March 1992 and October 1996. Ninety patients received primary radiotherapy alone and 21 were treated with surgery. TP53 mutations were found in 45 patients (39%) and in patients receiving radiotherapy, TP53 mutation was highly associated with poor prognosis. Loco-regional control rates (5-year actuarial values) for TP53 mutation was 29 vs. 54% for TP53 wildtype (PPTP53 mutation did not influence the response to surgery). **Conclusions:** A strong relationship was observed between TP53 mutation and poor prognosis (increased risk of loco-regional failure and death) in head and neck cancer patients given primary radiotherapy but not surgery.

Andreassen, C. N., J. Alsner, et al. "TGFB1 polymorphisms are associated with risk of late normal tissue complications in the breast after radiotherapy for early breast cancer." Radiotherapy and Oncology **In Press, Corrected Proof** <http://www.sciencedirect.com/science/article/B6TBY-4FPJ9MG-1/2/ecf6682f6739409e9ea55efce023b71d>

Recent studies suggest that normal tissue radiosensitivity is influenced by single nucleotide polymorphisms (SNPs) in certain genes. In order to seek a confirmation of these findings, this study investigated SNPs in genes TGFB1 (position -509, codon 10 and codon 25), SOD2 (codon 16), XRCC1 (codon 399), XRCC3 (codon 241), APEX (codon 148) and ATM (codon 1853) in 26 breast cancer patients with marked changes in breast appearance after radiotherapy and 26 matched controls. Statistically significant associations were found between the TGFB1 codon 10 Pro allele (P=0.005) as well as the TGFB1 position -509 T allele (P=0.018) and increased risk of altered breast appearance. No significant associations were found for the remaining SNPs.

Andreassen, C. N., J. Alsner, et al. (2003). "Prediction of normal tissue radiosensitivity from polymorphisms in candidate genes." Radiotherapy and Oncology **69**(2): 127.

<http://www.sciencedirect.com/science/article/B6TBY-4B3FV8Y-3/2/e4e6cbf00a759380c2c733bd03550ff5>

Background and purposeSingle nucleotide polymorphisms (SNPs) in genes related to the biological response to radiation injury may affect clinical normal tissue radiosensitivity. This study investigates whether seven selected SNPs in five candidate genes influence risk of subcutaneous

fibrosis and telangiectasia after radiotherapy. Patients and methods The 41 patients included in this study were given post-mastectomy radiotherapy in 1978-1982 and subsequently evaluated in detail with regard to several different normal tissue reactions. SNPs in TGFB1 (codons 10, 25 and position -509), SOD2 (codon 16), XRCC3 (codon 241), XRCC1 (codon 399) and APEX (codon 148) were analyzed by PCR and single nucleotide primer extension. Dose-response curves were established for subcutaneous fibrosis and telangiectasia in patients with different genotypes. Differences in radiosensitivity were quantified in terms of ED50 values and enhancement ratios. Results For TGFB1, the Pro/Pro genotype in codon 10 and the T/T genotype in position -509 correlated positively with risk of subcutaneous fibrosis. The SOD 2 codon 16 Val/Ala genotype was associated with increased risk of subcutaneous fibrosis when compared to the Val/Val genotype. The Thr/Thr genotype in XRCC3 codon 241 correlated with increased risk of subcutaneous fibrosis as well as telangiectasia. The Arg/Arg genotype in XRCC1 codon 399 was associated with increased risk of radiation-induced subcutaneous fibrosis. For these polymorphisms, enhancement ratios between 1.09 and 1.25 were found. Combined analysis of multiple SNPs demonstrated that the risk of subcutaneous fibrosis correlated with the number of risk alleles in such a manner that patients with few risk alleles exhibited a remarkable degree of radioresistance. Conclusion The present study established significant correlations between five SNPs and risk of radiation-induced normal tissue reactions. These findings support the assumption that clinical normal tissue radiosensitivity should be regarded as a phenomenon dependent on the combined effect of variation in several genes and indicate that models based on multiple genetic markers may have the potential to predict normal tissue responses after radiotherapy.

Andreassen, C. N., F. B. Sorensen, et al. (2004). "Optimisation and validation of methods to assess single nucleotide polymorphisms (SNPs) in archival histological material." Radiotherapy and Oncology **72**(3): 351.

<http://www.sciencedirect.com/science/article/B6TBY-4D1DKN6-1/2/b7648e36f23129015a9bff45d8d24ab7>

Background and purpose An increasing amount of evidence indicates that single nucleotide polymorphisms (SNPs) may affect a variety of oncology related phenotypes. Occasionally, it is convenient to base studies addressing genotype-phenotype relationships on historical patient cohorts, from which only archival specimens are available. This study was conducted to validate protocols optimised for assessment of SNPs based on paraffin embedded, formalin fixed tissue samples. Patients and methods In 137 breast cancer patients, three TGFB1 SNPs were assessed based on archival histological specimens. In 37 of these patients, the SNPs were also assessed using cultured fibroblasts and the assays were validated by direct comparison of the results. From the remaining 100 patients, only archival material was available. In these patients, the existence of a genetic linkage pattern between the assessed TGFB1 SNPs was used to provide an indirect validation of the genotyping results. Furthermore, two different methods for DNA extraction were compared (semi-automatic DNA extraction using the ABI Prism[trademark] 6100 Nucleic Acid PrepStation versus Proteinase K digestion for 5 days followed by boiling and DNA precipitation). Results Assessment of SNPs based on archival histological material is encumbered by a number of obstacles and pitfalls. However, these can be widely overcome by careful optimisation of the methods used for sample selection, DNA extraction and PCR. Within 130 samples that fulfil the criteria for analysis a highly reliable SNP assessment was observed. The study demonstrated that different 'down-stream applications' ('single nucleotide primer extension' or 'TaqMan[trademark]-based' real-time PCR) could be used as genotyping procedure. Conclusions Reliable assessment of SNPs in formalin-fixed paraffin-embedded specimens is possible but a number of precautions should be carefully taken.

Rube, C. E., F. Wilfert, et al. (2004). "Increased expression of pro-inflammatory cytokines as a cause of lung toxicity after combined treatment with gemcitabine and thoracic irradiation." Radiotherapy and Oncology **72**(2): 231.

<http://www.sciencedirect.com/science/article/B6TBY-4D0XNTC-5/2/f95cb8fc57834b1188890add2097160d>

Background and purpose Preclinical evidence suggesting gemcitabine potentiates the anti-tumor effects of irradiation has resulted in clinical trials to evaluate the treatment efficacy of gemcitabine and concurrent thoracic irradiation in non-small-cell lung cancer (NSCLC). Although these studies demonstrated favorable tumor response, this combined treatment modality was accompanied by severe treatment-related toxicities predominantly of the lung. In an attempt to elucidate the determinants of lung toxicity for gemcitabine, we analyzed the expression of the pro-inflammatory cytokines TNF-[alpha], IL-1[alpha] and IL-6 in the lung tissue of mice treated with gemcitabine and concurrent thoracic irradiation. **Materials and methods** Four study groups were defined: C57BL/6J mice that received neither irradiation nor gemcitabine (NT-group), those that received gemcitabine (120 mg/kg intraperitoneal, i.p.) but no irradiation (GEM-group), those that underwent thoracic irradiation (12 Gy) without gemcitabine (XRT-group), and those that received both gemcitabine (120 mg/kg i.p., 2 h before irradiation) and thoracic irradiation (GEM/XRT-group). The mice were sacrificed at 1 h, 1 and 3 days, 1, 2 and 4 weeks post-treatment (p.t.). The mRNA expression of TNF-[alpha], IL-1[alpha] and IL-6 in the lung tissue was quantified by competitive RT-PCR. The cellular origin of the cytokine expression was identified by immunohistochemistry. The cytokine expression was correlated with histopathological alterations. **Results** The TNF-[alpha], IL-1[alpha] and IL-6 expression in the lung tissue of the GEM/XRT mice was clearly higher at all assessment time points compared to the NT mice (statistically significant at 1 h, 1 and 3 days, 1, 2 and 4 weeks p.t.), XRT mice (statistically significant at 1 week p.t.) or GEM mice (statistically significant at 1 h, 1 and 2 weeks p.t.). Maximal treatment-induced cytokine expression in the lung tissue of the GEM/XRT mice occurred already at 1 week p.t. (TNF-[alpha]: 30.9+/-5.3/IL-1[alpha]: 28.3+/-5.0/IL-6: 4.9+/-0.1 times basal level), and coincides with pathohistologically discernable interstitial pneumonitis. The elevated levels of TNF-[alpha] and IL-1[alpha] have been found to correlate with immunohistochemical staining of the bronchiolar epithelium and predominantly of inflammatory cells. **Conclusions** Our data provide evidence that the increased expression of pro-inflammatory cytokines and the induction of a cytokine-triggered inflammatory response may be a determinant of the observed elevated lung toxicity after concurrent treatment with gemcitabine and thoracic irradiation.

Rube, C. E., F. Wilfert, et al. (2002). "Modulation of radiation-induced tumour necrosis factor [alpha] (TNF-[alpha]) expression in the lung tissue by pentoxifylline." Radiotherapy and Oncology **64**(2): 177.

<http://www.sciencedirect.com/science/article/B6TBY-45WGGWW-1/2/c76fc87afce66281f536f140a8ac4c50>

Purpose: The lung is the major dose-limiting organ for radiotherapy of cancer in the thoracic region. Immediate cellular damage after irradiation is supposed to result in cytokine-mediated multicellular interactions with induction and progression of inflammatory and fibrotic tissue reactions. Pentoxifylline (PTX) down-regulates the production of proinflammatory cytokines, particularly TNF-[alpha], in response to noxious stimuli and may therefore provide protection against radiation-induced, cytokine-mediated cellular damage. The purpose of this study was to investigate the temporal and spatial release of TNF-[alpha] in the lung tissue after thoracic irradiation with 12 Gy. In addition, we evaluated the ability of PTX to reduce the radiation-induced TNF-[alpha] release in this animal model of thoracic irradiation. **Materials and methods:** C57BL/6J mice were exposed to either sham irradiation or single fraction of 12 Gy delivered to the thorax. Four study groups were defined: those that received neither irradiation nor PTX (NT group), those

that received PTX but no irradiation (PTX group), those that underwent irradiation without PTX (XRT group) and those that received both PTX and irradiation (PTX/XRT group). Treated and sham-irradiated mice were sacrificed corresponding to the latent period and the pneumonic phase. The TNF-[alpha] mRNA expression in the lung tissue was quantified by 'real-time' quantitative reverse transcriptase polymerase chain reaction (RT-PCR). Immunohistochemical detection methods (alkaline phosphatase anti-alkaline phosphatase (APAAP)) and automated image analysis were used for objective quantification of TNF-[alpha] protein expression. Results: Following thoracic irradiation with a single dose of 12 Gy (XRT group), radiation-induced TNF-[alpha] mRNA release in the lung tissue was significantly increased during the acute phase of pneumonitis (PPConclusions: This study demonstrates a significant radiation-induced increase of TNF-[alpha] (on mRNA and protein level) in the lung tissue during the pneumonic phase. The predominant localisation of TNF-[alpha] in areas of inflammatory cell infiltrates suggests involvement of this cytokine in the pathogenesis of radiation-induced lung injury. In addition, we observed a pronounced reduction of the TNF-[alpha] mRNA and protein production in the study group that received both PTX and radiation (PTX/XRT group) as compared to the radiation-only group (XRT group). Therefore our results indicate that PTX down-regulates the TNF-[alpha] mRNA and protein production in the lung tissue in response to radiation.

Recent Prog. Horm. Res. (1)

Chen, L., C. Finnerty, et al. (2003). "Genomic Analysis of Glucocorticoid-regulated Promoters in Murine T-lymphoma Cells." *Recent Prog. Horm. Res.* **58**(1): 155-174.

<http://rphr.endojournals.org/cgi/content/abstract/58/1/155>

We have undertaken a high-throughput analysis to identify targets of glucocorticoid regulation in P1798 murine T-lymphoma cells. G1/S-arrested cultures were treated for 8 hours with 0.1 {micro}M dexamethasone (dex) in the presence and absence of 1 {micro}g/ml cycloheximide. Untreated cultures and cultures exposed to cycloheximide alone were prepared as controls. RNA was isolated and gene expression analyzed using Affymetrix MG-U74A oligonucleotide arrays (Gene Chips(R)). Three independent experiments were performed. The data were analyzed using a variety of statistical and analytical approaches in order to identify primary transcriptional targets of the glucocorticoid receptor. We identified 44 genes that increase by > 2-fold in both dex-treated and dex + cycloheximide-treated cultures (relative to control and cycloheximide-treated cultures) in three replicate experiments. Statistical analysis of control data indicate that the probability that a given probeset would, as a result of random error, increase > 2-fold both in the presence and absence of cycloheximide in two independent experiments is approximately 7×10^{-9} . We have retrieved from the Celera mouse genomic sequence 8 kb of promoter sequence, spanning 4 kb either side of the 5'-end of the cDNA from eight of the induced genes. These sequences were analyzed for potential glucocorticoid receptor binding sites. Five of these genes contain the sequence ACAnnnTGTnCT within 4 kb of the presumptive transcriptional start site. Eight control genes were selected at random and analyzed for the sequence ACAnnnTGTnCT. Two control genes had such sequences within 4 kb of the transcriptional start site.

Regulatory Peptides (24)

Albers, A. R., M. S. O'Dorisio, et al. (2000). "Somatostatin receptor gene expression in neuroblastoma." Regulatory Peptides **88**(1-3): 61.

<http://www.sciencedirect.com/science/article/B6T0S-3YRW08S-9/2/5790e44054927ad9ad53096e723209ae>

Somatostatin receptor expression is a favorable prognostic factor in human neuroblastoma. Somatostatin receptors have been demonstrated in vitro by pharmacologic analysis of tumor tissue and in vivo by diagnostic radioreceptor scintigraphy. However, which receptor subtypes (sst1, sst2, sst3, sst4, and sst5) are expressed in these tumors has not yet been delineated. We used RT-PCR to analyze expression of the five somatostatin receptor genes in 32 neuroblastoma tumor specimens. All 32 tumor specimens expressed mRNA for c-abl and sst1; sst2 mRNA was detected in 27/32 samples and somatostatin mRNA was detected in 30/32 tumor specimens. The remaining receptor subtypes, sst3, sst4, and sst5 were variably expressed. Receptor protein for sst1 and sst2 was visualized in tumor neuroblasts as well as in endothelial cells of tumor vessels using immunostaining with specific anti-receptor antibodies. The effect of high expression of somatostatin receptors on cell proliferation was examined in SKNSH neuroblastoma cells transfected with sst1 and sst2. SS14 binding to wild-type SKNSH cells was undetectable; but the native peptide bound with high affinity to the SKNSH/sst1 and SKNSH/sst2 neuroblastoma cell lines. Pharmacologic analysis of binding with two long-acting analogues, CH275 and octreotide, confirmed selective expression of sst1 and sst2 in stably transfected SKNSH cells. Formation of neuroblastoma xenograft tumors in nude mice was significantly delayed for both SKNSH/sst1 (Psst2) (Psst1 and sst2, are expressed in the majority of neuroblastomas at diagnosis; and (2) upregulation of functional sst1 or sst2 in neuroblastoma cell lines suppresses tumorigenicity in a xenograft model. These observations suggest that somatostatin receptors may be a useful therapeutic target in neuroblastoma.

Balster, D. A., M. S. O'Dorisio, et al. (2002). "Suppression of tumorigenicity in neuroblastoma cells by upregulation of human vasoactive intestinal peptide receptor type 1." Regulatory Peptides **109**(1-3): 155.

<http://www.sciencedirect.com/science/article/B6T0S-46YXPJB-M/2/0948d00705fbb19f9751449e2975adac>

We hypothesize that vasoactive intestinal peptide (VIP) promotes neural crest differentiation through VIP receptor type I (VPAC1). In order to test this hypothesis, SKNSH neuroblastoma cells were stably transfected with VPAC1 and receptor expression was verified by real-time RT-PCR. Overexpression of VPAC1 in SKNSH cells resulted in upregulation of endogenous retinoic acid receptor expression for both RAR[alpha] and RXR[alpha] with no change in expression of RAR[beta]. Transfected cells demonstrated high affinity binding of VIP (KD=0.2 nM) and VIP-mediated stimulation of adenylate cyclase and a shift in cell cycle kinetics to a near triploid DNA index in G1. SKNSH/VPAC1 cells treated with VIP were observed to express a more differentiated phenotype compared to wild type cells as characterized by an increase in tissue transglutaminase II and a decrease in bcl-2 immunostaining. VIP-induced differentiation effects were potentiated by retinoic acid. This differentiation resulted in decreased proliferative potential in a xenograft model. Whereas, wild type SKNSH cells induced tumor growth in 100% of nude mice within 13 days post-injection, SKNSH transfected with VPAC1 demonstrated no tumor formation in xenografts followed for 6 months. Taken together, these data support the hypothesis that VIP modulation of neural crest differentiation is mediated via VPAC1 and that high expression of VPAC1 induces differentiation in and decreases tumorigenicity of neuroblastoma cells.

Bjorkqvist, M., E. Lindstrom, et al. (1999). "Gastrin-induced gene expression in oxyntic mucosa and ECL cells of rat stomach." Regulatory Peptides **84**(1-3): 29.

<http://www.sciencedirect.com/science/article/B6T0S-3XG1FYF-4/2/e177cd6cf31659bdd6a0f583c7d49062>

The histamine-producing ECL cells are numerous in the acid-producing (oxyntic) mucosa. They respond to gastrin by secretion of histamine that acts on parietal cells to produce acid. In addition, gastrin has a trophic effect on the oxyntic mucosa which is exerted on stem cells and ECL cells. To elucidate the molecular actions of gastrin on the stomach we attempted to identify genes that are regulated by gastrin in oxyntic mucosa and in isolated ECL cells. Differential display polymerase chain reaction was used to identify mRNAs that are differentially expressed in rats that are hypergastrinemic after treatment with the proton pump inhibitor omeprazole for 48 h compared with rats that are hypogastrinemic after 24 h fasting. Differences in mRNA levels were confirmed by Northern blot analysis (comparing mRNA from fasted rats, omeprazole-treated rats and rats treated with omeprazole+the CCK2 (cholecystokinin) receptor antagonist YF476). The cDNAs were identified by sequencing followed by data base search. Hypergastrinemia induced by omeprazole treatment resulted in overexpression of mRNA for histidine decarboxylase, fetuin, pepsinogen and cytochrome P450 in the oxyntic mucosa. This was prevented by CCK2 receptor blockade. In isolated ECL cells gastrin upregulated mRNAs for histidine decarboxylase and synaptotagmin V as well as one mRNA transcript without known homology.

Borowsky, B., M. W. Walker, et al. (1998). "Molecular biology and pharmacology of multiple NPY Y5 receptor species homologs." Regulatory Peptides **75-76**: 45.

<http://www.sciencedirect.com/science/article/B6T0S-3Y90819-6/2/3d44a89766f59763afc12af15f7883f3>

NPY is a 36-amino acid peptide which exerts its physiological effects through the activation of a family of G-protein coupled receptors. In vivo and in vitro characterization of the recently cloned rat Y5 receptor suggests that it is a primary mediator of NPY-induced feeding (Gerald et al., Nature 1996;382:168-171). We now report the molecular cloning and pharmacological characterization of the human, dog and mouse homologs of the Y5 receptor. With the exception of a 21 amino acid repeat in the amino terminus of the mouse Y5 receptor, the sequence of the four species homologs appear to be highly conserved, with 88% to 97% amino acid identities between any two species. Similarly, the pharmacological profiles of the four species homologs as determined in porcine 125I-PYY binding assays show a great deal of conservation, with the following rank order of affinity: human or porcine NPY, PYY, [Leu31,Pro34]NPY, NPY2-36, human PP>human [-Trp32]NPY>rat PP, C2-NPY. Northern blot analysis reveals that the Y5 receptor is widely distributed in the human brain, with the strongest signals detected in the cortex, putamen and caudate nucleus. The chromosomal localization of the human Y5 receptor, previously shown to be overlapping and in the opposite orientation to the Y1 receptor, is determined to be 4q31, the same locus as previously demonstrated for the human Y1 receptor (Herzog et al., J Biol Chem 1993;268:6703-6707), suggesting that these receptors may be coregulated. These Y5 species homologs along with corresponding animal models may be useful in the search for novel therapeutics in the treatment of obesity and related feeding disorders.

Busto, R., A. V. Schally, et al. (2002). "Expression of mRNA for growth hormone-releasing hormone and splice variants of GHRH receptors in human malignant bone tumors." Regulatory Peptides **108**(2-

3): 47.

<http://www.sciencedirect.com/science/article/B6T0S-46NXSPV-2/2/db6fc9ab1941cd88396b03a542d32050>

Splice variants (SV) of receptors for growth hormone-releasing hormone (GHRH) have been found in several human cancer cell lines. GHRH antagonists inhibit growth of various human cancers, including osteosarcomas and Ewing's sarcoma, xenografted into nude mice or cultured in vitro and their antiproliferative action could be mediated, in part, through these SV of GHRH receptors. In this study, we found mRNA for the SV1 isoform of GHRH receptors in human osteosarcoma line MNNG/HOS and SK-ES-1 Ewing's sarcoma line. We also detected mRNA for GHRH, which is apparently translated into the GHRH peptide and secreted by the cells, as shown by the presence of GHRH-like immunoreactivity in the conditioned media of cell cultures. In proliferation studies in vitro, the growth of SK-ES-1 and MNNG/HOS cells was dose-dependently inhibited by GHRH antagonist JV-1-38 and an antiserum against human GHRH. Our study indicates the presence of an autocrine stimulatory loop based on GHRH and SV1 of GHRH receptors in human sarcomas. The direct antiproliferative effects of GHRH antagonists on malignant bone tumors appear to be exerted through the SV1 of GHRH receptors on tumoral cells.

Chan, V. Y. W., M. W. Y. Chan, et al. (2005). "Intestinal trefoil factor promotes invasion in non-tumorigenic Rat-2 fibroblast cell." *Regulatory Peptides* **127**(1-3): 87.

<http://www.sciencedirect.com/science/article/B6T0S-4F29HYR-1/2/f334f273a368b253e464f129dfc36946>

Intestinal trefoil factor (TFF3) is essential in regulating cell migration and maintaining mucosal integrity in gastrointestinal tract. We previously showed that TFF3 was overexpressed in gastric carcinoma. Whether TFF3 possesses malignant potential is not fully elucidated. We sought to investigate the effects of inducing TFF3 expression in a non-malignant rat fibroblast cell line (Rat-2) on the cell proliferation, invasion and the genes regulating cell invasion. Invasiveness and proliferation of transfected Rat-2 cell line were assessed using in vitro invasion chamber assay and colorimetric MTS assay. Differential mRNA expressions of invasion-related genes, namely, metalloproteinases (MMP-9), tissue inhibitors of metalloproteinases (TIMP-1), [beta]-catenin and E-cadherin, were determined by quantitative real-time polymerase chain reaction (PCR). We showed that TFF3 did not inhibit the proliferation of Rat-2 cells. We also demonstrated that transfection of TFF3 significantly promoted invasion of Rat-2 cells by 1.4- to 2.2-folds. There was an upregulation of [beta]-catenin (13.1-23.0%) and MMP-9 (43.4-92.2%) mRNA expression levels, and downregulation of E-cadherin (25.6-33.8%) and TIMP-1 (31.5-37.8%) in TFF3-transfected cells compared to controls during 48-h incubation. Our results suggested that TFF3 possesses malignant potential through promotion of cell invasiveness and alteration of invasion-related genes.

Collado, B., I. Gutierrez-Canas, et al. (2004). "Vasoactive intestinal peptide increases vascular endothelial growth factor expression and neuroendocrine differentiation in human prostate cancer LNCaP cells." *Regulatory Peptides* **119**(1-2): 69.

<http://www.sciencedirect.com/science/article/B6T0S-4C12BBG-1/2/2d7eeabb0d9d83ec3621ae4505802e18>

Vasoactive intestinal peptide (VIP) upregulates the expression of vascular endothelial cell growth

factor (VEGF189, VEGF165 and VEGF121) mRNAs in human prostate cancer LNCaP cells, as shown by reverse transcriptase-polymerase chain reaction (RT-PCR). Real-time RT-PCR indicated that the effect was maximal by 1-2 h and must be accounted for increased transcription since VIP decreased VEGF165 mRNA stability. VIP stimulated VEGF165 protein synthesis as measured by ELISA. VIP regulation of VEGF expression was mediated by VPAC1 receptor and was cAMP/protein kinase A (PKA) dependent. Phosphoinositide 3-kinase (PI3-K) and mitogen-activated protein kinase MEK1/2 systems may also be involved as shown with specific kinase inhibitors. These actions together with the observation of VIP-induced neuroendocrine differentiation in LNCaP cells suggest a proangiogenic potential of VIP in prostate cancer.

Girard, B. M., E. T. Keller, et al. (2004). "Pituitary adenylate cyclase activating polypeptide and PAC1 receptor signaling increase Homer 1a expression in central and peripheral neurons." Regulatory Peptides **123**(1-3): 107.

<http://www.sciencedirect.com/science/article/B6T0S-4CXYJW-3/2/54e45ab470cf79c36007e42fd8810885>

Pituitary adenylate cyclase activating polypeptides (PACAP) and PAC1 receptor signaling have diverse roles in central and peripheral nervous system development and function. In recent microarray analyses for PACAP and PAC1 receptor modulation of neuronal transcripts, the mRNA of Homer 1a (H1a), which encodes the noncrosslinking and immediate early gene product isoform of Homer, was identified to be strongly upregulated in superior cervical ganglion (SCG) sympathetic neurons. Given the prominent roles of Homer in synaptogenesis, synaptic protein complex assembly and receptor/channel signaling, we have examined the ability for PACAP to induce H1a expression in sympathetic, cortical and hippocampal neurons to evaluate more comprehensively the roles of PACAP in synaptic function. In both central and peripheral neuronal cultures, PACAP peptides increased transiently H1a transcript levels approximately 3.5- to 6-fold. From real-time quantitative PCR measurements, the temporal patterns of PACAP-mediated H1a mRNA induction among the different neuronal cultures appeared similar although the onset of sympathetic H1a transcript expression appeared protracted. The increase in H1a transcripts was accompanied by increases in H1a protein levels. Comparative studies with VIP and PACAP(6-38) antagonist demonstrated that the PACAP effects reflected PAC1 receptor activation and signaling. The PAC1 receptor isoforms expressed in central and peripheral neurons can engage diverse intracellular second messenger systems, and studies using selective signaling pathway inhibitors demonstrated that the cyclic AMP/PKA and MEK/ERK cascades are principal mediators of the PACAP-mediated H1a induction response. In modulating H1a transcript and protein expression, these studies may implicate broad roles for PACAP and PAC1 receptor signaling in synaptic development and plasticity.

Girard, B. M., V. May, et al. (2002). "Regulation of neurotrophic peptide expression in sympathetic neurons: quantitative analysis using radioimmunoassay and real-time quantitative polymerase chain reaction." Regulatory Peptides **109**(1-3): 89.

<http://www.sciencedirect.com/science/article/B6T0S-4700SF2-6/2/a426d9504496c492ddf07d8fe6b15d6b>

The regulated expression of the peptide and transcript levels of the neurotrophic peptides, pituitary adenylate cyclase-activating polypeptide (PACAP), galanin and vasoactive intestinal peptide (VIP) were examined in sympathetic neurons of the rat superior cervical ganglion (SCG). Real-time quantitative PCR methods were developed to assess modulation of neuronal peptide precursor protein transcript levels following experimental paradigms of neuropeptidergic plasticity. Oligonucleotide primer, fluorogenic probe and amplification conditions were optimized for

maximal assay sensitivity. Depolarization of primary cultured sympathetic neurons stimulated PACAP, galanin, and VIP peptide contents and releases with differing magnitudes and temporal profiles. The rank order of increased neuronal peptide content paralleled the augmented peptide release (VIP>galanin>PACAP). Maximal cellular PACAP and VIP levels were achieved by 72 and 96 h, respectively; galanin levels did not plateau during the treatment period. PACAP transcript elevation was rapid and transient; PACAP mRNA expression diminished at longer depolarization times, which diverged markedly from the sustained high peptide production levels. By contrast, VIP and galanin mRNAs reached maximal levels at later times, and appeared to correlate more closely with peptide production. We previously described multiple proPACAP mRNA variants resulting from alternative 3' untranslated region cleavage and polyadenylation. The shorter depolarization-induced PACAP transcripts exhibit longer half-lives, suggesting that the short proPACAP mRNA variant may function to impart PACAP translational efficiency and sustain PACAP peptide production.

Grant, M. B., S. Caballero, et al. (1993). "Inhibition of IGF-I and b-FGF stimulated growth of human retinal endothelial cells by the somatostatin analogue, octreotide: a potential treatment for ocular neovascularization." Regulatory Peptides **48**(1-2): 267.

<http://www.sciencedirect.com/science/article/B6T0S-47GGSM5-RN/2/d52facb2a3c6202c82989e19a0a5a7a9>

Karacay, B., M. S. O'Dorisio, et al. (2003). "VIP receptor 1 (VPAC1) promoter targets the expression of a reporter gene to cerebellum and adrenal medulla in transgenic mice." Regulatory Peptides **116**(1-3): 1.

<http://www.sciencedirect.com/science/article/B6T0S-49WGMRH-2/2/52a5f34873db4e52d562c3bc95a77b4b>

Vasoactive intestinal peptide (VIP) is a neurotransmitter with neurotropic effects. VIP functions through two distinct G-protein-coupled receptor subtypes (VPAC1 and VPAC2). We have demonstrated expression of VPAC1 in pediatric nervous system tumors, including medulloblastoma arising in the cerebellum and neuroblastoma arising in the adrenal medulla. More recently, we have reported the differentiation of neuroblastoma cells by upregulation of VIP type 1 receptor suggesting a role for VPAC1 in neuronal development. To understand the molecular mechanisms regulating VPAC1 expression in both cerebellum and adrenal medulla, we have cloned the human VPAC1 gene and sequenced 2.6-kb of the 5'-flanking sequence. Expression of the luciferase reporter gene under the control of this 2.6-kb human VPAC1 promoter was induced 35-fold in a human medulloblastoma cell line (DAOY) and 36-fold in a human neuroblastoma cell line (SKNSH). Analysis of 5'-unidirectional deletion derivatives of the 2.6-kb fragment demonstrated that a 241-bp sequence immediately upstream of the VPAC1 coding region retains high activity, suggesting that it contains the core promoter region. Quantitative RT-PCR analysis demonstrated that VPAC1 is expressed in mouse cerebellar and adrenal tissues. The VPAC1 promoter also directed expression of a reporter gene in cerebellum and adrenal medulla in transgenic mice. Along with our previous findings, these results suggest that VPAC1 may play a functional role in development of both cerebellum and adrenal medulla.

Lachal, S., J. Ford, et al. (2004). "PPAR[alpha] agonists stimulate progastrin production in human colorectal carcinoma cells." Regulatory Peptides **120**(1-3): 243.

<http://www.sciencedirect.com/science/article/B6T0S-4CB68MD-2/2/b2f1dce51806a0dc0bc5190b8fed9e9a>

The three subtypes of peroxisome proliferator activated-receptors (PPAR[alpha], [delta] and [gamma]) control the storage and metabolism of fatty acids. Treatment of rats with the PPAR[alpha] ligand ciprofibrate increases serum gastrin concentrations, and several lines of evidence suggest that non-amidated gastrins act as growth factors for the colonic mucosa. The aim of the present study was to investigate the expression of PPARs and the effect of PPAR ligands on gastrin production and cell proliferation in human colorectal carcinoma (CRC) cell lines. mRNAs for all three PPAR subtypes were detected by PCR in all CRC cell lines tested. The concentrations of progastrin, but not of glycine-extended or amidated gastrin, measured by radioimmunoassay in LIM 1899 conditioned media and cell extracts were significantly increased by treatment with the PPAR[alpha] ligand clofibrate. Similar increases in progastrin were seen following treatment with the PPAR[alpha] ligands ciprofibrate and fenofibrate, but not with bezafibrate, gemfibrozil or Wy 14643. The PPAR[gamma] agonist rosiglitazone had no significant effect on progastrin production. The PPAR[alpha] ligand clofibrate also stimulated proliferation of the LIM 1899 cell line. We conclude that some PPAR[alpha] ligands increase progastrin production by the human CRC cell line LIM 1899, and that clofibrate increases proliferation of LIM 1899 cells. These studies have revealed a relationship between PPARs and gastrin, two regulatory molecules implicated in the pathogenesis of CRC.

Malmstrom, R. E., T. Hokfelt, et al. (1998). "Characterization and molecular cloning of vascular neuropeptide Y receptor subtypes in pig and dog." Regulatory Peptides **75-76**: 55.

<http://www.sciencedirect.com/science/article/B6T0S-3Y90819-7/2/b351c2b8ef406bf3ecc452c70be7ee70>

Cloning with subsequent in vitro and in vivo characterization of vascular neuropeptide Y (NPY) receptor subtypes in porcine and canine peripheral tissues was performed. RT-PCR with Y1 and Y2 receptor-specific primers, indicated expression of Y1 receptors in both kidney and spleen of dog and pig, and expression of Y2 receptors in pig spleen. In pig kidney, expression of Y1 receptor mRNA was located to intrarenal arteries, as demonstrated with in situ hybridization using human probes. The cloned and sequenced canine Y1, porcine Y1 and Y2 receptors revealed high homologies to previously characterized mammalian NPY receptors. Membrane and autoradiographic receptor-binding studies showed specific high-affinity binding sites for the purported Y1-selective radioligands 125I-[Leu31Pro34]peptide YY (PYY) and 3H-BIBP 3226 in dog spleen, and for the putative Y2-selective 125I-PYY(3-36) in dog and pig spleen. In the pig in vivo, [Leu31Pro34]PYY, administered i.v., evoked vasoconstriction in spleen and kidney, actions that were potently inhibited by the non-peptide Y1 receptor antagonist SR 120107A. In contrast, PYY(3-36) evoked vasoconstriction only in spleen and this effect was not influenced by SR 120107A. NPY evoked renal and splenic vasoconstriction in the dog in vivo, vascular responses that were inhibited by both BIBP 3226 and SR 120107A. Furthermore, the Y1 receptor agonist [Leu31Pro34]NPY also caused vasoconstriction in dog kidney and spleen, whereas the putative Y2 agonist N-acetyl[Leu28Leu31]NPY(24-36) evoked no such vascular responses. It is concluded that the pig spleen is likely to contain Y1 and Y2 receptors, both involved in splenic vasoconstriction. In contrast, the Y1 receptor seems to be the sole vascular NPY receptor subtype in pig kidney. Moreover, Y1 receptors predominate in dog spleen and kidney. Furthermore, the cloned canine Y1 receptor and the porcine Y1 and Y2 receptors show great homologies to, and possess ligand requirement profiles in accordance with, the human forms.

Opgaard, O. S., L. Cantera, et al. (1996). "Endothelin-A and -B receptors in human coronary arteries and veins." Regulatory Peptides **63**(2-3): 149.

<http://www.sciencedirect.com/science/article/B6T0S-49G07X5-M/2/4bd8ae79797ce4c7bc40a466df47ae93>

Using reverse transcriptase-polymerase chain reaction, products corresponding to mRNA encoding endothelin-A and -B (ETA and ETB) receptors were demonstrated in human coronary arteries and veins with intact endothelium and in endothelium-denuded human coronary arteries. Vasomotor responses were studied on isolated segments of human epicardial coronary arteries and veins at resting tension and after precontraction with U46619. In both arteries and veins, endothelin-1 (ET) induced strong and potent contractions, and preincubation with different concentrations of the non-selective ETA/ETB receptor antagonist PD 145065 caused a rightward shift of the concentration-response curves without significantly changing maximum responses (pA_2 value 6.7 arteries, 7.4 veins). The ETB receptor agonist IRL 1620 induced no contraction of arteries or veins at resting tension, but induced weak relaxation of all arteries and most precontracted veins, the relaxation being endothelium-dependent in arteries. ET at low concentrations induced weak relaxations of most precontracted arteries, but no veins. In conclusion, mRNA encoding ETA and ETB receptors is present in human coronary arteries and veins, ETA receptors mediating contraction and ETB receptors mediating relaxation. In arteries, mRNA for both receptor types was detected in the media, but ETB receptor-mediated relaxation was endothelium-dependent.

Peng, J., B. Kimura, et al. (2002). "The predominant role of brain angiotensinogen and angiotensin in environmentally induced hypertension." *Regulatory Peptides* **110**(1): 25.

<http://www.sciencedirect.com/science/article/B6T0S-46RM1XR-1/2/59c86cad95957875435e37fb629e441a>

Rats exposed chronically to a cold environment (5 [deg]C/4 [deg]F) develop hypertension. This cold-induced hypertension (CIH) is a non-genetic, non-pharmacological, non-surgical model of environmentally induced hypertension in rats. The renin-angiotensin system (RAS) appears to play a role in both initiating and/or maintaining the high blood pressure in CIH. The goal of the present study was to evaluate the role of central and peripheral circulating RAS components, angiotensinogen (AGT), angiotensin-converting enzyme (ACE) and angiotensin (Ang) II, in CIH. Seventy-two Sprague-Dawley adult male rats were used. Thirty-six rats were kept in cold room at 5 [deg]C while the other 36 were at 24 [deg]C as controls for 5 weeks. Systolic blood pressure (SBP) was recorded by tail cuff. The SBP was increased in rats exposed to cold within 1 week, and this increase was significant for the next 2-5 weeks of the cold exposure ($p=12$) were sacrificed at 1, 3 and 5 weeks. The brain and liver were removed and plasma was saved. The AGT mRNA significantly increased in the hypothalamus and liver in cold-treated rats from the first week of exposure to cold, and was maintained throughout the time of exposure to cold ($n=4$, $pp>0.05$, $n=4$). The hypothalamic Ang II levels were significantly increased, whereas plasma Ang II levels significantly decreased, in the rats of 5 weeks of cold exposure ($n=8$, $ppn=12$). The results show differential regulation of RAS components, AGT, ACE and Ang II, between brain and periphery in cold-exposed rats. We conclude that the exposure to low temperature initially increases plasma RAS but with continuous exposure to cold, the brain RAS maintains the hypertension, probably by sustained sympathetic activation, which would provide increased metabolism but also vasoconstriction leading to hypertension.

Peng, J. and M. I. Phillips (2001). "Opposite regulation of brain angiotensin type 1 and type 2 receptors in cold-induced hypertension." *Regulatory Peptides* **97**(2-3): 91.

<http://www.sciencedirect.com/science/article/B6T0S-427JWNP-1/2/06c18d676e87f4130ff020677157a65b>

Rats exposed chronically to mild cold (5[deg]C/41[deg]F) develop hypertension. This cold-induced hypertension (CIH) is an environmentally induced, non-surgical, non-pharmacological and non-genetic model for studying hypertension in rats. The blood renin angiotensin system (RAS) appears to play a role in both initiating and maintaining the high blood pressure in CIH. The goal of the present study was to evaluate the role of brain angiotensin type 1 and type 2 receptors (AT1 R and AT2 R) in CIH. Sprague-Dawley adult male rats were used. Thirty-six rats were kept in a cold room at 5[deg]C and the other 36 were kept at 24[deg]C as controls. Systolic blood pressure (SBP) was recorded by tail cuff. The SBP was elevated in rats exposed to cold within 1 week ($n=12$, $P>0.05$), significantly increased at 3 weeks (PP1 R and AT2 R). The AT1 R mRNA was increased significantly in hypothalamus and brainstem after the first week in cold-treated rats and was maintained throughout the time of exposure to cold ($n=6$, P1 R binding significantly increased initially in hypothalamus and thereafter in brainstem). The mRNA and the receptor binding for AT2 R decreased significantly ($Pn=6$) in nucleus of inferior olive and locus coeruleus of brainstem in cold-treated rats after exposure to cold. The experiments show differential regulation of RAS components, AT1 R and AT2 R, in different brain areas in cold-exposed rats and provide evidence that up-regulated AT1 R and down-regulated AT2 R in different brain areas are involved in CIH. The opposing directions of expression of AT1 R and AT2 R suggest that they play counterbalancing roles in brain function.

Pinzani, P., C. Orlando, et al. (2001). "Type-2 somatostatin receptor mRNA levels in breast and colon cancer determined by a quantitative RT-PCR assay based on dual label fluorogenic probe and the TaqMan(TM) technology." Regulatory Peptides **99**(2-3): 79.

<http://www.sciencedirect.com/science/article/B6T0S-43439N1-2/2/39d7bd24f1a42611206a81e286ebc19b>

We reported previously that the expression of type 2 somatostatin receptor (sst2) was positively related to patient outcome in the childhood tumor neuroblastoma. To quantitate the expression of mRNA sst2 expression, we used a competitive RT-PCR assay. To improve the practicability of this measurement and its applicability to large groups of patients, we present here an original 'real-time' quantitative RT-PCR method, based on a dual-labeled fluorogenic probe and the TaqMan(TM) technology. By this method, we have measured sst2 mRNA expression in 24 breast cancer samples and 26 colon carcinomas as well as on the corresponding non-adjacent non-neoplastic tissue of the same patients. The proposed method has a dynamic range of 4×10^4 to 4×10^8 molecules of sst2 mRNA. The intra-assay precision of the test, evaluated as signal detection variability, was 2.4%. Accuracy, evaluated by the addition of standard RNA to unknown samples, provided a mean recovery of $98 \pm 2\%$. A significant correlation has been observed in a study performed in 24 neuroblastoma samples measured both with the proposed method and with a competitive RT-PCR assay ($r=0.913$, $p7 \pm 2.0 \times 10^7$ molecules/[μ g total RNA, cancer tissue $9.7 \times 10^7 \pm 4.2 \times 10^7$) and breast tumors (normal tissue $5.5 \times 10^8 \pm 2.0 \times 10^8$, cancer tissue $4.4 \times 10^8 \pm 3.7 \times 10^8$). However, in colorectal cancer, sst2 mRNA values of subjects with high circulating carcinoembryonic antigen (CEA) levels (>5 ng/ml) were statistically lower ($2.3 \times 10^7 \pm 6.2 \times 10^6$ molecules/[μ g total RNA; $p8 \pm 6.7 \times 10^7$). Also, the sst2 mRNA ratio between normal and tumor tissue (N/T ratio) resulted significantly inversely related to CEA levels. In breast cancer, a significant difference was found between the mean N/T ratio of negative (below 10 fmol/mg protein) and positive estrogen receptor tumors ($pp<0.05$). The proposed method is accurate, precise, sensitive and less labor-intensive than the competitive RT-PCR assay. For a correct evaluation of sst2 mRNA expression, it seems very important to measure the sst2 expression both in tumor and in the non-tumoral non-adjacent tumor specimens.

Sharma, P., S. K. S. Holmberg, et al. (1998). "Cloning and functional expression of the guinea pig neuropeptide Y Y2 receptor." Regulatory Peptides **75-76**: 23.

<http://www.sciencedirect.com/science/article/B6T0S-3Y90819-3/2/488dff2b7d1adf45cdc742c08030a012>

Five neuropeptide Y (NPY) receptor subtypes have been cloned in mammals. The degree of sequence conservation differs considerably between subtypes as well as between evolutionary lineages. To shed further light on this, we have cloned the five NPY receptors in the guinea pig. Here, we report the cloning of the guinea pig Y2 receptor. The Y2 receptor is generally highly conserved, with 90-95% identity between different orders of mammals, including the guinea pig. The guinea pig receptor has a divergent cytoplasmic tail, indicating possible differences in regulation of signalling and/or down regulation. COS-7 cells transiently transfected with the gpY2 receptor show saturable 125I-PYY binding with a $K_d=6$ pM. In displacement experiments, the gpY2 receptor was similar to the human and rat receptors with the following rank order of potencies: pNPY>pPYY>pNPY13-36=pNPY22-36>>[Leu31Pro34]NPY>BIBP3226. Thus, the guinea pig Y2 receptor is well conserved in comparison with human and rat with regard to both amino acid sequence and pharmacological profile.

Slagter, B. J., J. Kittilson, et al. (2005). "Expression of somatostatin receptor mRNAs is regulated in vivo by growth hormone, insulin, and insulin-like growth factor-I in rainbow trout (*Oncorhynchus mykiss*)."
Regulatory Peptides **128**(1): 27.

<http://www.sciencedirect.com/science/article/B6T0S-4F6MDHV-2/2/898d95d18a71de1a818c1ee2c4b45a41>

Somatostatins are a diverse family of peptide hormones that regulate various aspects of growth, development, and metabolism through interactions with numerous somatostatin receptor subtypes (SSTRs) on target tissues. In this study, we used rainbow trout to evaluate the effects of growth hormone (GH), insulin (INS), and insulin-like growth factor-I (IGF-I) on the expression of SSTR 1A, 1B and 2 mRNAs. GH regulated the expression of SSTRs in a subtype- and tissue-specific manner. GH reduced SSTR 1A, 1B, and 2 expression in optic tectum, reduced SSTR 1A and 1B expression in pancreas, reduced SSTR 1A expression in liver, and increased hepatic SSTR 1B expression. INS also regulated SSTR expression in a subtype- and tissue-specific manner. INS reduced SSTR 1B expression in optic tectum, increased SSTR 2 expression in pancreas, and increased SSTR 1B and 2 expression in liver. IGF-I generally decreased the expression of all SSTRs. These data indicate that GH, INS, and IGF-I modulate the expression of SSTRs and suggest that independent mechanisms may serve to regulate the various receptor subtypes.

Springer, J., D. Pleimes, et al. (2005). "Substance P mediates AP-1 induction in A549 cells via reactive oxygen species."
Regulatory Peptides **124**(1-3): 99.

<http://www.sciencedirect.com/science/article/B6T0S-4D2N0BC-1/2/743fb077ba36a09e36fab3b2f3bde81>

A common feature in asthma is the induction of reactive oxygen species (ROS) and the AP-1 transcription factor during the inflammatory process. AP-1 induction leads to an increased expression of pro-inflammatory cytokines. Also, higher levels of the pro-inflammatory neuropeptide substance P (SP) have been reported in bronchoalveolar-lavage fluid of asthmatics. Here, the role of SP on ROS induction and the downstream activation of AP-1 in A549 airway epithelial cells was investigated by dichlorofluorescein-diacetate method and reporter gene assays. The SP-mediated AP-1 induction was dependent on extracellular calcium and ROS. The likely source of ROS are the mitochondria as rotenone inhibited AP-1 induction and the

p47phox subunit of the NADPH oxidase complex, responsible for ROS generation in phagocytotic cells, was not expressed in A549 cells assayed by RT-PCR. This is consistent with results obtained from cells of murine bronchial epithelium, isolated by laser capture microdissection. In summary, this study provides evidence for an SP-mediated induction of AP-1, which may contribute to the expression of pro-inflammatory cytokines.

Takeda, M., R. Dubey, et al. (2002). "Effects of vasopressin on isolated rat adrenal chromaffin cells." Regulatory Peptides **106**(1-3): 55.

<http://www.sciencedirect.com/science/article/B6T0S-45C0618-3/2/5a09499a59f229921f40a3bd25616cdc>

It has been demonstrated that arginine vasopressin (AVP) is synthesized not only in specific hypothalamic nuclei, but also in the adrenal medulla where it is thought to regulate adrenal functions by autocrine and paracrine mechanisms. In order to further characterise the effects of AVP on rat adrenal chromaffin cells, we examined: (a) the mRNA expression for V1a and V1b AVP receptors in these cells; (b) the effects of AVP on the membrane potential and membrane currents measured with the whole-cell patch-clamp technique; and (c) effect of AVP on catecholamine release from single adrenal chromaffin cells measured with carbon fibre microelectrodes. Reverse transcription-polymerase chain reaction (RT-PCR) on tissue punch samples obtained from the adrenal medulla demonstrated message for both the V1a and V1b receptors, while material obtained from the adrenal cortex showed expression of the V1a receptor only. Single-cell RT-PCR conducted on acutely isolated chromaffin cells showed message for the V1a receptor in 84% of cells, while 38% of cells also contained message for the V1b receptor (n=45). Under current-clamp recording, responses to AVP application (4-40 [μM]) were variable; 22/34 (65%) tested cells were depolarised, 29% hyperpolarised, and the remaining cells showed a biphasic response. Changes in membrane potential of either direction were dose-dependent and accompanied by a decrease in cell membrane resistance. Under voltage-clamp (V_{hold}=-60 mV), AVP evoked inward current in 27/52 (52%) and outward current in 16/52 (31%) chromaffin cells. Both types of AVP-evoked responses were blocked by co-application of a nonselective V1a/V1b antagonist. Application of AVP evoked prolonged bursts of amperometric currents (indicative of catecholamine release) in 4/9 tested cells, but reduced the currents evoked by ACh application in all tested cells (n=7). These findings demonstrate a complex action of AVP on adrenal chromaffin cells, with individual adrenal chromaffin cells responding with either excitation or inhibition. This response pattern may be related to the expression of V1 receptor subtypes.

Teng, B., K. S. Murthy, et al. (1998). "Selective expression of vasoactive intestinal peptide (VIP)2/pituitary adenylate cyclase-activating polypeptide (PACAP)3 receptors in rabbit and guinea pig gastric and tenia coli smooth muscle cells." Regulatory Peptides **77**(1-3): 127.

<http://www.sciencedirect.com/science/article/B6T0S-3TVXFHH-M/2/c2dfec000c4512a7ca19c25394552a69>

In both functional and radioligand binding studies of gastric smooth muscle from rabbit and guinea pig, vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) show equal potency indicating that the receptor type is either a VIP1/PACAP2 or a VIP2/PACAP3 receptor. We have characterized the VIP/PACAP receptor expressed in freshly dispersed and cultured gastric and tenia coli smooth muscle cells of rabbit and guinea pig by reverse transcriptase-polymerase chain reaction (RT-PCR), Northern analysis, and cloning of the first extracellular domain. Specific primers based on cDNA sequences for rat VIP1/PACAP2, VIP2/PACAP3 and PACAP1 receptors were designed spanning the first extracellular domain. A 275 base pair product corresponding to VIP2/PACAP3 receptor was

amplified by RT-PCR in muscle cells from both species. No RT-PCR product was obtained with primers for VIP1/PACAP2 and PACAP1 receptors. The deduced amino acid sequences showed 90% similarity in rabbit and 77% in guinea pig to the sequence in rat. The location of the aspartate, tryptophan and glycine residues and all six N-terminal cysteines required for VIP binding were conserved. The sequence in guinea pig *tenia coli* differed from that in guinea pig stomach by two amino acid residues, Phe40 and Phe41. Northern analysis revealed a single 3.9 kilobase (kb) mRNA corresponding to VIP2/PACAP3 receptors in rabbit and a 2.1 kb mRNA in guinea pig gastric and *tenia coli* muscle cells. We conclude that only VIP2/PACAP3 receptors are expressed in smooth muscle cells of rabbit and guinea pig. The two amino acid difference in the sequence obtained from guinea pig *tenia coli* may reflect the distinct binding and functional properties of this tissue.

Wada, R., I. Sakata, et al. (2003). "Existence of ghrelin-immunopositive and -expressing cells in the proventriculus of the hatching and adult chicken." Regulatory Peptides **111**(1-3): 123.

<http://www.sciencedirect.com/science/article/B6T0S-47RJTH4-1/2/5699d96952eff09d08453afdb3809f67>

Ghrelin was isolated from the rat stomach as an endogenous ligand for the growth hormone secretagogue receptor (GHS-R) and has been found in the gastrointestinal tract of many vertebrates. Although the sequence and structure of chicken ghrelin has recently been determined, morphological characteristics of ghrelin cells in the chicken gastrointestinal tract are still obscure. In this study, we investigated ghrelin expression and distribution of ghrelin-producing cells in the hatching and adult chicken gastrointestinal tract by RT-PCR, immunohistochemistry and in situ hybridization. Ghrelin mRNA expression was observed mainly in the proventriculus in the hatching chicken and in the proventriculus, pylorus and duodenum of the adult chicken by RT-PCR. Ghrelin-immunopositive (ghrelin-ip) cells in the proventriculus were located at the mucosal layer but not in the myenteric plexus or smooth muscle layer. The number of ghrelin-ip cells in the adult chicken was greater than that in the hatching chicken. Interestingly, in the adult chicken, the number of ghrelin-ip cells were almost the same as that of ghrelin mRNA-expressing (ghrelin-ex) cells; however, in the hatching chicken, the number of ghrelin-ex cells was greater than that of ghrelin-ip cells. These results clearly demonstrate that ghrelin-producing cells exist in the chicken gastrointestinal tract, especially in the proventriculus, from hatching to adult stages of development, as well as in mammals.

Wortley, K. E., G.-Q. Chang, et al. (2004). "Cocaine- and amphetamine-regulated transcript in the arcuate nucleus stimulates lipid metabolism to control body fat accrual on a high-fat diet." Regulatory Peptides **117**(2): 89.

<http://www.sciencedirect.com/science/article/B6T0S-4B5044P-4/2/c9ef8e3df82308cc49198ba16c5afe33>

Previous studies have indicated a relationship between cocaine- and amphetamine-related transcript (CART) and leptin. The present study used quantitative PCR and in situ hybridization to examine this CART-leptin relationship in different animal models. With CART injection, the function of this pathway was also investigated. The results demonstrate that CART mRNA in the arcuate nucleus (ARC) was significantly increased in subjects fed a high-fat diet (HFD) compared to low-fat diet (LFD). It was also elevated in obese vs. lean rats and in normal-weight obesity-prone vs. obesity-resistant rats. In each group tested, CART mRNA in the ARC was positively correlated specifically with circulating levels of leptin. Its close association specifically with leptin was further supported by a stimulatory effect of this hormone on CART expression. This leptin-CART relationship in the ARC, in contrast, was less consistent or undetectable in the

paraventricular nucleus and lateral hypothalamus. Central injection of CART peptide (55-102) increased circulating non-esterified fatty acid levels and decreased lipoprotein lipase activity in adipose tissue. These results suggest that, on a fat-rich diet, this leptin-CART pathway originating in the ARC inhibits excessive body fat accrual by causing a shift from lipid storage toward lipid mobilization.

Reproduction (4)

Barcroft, L. C., S. E. Gill, et al. (2002). "The gamma-subunit of the Na-K-ATPase as a potential regulator of apical and basolateral Na⁺-pump isozymes during development of bovine pre-attachment embryos." Reproduction **124**(3): 387-397.

<http://www.reproduction-online.org/cgi/content/abstract/124/3/387>

Expression and activity of the Na-K-ATPase within the basolateral membrane domains of the trophoctoderm epithelium provide the driving force for accumulation of Na⁽⁺⁾ and Cl⁽⁻⁾ across the nascent epithelium, mediating fluid movement into the forming blastocoel. Within the trophoctoderm of the bovine blastocyst, multiple isozymes of the Na-K-ATPase are expressed. Immunolocalization has demonstrated that the alpha1-isozyme localizes within the basolateral membrane, whereas the alpha 3-isozyme localizes to the apical cell margins. Gene-specific RT-PCR and wholemount indirect immunofluorescence confocal laser scanning microscopy were used to examine expression of the Na-K-ATPase gamma-subunit (a regulatory subunit of the Na-K-ATPase) throughout development of bovine preattachment embryos in vitro. Expression of mRNA transcripts for the gamma-subunit was detected throughout bovine pre-attachment development from the fertilized one-cell embryo to the blastocyst stage. A similar pattern of expression was also observed for gamma-subunit protein, and immunofluorescence was detected within the membranes of embryonic blastomeres at all stages of development. In contrast to the expression patterns observed for the alpha-subunits, gamma-subunit proteins were detected in both the basolateral and apical cell margins of the trophoctoderm, and surrounding all cells of the inner cell mass. Co-localization studies demonstrated that gamma-subunit peptides are co-expressed with the alpha1-subunit in the basolateral domains of the trophoctoderm. These results indicate a role for the gamma-subunit of the Na-K-ATPase in modulating Na⁽⁺⁾-pump activity in both apical and basolateral margins of the trophoctoderm during formation and expansion of the bovine blastocyst, and adds a further level of complexity to Na⁽⁺⁾-pump regulation of cavitation.

Ben-Aharon, I., P. R. Brown, et al. (2005). "The expression of calpain 1 and calpain 2 in spermatogenic cells and spermatozoa of the mouse." Reproduction **129**(4): 435-442.

<http://www.reproduction-online.org/cgi/content/abstract/129/4/435>

There is some evidence suggesting that Ca²⁺ is involved in processes that occur during the development and function of spermatozoa. Calcium-dependent proteins, such as calmodulin, are expressed during mammalian spermatogenesis further suggesting that Ca²⁺ takes part in its regulation. However, the precise roles of Ca²⁺ in spermatogenesis remain to be elucidated. Calpains are a family of Ca²⁺-dependent cysteine proteases whose members are expressed ubiquitously or in a tissue-specific manner. Calpain has been demonstrated to mediate specific

Ca²⁺-dependent processes including cell fusion, mitosis and meiosis. We herein followed the expression pattern of calpain's ubiquitous isoforms, 1 and 2, throughout spermatogenesis at the RNA and protein levels by RT-PCR and Western blotting analysis. Both RNA and protein studies revealed that these isoforms are expressed in all spermatogenic cells. The expression of calpain 1 levels is slightly higher in spermatocytes entering the meiotic phase. Both calpain isoforms are also expressed in mouse spermatozoa and are localized to the acrosomal cap. Inducing capacitated spermatozoa to undergo the acrosome reaction in the presence of a selective calpain inhibitor significantly reduced the acrosome reaction rate in a dose-dependent manner. Thus, calpain, a pluripotential protease with numerous substrates, may serve as an effector in more than one pathway in the complex process of spermatogenesis and in the events preceding fertilization, such as the acrosome reaction.

Klipper, E., T. Gilboa, et al. (2004). "Characterization of endothelin-1 and nitric oxide generating systems in corpus luteum-derived endothelial cells." *Reproduction* **128**(4): 463-473.

<http://www.reproduction-online.org/cgi/content/abstract/128/4/463>

Endothelium-derived endothelin-1 (ET-1) and nitric oxide (NO) are pivotal regulators of corpus luteum (CL) function. To have a better insight into their synthesis and action, members of the ET system (ET-1, ET converting enzyme (ECE-1) isoforms a-d, ETA and ETB receptors) along with NO synthase (NOS) isoforms - endothelial (e)NOS and inducible (i)NOS - were quantified in CL-derived endothelial cells (CLEC). The expression of these genes in microvascular CLEC, obtained by lectin-coated magnetic beads, was compared with cells removed from the luteal microenvironment and maintained in culture for different durations, and with endothelial cells (EC) derived from a large blood vessel (i.e. bovine aortic endothelial cells, BAEC). The profile of gene expression in the different EC types was determined by quantitative real-time PCR. Freshly isolated EC from mid-cycle CL exhibited high ET-1 receptor expression (both ETA and ETB), low ET-1 synthesizing ability (both prepro (pp) ET-1 and ECE-1), but elevated iNOS - the high throughput NOS isoform. The distinct phenotype of CLEC was lost soon after an overnight culture. ETA and ETB receptor levels declined, ppET-1 levels increased while iNOS was reduced. These changes were extenuated during long-term culture of CLEC. The general pattern of gene expression in BAEC and long-term cultured CLEC was similar yet some differences, reminiscent of freshly isolated CLEC, remained: ECE-1c, ETB receptor and NOS isoforms were expressed differently in BAEC as compared with lines of CLEC. This study suggests that the luteal microenvironment is necessary to sustain the selective phenotype of its resident endothelial cells. The inverse relationship between ppET-1 and iNOS observed in freshly isolated CLEC and in cultured cells is physiologically significant and suggests that ET-1 and NO may modulate the production of each other.

Spanos, S., S. Rice, et al. (2002). "Caspase activity and expression of cell death genes during development of human preimplantation embryos." *Reproduction* **124**(3): 353-363.

<http://www.reproduction-online.org/cgi/content/abstract/124/3/353>

It has been observed that apoptosis occurs in human blastocysts. In other types of cell, the characteristic morphological changes seen in apoptotic cells are executed by caspases, which are regulated by the BCL-2 family of proteins. This study investigated whether these components of the apoptotic cascade are present throughout human preimplantation development. Developing and arrested two pronucleate embryos at all stages were incubated with a fluorescently tagged caspase inhibitor that binds only to active caspases, fixed, counterstained with 4,6-diamidino-2-phenylindole (DAPI) to assess nuclear morphology and examined using confocal microscopy. Active caspases were detected only after compaction, at the morula and

blastocyst stages, and were frequently associated with apoptotic nuclei. Occasional labelling was seen in arrested embryos. Expression of proapoptotic BAX and BAD and anti-apoptotic BCL-2 was examined in single embryos using RT-PCR and immunohistochemistry. BAX and BCL-2 mRNAs were expressed throughout development, whereas BAD mRNA was expressed mainly after compaction. Simultaneous expression of BAX and BCL-2 proteins within individual embryos was confirmed using immunohistochemistry. The onset of caspase activity and BAD expression after compaction correlates with the previously reported appearance of apoptotic nuclei. As in other types of cell, human embryos express common molecular components of the apoptotic cascade, although apoptosis appears to be suppressed before compaction and differentiation.

Reproductive Toxicology (1)

Spencer, F., L. Chi, et al. (2001). "A mechanistic assessment of 1,3-butadiene diepoxide-induced inhibition of uterine decidual proliferation in pseudopregnant rats." Reproductive Toxicology **15**(3): 253.

<http://www.sciencedirect.com/science/article/B6TC0-435CSWR-5/2/9f3b5782034320e7912b5a9a4928cad0>

Butadiene diepoxide (BDE), a reactive metabolite of 1,3-butadiene that is an important industrial chemical used in synthetic rubber production causes a dose-dependent inhibition of decidual development in pseudopregnant Sprague-Dawley rats. This study used 4 daily i.p. BDE doses of 0.20, 0.25, 0.30, 0.35, or 0.40 to characterize mechanisms that may be responsible for the antidecidual effect. Pseudopregnant rats were treated either before (pseudopregnancy [PPG] days 1-4) or after (PPG days 5-9) decidual induction by endometrial trauma with a blunt needle. Animals were killed on PPG day 9 and evaluated for serum progesterone and endometrial protein and DNA. RT-PCR was used to measure message for estrogen receptor (ER) [alpha] and pituitary adenylate cyclase-activating polypeptide (PACAP). Substrate zymography and Western blotting were used respectively to measure matrix metalloproteinase (MMP)-9 and inducible nitric oxide synthase. The antidecidual effects of BDE were associated with decreases in endometrial weight, protein, and DNA, with decreases in serum progesterone, and with decreases in PACAP message and MMP-9. A reduction in NOS was identified at the highest dose of BDE. Message for estrogen receptor (ER) [alpha] was not affected at any dose. We conclude that the reduction in decidual proliferation was direct and appeared to be associated with either 1) a decrease in the effectiveness of the decidualogenic stimulation and/or a weakened endometrial sensitivity to the stimulus; or 2) an effect on decidual development. Molecular mechanisms that apparently contributed to BDE inhibition of decidual metabolism included the synthesis of protein and DNA involved in decidual growth, the synthesis and activation of a matrix metalloproteinase for degradation of the extracellular matrix that is essential for tissue remodeling during decidual development, and the nitric oxide/nitric oxide synthase and pituitary adenylate cyclase-activating peptide systems that are involved in promoting vasodilation and increased vascular permeability to enhance the availability of substrates for maximal decidual growth. The ovotoxicity of BDE, which has previously been established, may indirectly affect decidual proliferation by reducing progesterone, the preeminent endocrine regulator of decidual development. The findings also suggest that BDE may possess no estrogenic action since it was associated with endometrial weight loss and unaltered levels of the estrogen receptor [alpha] mRNA expression.

de Lamballerie, X., C. Zandotti, et al. (1992). "A one-step microbial DNA extraction method using "Chelex 100" suitable for gene amplification." Research in Microbiology **143**(8): 785.

<http://www.sciencedirect.com/science/article/B6VN3-47DDBMR-1C2/701a8333b92d149bcfac19d01fdfeaaa>

"Chelex 100" chelating resin has been previously proposed for the rapid extraction of human DNA for polymerase chain reaction. Protocols are given for the rapid extraction of bacterial and viral DNA from cultures or clinical samples. The DNA samples obtained were suitable for use in polymerase chain reaction.

Janvier, B., C. Constantinidou, et al. (1998). "Characterization and gene sequencing of a 19-kDa periplasmic protein of *Campylobacter jejuni/coli*." Research in Microbiology **149**(2): 95.

<http://www.sciencedirect.com/science/article/B6VN3-3V7PB37-9/2/959942c811704a88371e01edf07d6abc>

In order to study a 19-kDa protein (p19) of *Campylobacter jejuni*, we purified this protein to homogeneity from *C. jejuni* strain 81176 by anion exchange chromatography. The molecular weight of the native protein is 19,000 daltons. P19 was found to be acidic with an isoelectric point of 4.8 and was located in the periplasmic space of the bacteria. The 20 N-terminal amino acids were sequenced and no significant similarities with known proteins were shown. A monoclonal antibody showed that p19 is conserved in the 2 species *C. jejuni* and *C. coli*. Analysis of sera from 23 patients with a *Campylobacter*-related infection indicated that p19 is not immunogenic during natural infection in man. The gene encoding p19 was cloned and no strong homologies with known sequences were identified. The preparation of a knockout mutant in p19 will enable the investigation of the function of this cell wall component of *Campylobacter*.

Lagatolla, C., A. Lavenia, et al. (1998). "Characterization of oligonucleotide probes for the identification of *Acinetobacter* spp., *A. baumannii* and *Acinetobacter* genomic species 3." Research in Microbiology **149**(8): 557.

<http://www.sciencedirect.com/science/article/B6VN3-3XXDWSF-3/2/133e5f862e14c8665a31fb87cfaa233d>

The 16S-23S intergenic spacer regions of four *Acinetobacter* genomic species belonging to the *A. calcoaceticus*-*A. baumannii* (Acb) complex, i.e. genomic species 1 (*A. calcoaceticus*), genomic species 2 (*A. baumannii*), genomic species 3 and Tjernberg and Ursing (TU) genomic species 13, have been cloned and sequenced. Sequence analysis led to the discovery of a single copy of Ile and Ala tRNA genes within each spacer. Sequence comparison allowed the identification of a 192-base-pair long highly conserved sequence between the 3' end of the 16S rRNA and the 5' end of the tRNA^{Ala} genes. Moreover, two short regions, which were specific to, respectively, genomic species 2 and 3, could be identified. Oligonucleotides corresponding to these sequences were constructed and tested for the ability to hybridize with chromosomal DNA extracted from *Acinetobacter* belonging to different genomic species and with chromosomal DNA of other

bacterial genera. One of these oligonucleotides was demonstrated to be useful as a sensitive and specific probe for *A. baumannii*. A less sensitive probe for *Acinetobacter* genomic species 3 was also developed.

Paffetti, D., C. Barberio, et al. (1995). "DNA fingerprinting by random amplified polymorphic DNA and restriction fragment length polymorphism is useful for yeast typing." Research in Microbiology **146**(7): 587.

<http://www.sciencedirect.com/science/article/B6VN3-3Y5FMKC-8/2/bffca24f68b2b5eb3602432c4911f1c>

Random amplified polymorphic DNA (RAPD) analysis was applied to genomic DNA from nineteen yeast strains belonging to the genera *Saccharomyces* and *Zygosaccharomyces*. Results obtained with five primers indicated that this technique is a powerful tool for yeast differentiation and identification. The data were consistent with those derived from restriction fragment length polymorphism (RFLP) using two *S. cerevisiae* DNA probes. We conclude that RAPD fingerprinting, combined with the analysis of RFLP, can provide unambiguous type assignment in yeasts.

Schvoerer, E., F. Bonnet, et al. (2000). "PCR detection of human enteric viruses in bathing areas, waste waters and human stools in southwestern France." Research in Microbiology **151**(8): 693.

<http://www.sciencedirect.com/science/article/B6VN3-41GWN7Y-9/2/10b1617605570d31fc47b17aa77edfab>

Detection of human pathogenic viruses by molecular techniques might be suitable for identifying viral pollution in environmental waters and for improving diagnosis in patients. Environmental samples were taken from bathing areas and sewage treatment plants in southwestern France. Small volume samples (50 [μ]L) were tested. Five groups of enteric pathogenic viruses were studied: enteroviruses, Norwalk-like viruses (NLVs), hepatitis A virus, rotaviruses and adenoviruses. Moreover, human samples were tested for NLV. After extraction of viral nucleic acids (Boom's procedure), a nested polymerase chain reaction was conducted before hybridization. Five bathing waters out of 26 were positive for one viral group, without systematic association with bacterial contamination. Eight sewage plant samples out of 13 were positive for at least one viral group. Seven patients out of 45 were NLV-positive. Molecular techniques allow efficient screening of viral contamination in environmental waters and the study of NLV molecular epidemiology.

Wang, J., V. Michel, et al. (1998). "Cloning of the J gene of bacteriophage lambda, expression and solubilization of the J protein: first in vitro studies on the interactions between J and LamB, its cell surface receptor." Research in Microbiology **149**(9): 611.

<http://www.sciencedirect.com/science/article/B6VN3-3WB7RD8-C/2/01c1e3e7eeab351a96d8cce2c36ec84f>

Bacteriophage [lambda] adsorbs to its *Escherichia coli* K12 host by interacting with a specific cell surface receptor, the outer membrane protein LamB. Previous genetic analyses led us to define a set of residues at the surface of LamB, which belong to the [lambda] receptor site. Further genetic studies indicated that the C-terminal portion of J, the tail fibre protein of [lambda], was directly

involved in the recognition of the receptor site. The present work describes first in vitro studies on the interactions between J and LamB. The J gene of [lambda] was cloned into a plasmid vector under ptac promoter control and expressed in E. coli. We showed that J could be expressed at high levels (up to 28% of whole cell proteins), in an insoluble form. Anti-J antibodies, induced in rabbits immunized with insoluble J extracts, appeared to specifically neutralize [lambda] infection. Under defined conditions of extraction, the J protein was obtained in a soluble form. We showed that solubilized J was able to interact with LamB trimers in vitro. Implications for future studies on the interactions between LamB and J are discussed.

Research in Veterinary Science (7)

Amin, A. S. (2003). "Application of touchdown enzyme time release (TETR)-PCR for diagnosis of Chlamydomphila abortus infection." Research in Veterinary Science **74**(3): 213.

<http://www.sciencedirect.com/science/article/B6WWR-48BC03B-1/2/0a4dcfd9bb9df501470d11070e2d5bea>

Chlamydomphila abortus-DNA was detected using a touchdown enzyme time-release (TETR)-polymerase chain reaction (PCR) assay as an improved test for sensitive and rapid diagnosis of abortion in small ruminants. Two hundred and fifty two placentae, liver or spleen tissue samples from aborting ewes and goats or aborted lambs and kids in which C. abortus infection was suspected were examined by TETR-PCR and the results were compared with cell culture. Sixty-five tissue samples were found to be TETR-PCR positive while only 56 samples were cell culture-positive. After resolution of discrepant samples with a confirmatory nested PCR assay, TETR-PCR had a sensitivity of 97% and a specificity of 99.5% while culture had a sensitivity of 84.8% and a specificity of 100%. The analytical sensitivity of the TETR-PCR assay was determined with DNA extracted from 4-fold serial dilution of C. abortus B577 culture and found to be 0.25 inclusion-forming unit per PCR. No reduction in the analytical sensitivity was noted when the assay was tested with mouse liver samples spiked with 4-fold serial dilution of C. abortus B577 culture. No target product was amplified when DNA from Chlamydomphila pecorum was tested. TETR-PCR used in this study is a practical, rapid, sensitive and specific assay that could be used for the detection of C. abortus in infected tissue samples. We recommend the use of this assay as a supplemental diagnostic tool for detection of C. abortus in infected tissue samples.

Awan, A. R., M. Baxi, et al. (1995). "EHV 1-induced abortion in mice and its relationship to stage of gestation." Research in Veterinary Science **59**(2): 139.

<http://www.sciencedirect.com/science/article/B6WWR-4CWRXMS-1S/2/db52f8ccd300a9478561256682d0142f>

The most important consequence of equine herpesvirus-1 (-1) infection is abortion. The object of the present study was to characterise further a murine -1 abortion model and to make comparisons with the natural host with particular reference to the stage of gestation during which the infection occurs. /c mice at different stages of pregnancy were infected intranasally with -1 (strain A134); they suffered respiratory distress, weight loss, and other constitutional signs of infection. When the virus was inoculated in the late second or early third week of gestation dead or dying fetuses were aborted, whereas infection between seven and nine days of pregnancy led

to fetal death and resorption. During the process of resorption, complications were observed. Virus was frequently isolated from the placentas and occasionally from the tissues of the aborting fetuses, depending on the severity of the infection of the placentas. In some cases, therefore, the inoculation resulted in abortion although the infection was restricted to the placenta. Virus antigen was detected in the placentas, lungs and occasionally in other tissues of the aborting fetuses. The potential of this murine model for testing methods for the diagnosis and control of equine abortion is discussed.

Bashiruddin, J. B., P. de Santis, et al. (2005). "Detection of *Mycoplasma mycoides* subspecies *mycoides* SC in bovine lung and lymph node tissues by culture, sandwich ELISA and polymerase chain reaction systems." Research in Veterinary Science **78**(3): 199.

<http://www.sciencedirect.com/science/article/B6WWR-4DTKYFF-2/2/0f0eea5bfe5de3998c699c64f39b486e>

Cattle from Northern Portugal, many with pulmonary lesions typical of contagious bovine pleuropneumonia, were investigated for the presence of *Mycoplasma mycoides* subspecies *mycoides* small colony (MmmSC), which is the causative agent of CBPP, with several detection tests. Sandwich ELISA that included a culture enrichment stage, and 2 different PCR diagnostic systems were used to detect MmmSC in lung and mediastinal lymph node tissues from these animals. The comparison of typical CBPP pathology with the results of detection revealed that no single one of these methods provided a perfect match to the pathological data. Best performing tests were the PCR with laser induced fluorescence and PCR with pleuroTRAP kit (Chemicon, Australia), which are diagnostic systems based on amplification of genomic MmmSC DNA followed by sensitive detection of the amplified products. These were followed by the broth-enriched sandwich ELISA, which uses a monoclonal antibody specific to the *M. mycoides* cluster, to capture the antigen.

Bucher, K., G. Szalai, et al. (1996). "Tumour suppressor gene p53 in the horse: identification, cloning, sequencing and a possible role in the pathogenesis of equine sarcoid." Research in Veterinary Science **61**(2): 114.

<http://www.sciencedirect.com/science/article/B6WWR-4D8W9G1-V/2/80646178c1bdb5a3c3dd257872096696>

The tumour suppressor protein p53 enhances the genetic stability of the cell and plays a critical role in tumour suppression. Equine p53 was analysed by sequencing exons 5 to 9, a region which includes most known mutations and all the mutational hotspots in the species that have been investigated. The fragment was amplified, cloned and sequenced from genomic and complementary DNA. A comparison of the predicted amino acid sequences between the horse and other species resulted in identities between 66 per cent with the clawed frog and 92 per cent with the cat. Using the single strand conformation polymorphism technique, exons 5 to 8 amplified from sarcoid tissue and peripheral leucocytes of 28 sarcoid-affected and 11 healthy horses were screened for mutations. No mutations were identified, suggesting that the frequency of p53 mutations in equine sarcoid might be low. However, the high incidence of bovine papillomavirus infection in equine sarcoid may indicate the functional inactivation of p53 by - encoded E6 protein.

Davidson, A. J., J. E. Hodgkinson, et al. (2005). "Cytokine responses to Cyathostominae larvae in the

equine large intestinal wall." Research in Veterinary Science **78**(2): 169.

<http://www.sciencedirect.com/science/article/B6WWR-4D7K29M-1/2/60a6ad3c68e341cdab3a0a6c3ee3d827>

To investigate cytokine responses in cyathostomin infection, we quantified mucosal interleukin-4 (IL-4), interleukin-10 (IL-10), tumour necrosis factor (TNF)-[alpha] and interferon (IFN)-[gamma] by reverse transcriptase-competitive polymerase chain reaction. The analysis was performed on large intestinal wall samples obtained from six anatomical sites spanning the caecum and colon of 17 naturally exposed horses. The numbers of developing larvae (DL) and early third stage larvae (EL3) were ascertained using transmural illumination and pepsin digestion techniques, respectively. Levels of each cytokine transcript were correlated with local intestinal wall burdens of Cyathostominae larvae. IL-4 and IL-10 levels showed significant correlations with EL3 and DL burdens at several sites. No significant correlations were observed with IFN[gamma]. A pro-inflammatory response, typified by detection of TNF[alpha] transcript, was observed at a few sites in some horses with inflammatory enteropathy associated with emerging or emerged larvae. However, this cytokine was measured at an insufficient number of sites to enable statistical analysis. Levels of IL-4, IL-10 and IFN[gamma] transcript were compared between two groups: one group consisting of horses with low to high mucosal burdens (Group A) and the other, of horses with negative/negligible mucosal burdens (Group B). Significant differences in IL-4 ($P < 0.001$) transcript levels were observed between the groups, with higher levels observed in Group A. No significant differences in IFN[gamma] were observed. Taken together, these results indicate that Th2 responses predominate in mucosal Cyathostominae infection prior to larval reactivation.

Johansson, K. E., B. Pettersson, et al. (1995). "Identification of the causative agent of granulocytic ehrlichiosis in Swedish dogs and horses by direct solid phase sequencing of PCR products from the 16S rRNA gene." Research in Veterinary Science **58**(2): 109.

<http://www.sciencedirect.com/science/article/B6WWR-4CWRXPY-27/2/f0b32412d18efb87350857aea17eb9ea>

Seven Swedish isolates of Ehrlichia species from the blood of four dogs and three horses with clinical granulocytic ehrlichiosis, were identified by direct solid phase DNA sequencing of polymerase chain reaction (PCR) products from the 16S rRNA gene. The amplified DNA fragments were produced with primers complementary to the universal regions, U1, U2, U5 and U8 of the 16S rRNA molecule. Identical sequences were obtained from all seven isolates. This nucleotide sequence was similar to the sequences deposited in GenBank for Ehrlichia phagocytophila and E. equi. The sequence of the Swedish ehrlichiae differed in two nucleotide positions from the E. phagocytophila sequence and in three positions from the E. equi sequence, and it is tentatively proposed that it is a subspecies of one of these two. The alignment of the sequence of the Swedish isolates with a recently deposited sequence from human cases of ehrlichiosis in the USA revealed 100 per cent identity in a segment of about 1400 bp.

Laamanen, U. I., E. P. Neuvonen, et al. (1997). "Comparison of assay and virus isolation in cell cultures for the detection of bovine viral diarrhoea virus in field samples." Research in Veterinary Science **63**(3): 199.

<http://www.sciencedirect.com/science/article/B6WWR-4CWRYJJ-BC/2/a4d7ef34cfb1f42b3e7e343f110e0244>

The virus isolation-immunoperoxidase test on cell cultures and the reverse transcription-polymerase chain reaction assay were compared for the detection of bovine viral diarrhoea virus directly in serum samples. Material for this study consisted of 403 sera originating from cattle in 41 -infected Finnish dairy herds and one suckler cow herd. The presence of virus was demonstrated in 48 samples by both assays. In addition, two more samples were found to be positive by the assay. Both methods proved to be extremely sensitive, detecting pestiviruses even in high serum dilutions, and thus to be suitable for demonstrating the occurrence of persistently infected cattle. In conclusion, the method used had the advantage of ascertaining nucleic acid sequences in samples in which the virus had been inactivated, eg during transport or due to the presence of neutralising antibodies.

Respiration Physiology (1)

Okabe, S., M. Mackiewicz, et al. (1997). "Serotonin receptor mRNA expression in the hypoglossal motor nucleus." Respiration Physiology **110**(2-3): 151.

<http://www.sciencedirect.com/science/article/B6T3J-3RSFWSR-8/2/1675acece557c33cefd7b3f1b960e27b>

Brainstem serotonin (5-HT)-containing cells are remarkable for their widespread axonal projections and having their highest activity during wakefulness and lowest during rapid eye movement sleep. One important site of action of 5-HT is on upper airway motoneurons. However, which of the 14 known 5-HT receptors mediate the effects is uncertain. We used the reverse transcriptase/polymerase chain reaction to detect mRNA for six distinct 5-HT receptors (1A, 1B, 2A, 2C, 3 and 7) in 50 nl micro-punches collected from the hypoglossal (XII) motor nucleus and, for comparison, from the viscerosensory nucleus of the solitary tract (NTS) in adult rats. The relative abundance of the distinct mRNAs was characterized by the minimal number of amplification cycles (25-40) necessary to detect a given mRNA. In the XII nucleus, mRNA for type 1B, 2A and 2C receptors was detectable after 29-31 cycles, detection of type 3 and 7 receptor mRNA required 33-35 cycles; and type 1A receptor mRNA was not detected. In the NTS, detection of mRNA for type 1B, 2C and 7 receptors required 31-33 cycles; type 1A receptor mRNA required 39 cycles; and type 2A receptor mRNA was not detected. The data from the XII nucleus demonstrate that not only the previously recognized type 1B, 2A and 2C receptors, but also type 3 and 7 receptors have the potential to mediate serotonergic effects in XII motoneurons.

Respiratory Medicine (2)

Bjerke, T., M. Gaustadnes, et al. (1996). "Human blood eosinophils produce and secrete interleukin 4." Respiratory Medicine **90**(5): 271.

<http://www.sciencedirect.com/science/article/B6WWS-4CDJ15V-2/2/19320bdfd9b2c4d9546cd3d7346d6453>

Interleukin 4 (IL-4) is an immunoregulatory cytokine which induces T-cell proliferation and differentiation into a Th2 phenotype, and is of particular importance for the induction of IgE synthesis. In the present study, the capability of human peripheral blood eosinophils from allergic and non-allergic donors to produce IL-4 was examined. Using reverse transcribed polymerase chain reaction (RT-PCR), it was shown that highly purified eosinophils from allergic patients express mRNA for IL-4. Resting eosinophils also gave specific immunoreactivity with anti-IL-4 antibodies, consistent with translation of IL-4 mRNA. Light and electron microscopic immunocytochemistry revealed that IL-4 was prestored in the eosinophilic granules. These results were confirmed by IL-4 specific ELISA which showed that IL-4 production could be upregulated in the eosinophils and released from the eosinophils following stimulation with the calcium ionophore A23187. These data indicate that eosinophils may be an important source of IL-4 at sites of allergic inflammation. Thus, eosinophils may act as immunomodulatory cells enhancing the allergic response through formation of Th2-cells and inducing the isotype switching to IgE in human B-cells.

Miyashita, N., A. Saito, et al. (2004). "Multiplex PCR for the simultaneous detection of Chlamydia pneumoniae, Mycoplasma pneumoniae and Legionella pneumophila in community-acquired pneumonia." *Respiratory Medicine* **98**(6): 542.

<http://www.sciencedirect.com/science/article/B6WWS-4BKN11H-1/2/1c543489788fe1a5d2b3e8c6b7cc7265>

A multiplex polymerase chain reaction (PCR) was developed for the simultaneous detection of Chlamydia pneumoniae, Mycoplasma pneumoniae and Legionella pneumophila. Oligonucleotide primers for the amplification of the DNA of these three organisms were optimized for use in combination in the same reaction. PCR products were detected by the Micro-Chip Electrophoresis Analysis System. Clinical samples were obtained from 208 community-acquired pneumonia (CAP) patients who were participants in a multicenter CAP surveillance study performed at seven medical schools and their affiliate hospitals in Japan. No significant differences in the sensitivity of each primer set were observed when tested in both the multiplex and monoplex PCR assays. Our multiplex PCR was able to reliably detect 10 copies/100 [μl] of each of the three pathogen DNAs. Of the panel of 208 samples, 14 of 15 C. pneumoniae, 10 of 10 M. pneumoniae, eight of eight L. pneumophila and 165 of 176 negative samples were correctly identified. Eleven cases who were the multiplex PCR positive and conventional method negative were observed. The PCR findings were of possible significance in at least four of these patients. Our multiplex PCR assay could potentially be used as a diagnostic and epidemiological tool. Further prospective studies are needed to establish its clinical usefulness.

Reviews in Molecular Biotechnology (1)

Lovatt, A. (2002). "Applications of quantitative PCR in the biosafety and genetic stability assessment of biotechnology products." *Reviews in Molecular Biotechnology* **82**(3): 279.

<http://www.sciencedirect.com/science/article/B6VR0-44VW8G-8/2/70bb7db05733e41574524ec8dffa285>

High throughput screening, increased accuracy and the coupling of real-time quantitative PCR

(Q-PCR) to robotic set-up systems are beginning to revolutionise biotechnology. Applications of Q-PCR within biotechnology are discussed with particular emphasis on the following areas of biosafety and genetic stability testing: (a) determination of the biodistribution of gene therapy vectors in animals; (b) quantification of the residual DNA in final product therapeutics; (c) detection of viral and bacterial nucleic acid in contaminated cell banks and final products; (d) quantification of the level of virus removal in process validation viral clearance studies; (e) specific detection of retroviral RT activity in vaccines with high sensitivity; and (f) transgene copy number determination for monitoring genetic stability during production. Methods employed for Q-PCR assay validation as required in ICH Topic Q2A Validation of Analytical Methods: Definitions and Terminology (1st June 1995) are also reviewed.

Revue Francaise des Laboratoires (1)

Decre, D., B. Gachot, et al. (2000). "Surveillance epidemiologique des souches de *Klebsiella pneumoniae* productrices de [beta]-lactamase a spectre etendu (KpBLSE) dans un service de reanimation." Revue Francaise des Laboratoires **2000**(320): 31.

<http://www.sciencedirect.com/science/article/B6VRF-46SVDDDB-9T/2/ed22165554b2e41f0a5b621ff1a7a462>

ResumeL'epidemiologie des souches de *Klebsiella pneumoniae* productrices de [beta]-lactamase a spectre etendu (KpBLSE), isolees durant une periode de 16 mois dans un service de reanimation, a ete etudiee. Un programme associant le renforcement des mesures d'isolement, le depistage systematique des patients a l'admission puis une fois par semaine et une decontamination digestive (DDS) a ete instaure en 1992. Afin d'analyser les transmissions croisees, differents marqueurs phenotypiques et genotypiques (contenu plasmidique, profils d'ADN total restreint par electrophorese en champ pulse, rep-PCR) ont ete utilises pour comparer 138 souches de KpBLSE isolees chez 64 patients. L'incidence des colonisations et/ou des infections etait de 11,9%. Cinquante cinq cas ont ete consideres comme acquis dans l'unite et 9 cas ont ete importes. Parmi les 45 infections observees chez 32 patients, les infections urinaires ont ete les plus frequentes. L'utilisation d'une DDS n'a pas permis de reduire l'acquisition de KpBLSE. L'utilisation combinee de plusieurs marqueurs s'est averee necessaire pour la differenciation des souches. Un clone producteur de [beta]-lactamase de type SHV-4 a ete a l'origine de 85% des cas acquis dans l'unite. Des cas sporadiques lies a des souches de KpBLSE productrices de [beta]-lactamases variees (TEM-3, SHV-2, SHV-3 et SHV-5) ont ete observes.

Rheumatology (5)

Bayley, J. P., A. M. Bakker, et al. (2003). "Association of polymorphisms of the tumour necrosis factor receptors I and II and rheumatoid arthritis." Rheumatology **42**(8): 969-971.

<http://rheumatology.oupjournals.org/cgi/content/abstract/42/8/969>

Objective. To assess the role of polymorphisms of the tumour necrosis factor (TNF) receptors, TNF-RI (p55) and TNF-RII (p75) in the susceptibility to and severity of rheumatoid arthritis (RA) in Dutch patients. **Methods.** A total of 319 consecutive RA patients, and a cohort of 90 female RA patients with detailed 12-yr follow-up were genotyped for the TNF-RI exon 1 (+36 A to G) and TNF-RII 3' UTR (+1690 T to C) polymorphisms. **Results.** The frequencies of the TNF-RI and TNF-RII polymorphisms were determined in both patient groups and healthy controls, but no significant differences were found. To determine the relationship of these polymorphisms to disease severity, the extent of joint damage in the cohort of 90 female RA patients was analysed. No differences in severity were observed. **Conclusion.** These TNF-RI and TNF-RII polymorphisms were not found to be associated with susceptibility to or severity of RA in the Dutch population.

Helweg-Larsen, J., B. Tarp, et al. (2002). "No evidence of parvovirus B19, Chlamydia pneumoniae or human herpes virus infection in temporal artery biopsies in patients with giant cell arteritis." Rheumatology **41**(4): 445-449.

<http://rheumatology.oupjournals.org/cgi/content/abstract/41/4/445>

Objectives. Recent studies have suggested that infective agents may be involved in the pathogenesis of giant cell arteritis (GCA), in particular Chlamydia pneumoniae and parvovirus B19. We investigated temporal arteries from patients with GCA for these infections as well as human herpes viruses using the polymerase chain reaction (PCR). **Methods.** Thirty temporal artery biopsies from 30 patients suspected of having GCA within a period of 1 yr were examined. Thirteen patients had classical GCA, two had biopsy-negative GCA, 10 patients had polymyalgia rheumatica and five patients had other conditions. DNA was extracted from frozen biopsies and PCR was used to amplify genes from Chlamydia pneumoniae, parvovirus B19 and each of the eight human herpes viruses: herpes simplex viruses HSV-1 and 2, Epstein-Barr virus, cytomegalovirus, varicella zoster virus and human herpes viruses HHV-6, -7 and -8. **Results.** In all 30 biopsies, PCR was negative for DNAs of parvovirus B19, each of the eight human herpes viruses and C. pneumoniae. **Conclusions.** We found no evidence of DNA from parvovirus B19, human herpes virus or C. pneumoniae in any of the temporal arteries. These agents do not seem to play a unique or dominant role in the pathogenesis of GCA.

Ida, H., E. Kawasaki, et al. (2004). "A novel mutation (T61I) in the gene encoding tumour necrosis factor receptor superfamily 1A (TNFRSF1A) in a Japanese patient with tumour necrosis factor receptor-associated periodic syndrome (TRAPS) associated with systemic lupus erythematosus." Rheumatology **43**(10): 1292-1299.

<http://rheumatology.oupjournals.org/cgi/content/abstract/43/10/1292>

Objective. To identify potential mutations in the tumour necrosis factor receptor superfamily 1A gene (TNFRSF1A) in a Japanese female patient with recurrent fever complicated by systemic lupus erythematosus (SLE), and in her family members. **Methods.** DNA sequencing of exons 1-10 of the TNFRSF1A gene was performed to determine mutations that might be associated with the tumour necrosis factor receptor-associated periodic syndrome (TRAPS). Moreover, the TNFRSF1A gene was examined in Japanese patients with autoimmune diseases, including SLE, rheumatoid arthritis (RA), mixed connective tissue disease (MCTD) and Behcet's disease, and in healthy Japanese controls. Enzyme-amplified sensitivity immunoassay (EASIA) analysis was used to assess serum levels of TNF, the 55-kDa TNF receptor (TNFRSF1A) and the 75-kDa TNF receptor (TNFRSF1B). Membrane TNFRSF1A expression was analysed on the surface of peripheral blood mononuclear cells by flow cytometry. **Results.** A novel mutation, a heterozygous C to T transition in exon 3 which substitutes an isoleucine for a threonine at position 61 (T61I) was detected in the TNFRSF1A gene derived from the genomic DNA of a Japanese female

TRAPS patient. Two nieces and one nephew, all with a similar clinical phenotype, also possessed the same TNFRSF1A mutation. We further demonstrated the same mutation in five of 60 SLE patients (8.3%) and in five of 120 healthy individuals (4.2%), with no significant differences. Although high titres of serum TNF and soluble TNFRSF1B protein were observed in this patient, low titres of soluble TNFRSF1A protein were detected. However, a defect in TNFRSF1A shedding in vitro was not observed in monocytes derived from this patient. Conclusion. This is the first report of a TRAPS patient associated with SLE with a novel TNFRSF1A mutation (T61I).

Koyama, T., H. Tsukamoto, et al. (2003). "A novel polymorphism of the human APRIL gene is associated with systemic lupus erythematosus." *Rheumatology* **42**(8): 980-985.

<http://rheumatology.oupjournals.org/cgi/content/abstract/42/8/980>

Objective. We investigated the association of gene polymorphisms in APRIL, a new member of the TNF family, with systemic lupus erythematosus. **Methods.** To detect polymorphisms of the human APRIL gene by exon-specific polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) analysis, we first determined the structure of the human APRIL gene. We designed exon-specific oligonucleotide primers according to the genomic DNA sequence of APRIL. All of the coding regions in exons of the APRIL gene were analysed by exon-specific PCR-SSCP in 148 SLE patients and 146 unaffected controls, then the nucleotide sequences of exons that displayed aberrant bands were determined. **Results.** The human APRIL gene comprised at least six exons with five introns, spanning approximately 2.8 kilobases of the genomic DNA. By exon-specific PCR-SSCP, we identified two novel polymorphisms at codons 67 and 96. Both had amino acid substitutions: G67R and N96S respectively. Only the 67G allele was associated with SLE in 148 Japanese SLE patients, with allele frequency 0.662 compared with 0.575 for 146 unaffected controls ($P=0.0302$). The frequency of the individuals who possessed at least one 67G allele in SLE patients (91.9%) was significantly higher than that in the unaffected controls (80.1%) ($P=0.0036$). **Conclusion.** The 67G allele of APRIL may be a contributing factor in the pathogenesis of SLE.

Schotte, H., M. Gaubitz, et al. (2004). "Interleukin-10 promoter microsatellite polymorphisms in systemic lupus erythematosus: association with the anti-Sm immune response." *Rheumatology* **43**(11): 1357-1363.

<http://rheumatology.oupjournals.org/cgi/content/abstract/43/11/1357>

Objectives. Overproduction of interleukin-10 (IL-10) is a pivotal feature in the pathophysiology of systemic lupus erythematosus (SLE). In vitro IL-10 secretion has previously been related to haplotypes of the IL-10 promoter microsatellite polymorphisms IL10.R and IL10.G. Published data concerning the association of IL10.G alleles with susceptibility to SLE are inconsistent in different ethnic populations. We analysed the association of IL-10 promoter microsatellite polymorphisms with disease susceptibility and manifestations in German Caucasian patients with SLE. **Methods.** Two hundred and ten (210) SLE patients fulfilling the 1997 revised ACR criteria and 158 ethnically, age- and sex-matched healthy controls were genotyped for the IL-10 promoter microsatellite polymorphisms by fragment length analysis. Haplotypes were reconstructed using a Bayesian coalescent theory-based method with PHASE software. Allele and haplotype distributions were compared between patients and controls and between subgroups of patients with different clinical and immunopathological findings. **Results.** In the study population no significant associations of individual IL10.R and G alleles or their haplotypes with susceptibility to SLE or major clinical manifestations were observed. By contrast, alleles G14 and G15 and haplotypes R2-G14 and R2-G15 were significantly over-represented in anti-Sm antibody-positive patients. **Conclusions.** The IL-10 promoter microsatellite polymorphisms and their haplotypes do

not constitute a major risk factor for SLE in German Caucasians. However, the identification of genetic markers such as the IL-10 high-response haplotype R2-G14 predisposing for the production of anti-Sm antibodies may help to elucidate the conditions that lead to the development of SLE.

RNA (3)

Bertrand, C., M. F. Prere, et al. (2002). "Influence of the stacking potential of the base 3' of tandem shift codons on -1 ribosomal frameshifting used for gene expression." *RNA* **8**(1): 16-28.

<http://www.rnajournal.org/cgi/content/abstract/8/1/16>

Bolton, E. C., C. Coombes, et al. (2005). "Identification and characterization of critical cis-acting sequences within the yeast Ty1 retrotransposon." *RNA* **11**(3): 308-322.

<http://www.rnajournal.org/cgi/content/abstract/11/3/308>

The yeast long terminal repeat (LTR) retrotransposon Ty1, like retroviruses, encodes a terminally redundant RNA, which is packaged into virus-like particles (VLPs) and is converted to a DNA copy by the process of reverse transcription. Mutations predicted to interfere with the priming events during reverse transcription and hence inhibit replication are known to dramatically decrease transposition of Ty1. However, additional cis-acting sequences responsible for Ty1 replication and RNA dimerization and packaging have remained elusive. Here we describe a modular mini-Ty1 element encoding the minimal sequence that can be retrotransposed by the Ty1 proteins, supplied in trans by a helper construct. Using a mutagenic screening strategy, we recovered transposition-deficient modular mini-Ty1-HIS3 elements with mutations in sequences required in cis for Ty1 replication and integration. Two distinct clusters of mutations mapped near the 5'-end of the Ty1 RNA. The clusters define a GAGGAGA sequence at the extreme 5'-end of the Ty1 transcript and a complementary downstream UCUCUC sequence, 264 nt into the RNA. Disruption of the reverse complementarity of these two sequences decreased transposition and restoration of complementarity rescued transposition to wild-type levels. Ty1 cDNA was reduced in cells expressing RNAs with mutations in either of these short sequences, despite nearly normal levels of Ty1 RNA and VLPs. Our results suggest that the intramolecular interaction between the 5'-GAGGAGA and UCUCUC sequences stabilizes an RNA structure required for efficient initiation of reverse transcription.

Khabar, K. S. A., M. Dhalla, et al. (2004). "Selection of AU-rich transiently expressed sequences: Reversal of cDNA abundance." *RNA* **10**(4): 747-753.

<http://www.rnajournal.org/cgi/content/abstract/10/4/747>

Study of early and transient response gene expression is important for understanding the mechanisms of response to growth stimuli and exogenous agents such as microbes, stress, and radiation. Many of the cytokines, proto-oncogenes, and other transiently expressed gene products are encoded by mRNAs that contain AU-rich elements (AREs) in their 3' untranslated

regions (UTRs). In this article, we describe an approach to selectively synthesize ARE-containing cDNA (ARE-cDNA) using an innovative combination of culture treatment, thermostabilization of reverse transcriptase (RT) by the disaccharide trehalose, and use of optimized ARE-specific oligomers. The monocytic cell line, THP-1, was treated with cycloheximide and endotoxin to enrich for ARE-mediated gene expression followed by the RT procedure. Selection of ARE-cDNA with simultaneous suppression of abundant cDNA was made possible using the procedure as monitored by the preferential expression of IL-8, an ARE-cDNA molecule, over the abundant housekeeping cDNA, {beta}-actin. The use of trehalose dramatically reversed cDNA abundance, resulting in almost complete suppression of housekeeping cDNA. Finally, construction of specialized ARE-cDNA libraries confirmed the selectivity of ARE-cDNAs and the presence of rare genes. The ability to reverse the abundance of housekeeping and other highly expressed genes toward ARE genes facilitates the discovery and study of rare early response and transiently expressed genes.

Schizophrenia Research (6)

Chen, Q.-Y., Q. Chen, et al. (2005). "Case-control association study of the close homologue of L1 (CHL1) gene and schizophrenia in the Chinese population." Schizophrenia Research **73**(2-3): 269.

<http://www.sciencedirect.com/science/article/B6TC2-4CVX6Y2-1/2/47cf7fe62ff4557b67d675d0c2be1deb>

The close homolog of L1 (CHL1), located on human chromosome 3p26.1, is a newly identified member of the L1 family of cell adhesion molecules which play important roles in cell migration, axonal growth, and synaptic remodeling. A positive association has been reported between a missense polymorphism in CHL1 gene and schizophrenia in the Japanese population [Sakurai, K., Migita, O., Toru, M., Arinami, T., 2002. An association between a missense polymorphism in the close homologue of L1 (CHL1, CALL) gene and schizophrenia. *Mol. Psychiatry* **7**, 412-415]. An association between a missense polymorphism in the close homologue of L1 (CHL1, CALL) gene and schizophrenia. In order to test this finding, we genotyped four SNPs in the gene in the Han Chinese population using a sample of 560 cases and 576 controls. Analysis of allele frequencies in both samples also showed strong association between SNP rs2272522 (the same marker studied by K. Sakurai) and the disease ($X^2=31.591$, PCHL1 gene and schizophrenia and indicate that CHL1 may be involved in the etiology of schizophrenia.

Masellis, M., V. S. Basile, et al. (2001). "Lack of association between the T->C 267 serotonin 5-HT6 receptor gene (HTR6) polymorphism and prediction of response to clozapine in schizophrenia." Schizophrenia Research **47**(1): 49.

<http://www.sciencedirect.com/science/article/B6TC2-4233NBT-5/2/ac0a993e99569ff2de5f27e81e294b34>

Sanders, A. R., D. E. Rincon-Limas, et al. (1993). "Association between genetic variation at the porphobilinogen deaminase gene and schizophrenia." Schizophrenia Research **8**(3): 211.

<http://www.sciencedirect.com/science/article/B6TC2-45RCDJT-66/2/3e31a09c3e949aca5ec16098b4e5bd07>

There is growing evidence that some genetic predisposition is important in the etiology of schizophrenia. We have sought to implicate a major gene by performing a candidate gene association study comparing the allele frequencies of seven restriction fragment length polymorphisms (RFLPs) at six loci in both a psychiatrically normal control group (N = 51) and an affected (schizophrenia or schizoaffective disorder) group (N = 55). Each group comprised Caucasians of northern European origin. The candidate areas (D5S39, D5S78, dopamine receptor D2 (DRD2), D11S29, porphobilinogen deaminase (PBGD), and D11S84) were selected on the basis of prior cytogenetic findings in schizophrenics, linkage studies, and/or implicated gene products. The presence of a polymorphic ApaI site within the PBGD gene showed a significant association with the presence of illness ($P = 0.02$). The relative risk of possessing the allele with the ApaI site was 2.10. No significant association was found with any of the six other RFLPs. Our data suggests that either the PBGD gene itself or an unknown gene linked to and/or in linkage disequilibrium with the PBGD locus predisposes some individuals to schizophrenia. Independent replication of these findings will be required to determine their relevance to schizophrenia.

Sasaki, T., S. Kuwata, et al. (1994). "HLA-DR types in Japanese schizophrenics: analysis by group-specific PCR amplification." Schizophrenia Research **14**(1): 9.

<http://www.sciencedirect.com/science/article/B6TC2-46040G3-3/2/414019163a1b1f35cac7befc803f96a7>

An association of HLA-DR8 and DR1 with DSM-III schizophrenia has been reported in Japan (Miyanaga et al. (1984) Biol. Psychiatr. 19, 121-129). To further investigate this preliminary finding, we compared HLA-DR types in 44 unrelated Japanese schizophrenics (DSM-III-R) with those in 51 unrelated, healthy Japanese volunteers. Group-specific PCR amplification was used in the determination of HLA-DR in the patients. No significant difference was observed in the frequency of any DR types between patients and controls, after statistical correction for multiple testing. However, the frequency of DR1 in our patients (23%) and controls (10%) was almost the same as those in the previous report (22% vs. 10%), which means that there is a suggestive trend which could become significant if numbers were larger. It is argued that an exact determination of HLA-DR by DNA typing is important in current HLA studies of schizophrenia.

Tani, A., R. Kikuta, et al. (2002). "Polymorphism analysis of the upstream region of the human N-methyl--aspartate receptor subunit NR1 gene (GRIN1): implications for schizophrenia." Schizophrenia Research **58**(1): 83.

<http://www.sciencedirect.com/science/article/B6TC2-455VRG4-3/2/f7c402c7152e29e986bca22cd6ec90ad>

Dysfunction of the gene for the NR1 subunit of the N-methyl--aspartate (NMDA) receptor (GRIN1) has been implicated in the pathogenesis of schizophrenia. In support of this hypothesis are behavioral abnormalities reminiscent of schizophrenia in mice with an attenuated expression of the NR1 subunit receptor and the reduced level of NR1 mRNA in postmortem brains of patients with schizophrenia. We screened single nucleotide polymorphisms (SNPs) in the upstream region between +51 and -941 from the translation initiation codon of GRIN1 and identified 17 SNPs, 10 of which were located within the region containing the Sp1 motif and the GSG motifs. As genotyping of 191-196 Japanese patients with schizophrenia and 202-216 controls revealed no

significant association between schizophrenia and the SNPs in the upstream region of GRIN1, these SNPs apparently do not play a critical role in the pathogenesis of schizophrenia in the Japanese population.

Yang, Y. F., W. Qin, et al. "Possible association of the MAG locus with schizophrenia in a Chinese Han cohort of family trios." Schizophrenia Research **In Press, Corrected Proof**
<http://www.sciencedirect.com/science/article/B6TC2-4F1SVRK-1/2/0b391792f1f7da7cf1df6483a38b6e20>

Neurotransmitter-based hypotheses have so far led to only moderate success in predicting new pathogenetic findings in etiology of schizophrenia. On the other hand, the more recent oligodendroglia hypotheses of this disorder have been supported by an increasing body of evidence. For example, the expression level of the myelin associated glycoprotein (MAG) gene has been shown to be significantly lower in schizophrenia patient groups compared to control groups. Such an effect might be a result of genetic variations of the MAG gene. In order to test this hypothesis, we genotyped four markers within the MAG locus in 413 trios sample of the Han Chinese using allele-specific PCR. None of the four markers revealed noticeable allelic significance. However, the four-marker and two-marker haplotypes covering components rs720309 and rs720308 were observed to be significantly associated with schizophrenia (PP=0.0001). The results demonstrated MAG might play a role in genetic susceptibility to schizophrenia. Furthermore, our finding of a possible association between the MAG locus and schizophrenia is in agreement with the hypotheses of oligodendroglial and myelination dysfunction.

Science (2)

Barnes, I., P. Matheus, et al. (2002). "Dynamics of Pleistocene Population Extinctions in Beringian Brown Bears." Science **295**(5563): 2267-2270.

<http://www.sciencemag.org/cgi/content/abstract/295/5563/2267>

Krishnan, M., V. M. Ugaz, et al. (2002). "PCR in a Rayleigh-Benard Convection Cell." Science **298**(5594): 793-.

<http://www.sciencemag.org>

Seminars in Arthritis and Rheumatism (1)

Simonini, G., B. Porfirio, et al. (2004). "Lack of association between the HLA-DRB1 locus and post-streptococcal reactive arthritis and acute rheumatic fever in Italian children." Seminars in Arthritis

and Rheumatism **34**(2): 553.

<http://www.sciencedirect.com/science/article/B6WWX-4DKVCRG-9/2/3d014d9a50908cfdcefbcc85ce8c4fc1>

Objective Post-streptococcal reactive arthritis (PSReA) may be a variant of acute rheumatic fever (ARF), but there still is debate on the relationship between the 2 entities. Possible associations with HLA class II antigens of PSReA (DRB1*01) and ARF (DRB1*16) were described previously in white Americans. To confirm these findings, we studied DRB1 alleles in a group of Italian children with PSReA and ARF. Methods We performed low-resolution HLA-DRB1 typing by a sequence-specific primer polymerase chain reaction method in 33 children with PSReA and 25 children with ARF. We also compared the DRB1 genotypes of our patients with 200 normal subjects from the same geographic area and typed in the same laboratory with the same methods. Results The allele distributions at the DRB1 locus observed in PSReA patients, ARF patients, and controls were not significantly different from each other (chi-square test with small numbers, $P = .65$). The positivity for each of the 13 HLA-DRB1 alleles was compared in disease groups (PSReA and ARF) and controls, and failed to show any significant association. Comparisons of the frequency of the DRB1*01 allele among PSReA, ARF, and controls did not show any statistical differences. No significant difference in the frequency of DRB1*16 was present between ARF vs the control group, between ARF vs PSReA, and in PSReA patients when compared with controls. Conclusions Our data do not confirm in Italian patients the previously reported associations of DRB1*01 and DRB1*16 with PSReA and ARF, respectively.

Sensors and Actuators A: Physical (1)

El-Ali, J., I. R. Perch-Nielsen, et al. (2004). "Simulation and experimental validation of a SU-8 based PCR thermocycler chip with integrated heaters and temperature sensor." Sensors and Actuators A: Physical **110**(1-3): 3.

<http://www.sciencedirect.com/science/article/B6THG-4B3JT2Y-1/2/d3b62fa144d94b26897d69b0e27305da>

We present a SU-8 based polymerase chain reaction (PCR) chip with integrated platinum thin film heaters and temperature sensor. The device is fabricated in SU-8 on a glass substrate. The use of SU-8 provides a simple microfabrication process for the PCR chamber, controllable surface properties and can allow on chip integration to other SU-8 based functional elements. Finite element modeling (FEM) and experiments show that the temperature distribution in the PCR chamber is homogeneous and that the chip is capable of fast thermal cycling. With heating and cooling rates of up to 50 and 30 [deg]C/s, respectively, the performance of the chip is comparable with the best silicon micromachined PCR chips presented in the literature. The SU-8 chamber surface was found to be PCR compatible by amplification of yeast gene ribosomal protein S3 and *Campylobacter* gene cadF. The PCR compatibility of the chamber surfaces was enhanced by silanization.

Sensors and Actuators B: Chemical (3)

Liu, R. H., J. Bonanno, et al. (2004). "Single-use, thermally actuated paraffin valves for microfluidic applications." Sensors and Actuators B: Chemical **98**(2-3): 328.

<http://www.sciencedirect.com/science/article/B6THH-4BJ77MJ-7/2/32fa825241fe10a15aec5dee54473307>

A new thermally actuated valving concept using paraffin as single-use valving material was developed. The paraffin undergoes a phase transition in response to changes in temperature. A variety of single-use paraffin-based microvalves, including "close-open," "open-close-open," "T," and toggle designs, were demonstrated. Fluidic experiments showed that these microvalves had zero leakage and a maximum hold-up pressure of 40 psi in a "closed" position. A DNA polymerase chain reaction microdevice containing paraffin-based microvalves to enclose the sample solution in the reaction chamber during the thermal cycling was demonstrated. The paraffin-based microvalving technique has advantages over many existing active microvalve approaches, including a simple design, ease of fabrication, low cost, and ease of integration into complex microfluidic systems. Moreover, this technique is particularly attractive for single-use and disposable microfluidic devices.

Sethu, P. and C. H. Mastrangelo (2004). "Cast epoxy-based microfluidic systems and their application in biotechnology." Sensors and Actuators B: Chemical **98**(2-3): 337.

<http://www.sciencedirect.com/science/article/B6THH-4BHCMJ5-8/2/d8cafa127afa7cf1e607cc519ccd8039>

This paper reports the development of epoxy microcasting technologies for the fabrication of plastic microfluidic platforms. Two new techniques one involving embedding active silicon devices in plastic microsystems using a polymer flip chip process and another involving surface micromachining to build active components like actuators for use in applications like pumping are discussed and explained. Application devices were fabricated for polymerase chain reaction (PCR) and capillary electrophoresis (CE).

Sun, K., A. Yamaguchi, et al. (2002). "A heater-integrated transparent microchannel chip for continuous-flow PCR." Sensors and Actuators B: Chemical **84**(2-3): 283.

<http://www.sciencedirect.com/science/article/B6THH-4534CH9-1/2/a11eed4240d2fb663113e9e986293188>

A microchannel chip for continuous-flow polymerase chain reaction (PCR) was developed using transparent materials. The microchannel was fabricated on a quartz glass substrate using standard photolithography and wet-etching techniques and was sealed by another quartz glass substrate. Two indium-tin-oxide (ITO) films were deposited on the etched substrate as a thermal source. To confirm the temperature distribution in the microchannel, we measured the fluorescence spectra of an aqueous solution of 1-pyrenesulfonic acid sodium salt (PS-Na), which is a temperature-indicator dye, in the microchannel under a continuous solution flow. The results confirm that the temperature distribution on the microchannel's ITO films was almost uniform (within ± 2 [deg]C) under two flow rates (56 and 152 nl/min). The slightness of this deviation indicates that the ITO films integrated into the microchannel chip can be very useful as a thermal source for PCR. An amplification of a 450 bp segment of Escherichia coli HB101 was successfully performed by two-stage (94 and 67 [deg]C) thermal cycling on the chip device.

Serodiagnosis and Immunotherapy in Infectious Disease (2)

Chan, F. T. H., N. Stewart, et al. (1994). "Detection of verotoxin-producing *Escherichia coli* in stool specimens by the polymerase chain reaction." Serodiagnosis and Immunotherapy in Infectious Disease 6(4): 185.

<http://www.sciencedirect.com/science/article/B6TC4-4C82FVF-4J2/f0a4e01d90393a4ac1683e5cc5d4c260>

In this retrospective study a total of 404 stools kept at -70[deg]C were tested for the presence of verotoxin-producing *Escherichia coli* (VTEC) by the polymerase chain reaction (PCR). Thirteen positive samples from 11 patients were identified by PCR which correlated with previous isolation of *E. coli* O157:H7. There was no failure to detect VTEC by PCR but PCR did not identify further VTEC isolates. We concluded that (a) the occurrence of VTEC other than serotype O157:H7 is rare in our demographic area, (b) PCR is effective in the identification of *E. coli* O157:H7, and (c) PCR has the additional advantage over conventional culture methods of identifying VTEC, including sorbitol-fermenting serotypes, which might have been detected if we had extended our sample size.

Iwamoto, M., H. Koga, et al. (1995). "Detection of *Legionella* species by polymerase chain reaction." Serodiagnosis and Immunotherapy in Infectious Disease 7(3): 99.

<http://www.sciencedirect.com/science/article/B6TC4-40079HF-B/2/286da697c44561a29832bea389877318>

The nested polymerase chain reaction (PCR) was clinically investigated to detect the *mip* gene of *Legionella* species. PCR detected 20 clinically important *Legionella* species, such as *L. pneumophila*, *L. micdadei*, and *L. bozemanii*. Eight species of Gram-positive and -negative bacteria other than *Legionella* species were negative for PCR. The sensitivity of PCR determined by the detection limit of DNA quantity was 1.0 pg for the first PCR, but the sensitivity increased approximately 100-fold to 10 fg following the second PCR. The nested PCR was applied to detect *L. pneumophila* in the sputum and pleural effusion fluid obtained from a patient with Legionnaires' disease. Both fluids were PCR positive, but culture was negative for *L. pneumophila*. Our results indicated that the nested PCR may be a useful tool for the rapid detection of *L. pneumophila* and the clinical diagnosis of Legionnaires' disease.

Sex. Transm. Inf. (4)

Crucitti, T., E. Van Dyck, et al. (2003). "Comparison of culture and different PCR assays for detection of *Trichomonas vaginalis* in self collected vaginal swab specimens." Sex. Transm. Inf. 79(5): 393-398.

<http://sti.bmjjournals.com/cgi/content/abstract/79/5/393>

Objectives: DNA amplification techniques have become widely used for the diagnosis of sexually transmitted infections. For the detection of *Trichomonas vaginalis*, PCR techniques are not yet widely used despite the publication of several assays. The sensitivity and specificity of five independent primer sets were determined on self collected vaginal specimens obtained from female commercial sex workers. **Methods:** Self collected specimens were obtained from symptomatic and asymptomatic women attending a female sex workers clinic in Abidjan, Cote d'Ivoire. Two vaginal specimens were collected, the first one was processed for culture and the second was processed for PCR analysis. PCR techniques for trichomonads were performed, using the primers as reported by Riley (TVA5/TVA6), Kengne (TVK3/TVK7), Madico (BTUB 9/BTUB 2), Shiao (IP1/IP2), and Mayta (TV1/TV2). An EIA amplicon detection method was designed for each of the primer sets. **Results:** True positive specimens were defined as culture positive and/or two positive PCR results with EIA amplicon detection in any combination. According to this definition a prevalence of 20% was obtained compared to 7% obtained by culture. The PCR primer set TVK3/TVK7 gave the highest sensitivity (89.2%). Poor sensitivities were obtained with the primer sets TV1/TV2 (60.2%) and TVA5/TVA6 (63.9%). PCR showed a sensitivity improvement of 2.4% up to 12% when EIA was used for amplicon detection. **Conclusions:** Overall, the sensitivities of the different PCR assays resulting from this study were lower than those previously described. These findings could be the result of the nature of the specimen population and suggests a strain variability.

Lai, W., C. Y. Chen, et al. (2003). "Increasing relative prevalence of HSV-2 infection among men with genital ulcers from a mining community in South Africa." *Sex. Transm. Inf.* **79**(3): 202-207.

<http://sti.bmjjournals.com/cgi/content/abstract/79/3/202>

Objectives: To determine the aetiology of genital ulcer disease (GUD) and its association with HIV infection in the mining community of Carletonville, South Africa, from two cross sectional surveys of consecutive men presenting with genital lesions during October 1993 to January 1994 and July to November 1998. **Methods:** A multiplex polymerase chain reaction (M-PCR) assay combined with amplicon detection was used to identify DNA specific sequences of *Treponema pallidum*, herpes simplex virus (HSV), and *Haemophilus ducreyi*. A real time PCR assay was used to differentiate between HSV-1 and HSV-2. **Results:** M-PCR detected *T. pallidum*, HSV, and *H. ducreyi* in 10.3%, 17.2%, and 69.4% of 232 GUD patients during 1993-4 and in 12.4%, 36.0%, and 50.5% of 186 GUD patients in 1998. The proportion of patients with more than one agent increased significantly from 7.3% (17/232) in 1993-4 to 16.7% (31/186) in 1998 ($p < 0.01$). HSV-2 was detected in a higher proportion of ulcer specimens from HIV infected patients than in specimens from HIV uninfected patients during both time periods (1993-4: 26.2% v 6.7%, $p < 0.001$; 1998: 42.1% v 29.6%, $p > 0.09$). **Conclusions:** Based on two cross sectional surveys, 4 years apart, chancroid remained the leading cause of GUD in men who presented at the STD clinic with genital ulcers in the mining community of Carletonville, South Africa. The relative prevalence of primary syphilis has remained low. However, HSV-2 has emerged as a more significant cause of GUD and the proportion of GUD patients infected with more than one agent also increased significantly. HSV-2 DNA was detected in a significantly higher proportion of ulcer specimens from HIV positive patients than from HIV negative patients. No association was found between HIV infection status and the relative prevalence of chancroid or syphilis.

Molano, M., E. Weiderpass, et al. (2003). "Prevalence and determinants of *Chlamydia trachomatis* infections in women from Bogota, Colombia." *Sex. Transm. Inf.* **79**(6): 474-478.

<http://sti.bmjjournals.com/cgi/content/abstract/79/6/474>

Objectives: Chlamydia trachomatis infection in the cervix and uterus has been hypothesised to be a co-factor for cervical cancer. We performed a cross sectional study in Bogota, Colombia, where cervical cancer rates are high, to determine the prevalence and determinants of C trachomatis infection, and in particular its association with human papillomavirus (HPV). Methods: 1829 low income sexually active women were interviewed and tested for C trachomatis, using an endogenous plasmid PCR-EIA, and for 37 HPV types, using a general primer GP5+/6+ mediated PCR-EIA. Results: The overall prevalence of C trachomatis was 5.0%, and it did not differ substantially between women with normal (5.0%) and those with abnormal (5.2%) cervical cytology. Women infected with any HPV type (15.1%) had a slightly increased risk of being simultaneously infected with C trachomatis (adjusted OR 1.3, 95% CI: 0.8 to 2.4). This association was stronger when multiple HPV infections (adjusted OR 2.5, 95% CI: 1.1 to 5.9) were present. No other lifestyle or reproductive characteristics were clearly associated with risk of C trachomatis infection. Conclusions: HPV infected women, particularly women with multiple HPV infections, are at increased risk of being infected with C trachomatis.

Rodriguez, A. C., P. E. Castle, et al. (2003). "A population based study of herpes simplex virus 2 seroprevalence in rural Costa Rica." Sex. Transm. Inf. **79**(6): 460-465.

<http://sti.bmjjournals.com/cgi/content/abstract/79/6/460>

Objectives: To determine seroprevalence and determinants of herpes simplex virus 2 (HSV-2) seropositivity, in a random sample of a population based cohort of 10 049 women of Guanacaste, Costa Rica, using a highly sensitive and specific serological assay. Methods: Seroprevalence was determined by a type specific HSV-2 ELISA assay in an age stratified random sample of 1100 women. Univariate and multivariate logistic regression was used to calculate odds ratios and 95% confidence intervals for risk factors of seropositivity. Results: Overall age adjusted HSV-2 seroprevalence was 38.5% (95% CI, 37.5 to 39.5), and it was strongly associated with increasing age (pTrend<0.0001), both among monogamous women and women with multiple sexual partners. A greater number of lifetime sexual partners increased the risk of seropositivity, with a 28.2% (95% CI, 24.4 to 32.2) seroprevalence among monogamous women and 75% (95% CI, 65.6 to 83.0) seroprevalence for those with four or more partners (OR = 7.6 95% CI, 4.7 to 12.4 pTrend<0.0001). Barrier contraceptive use was negatively associated with HSV-2 seropositivity (OR 0.54, 95% CI, 0.31 to 0.94). Women with antibodies against HPV 16, 18, or 31 were 1.6 times more likely to be HSV-2 seropositive (OR 1.6, 95% CI, 1.2 to 2.1). Conclusions: HSV-2 infection is highly endemic in Guanacaste, even among lifetime monogamous women, suggesting a role of male behaviour in the transmission of the infection. Until vaccination against HSV-2 is available, education to prevent high risk sexual behaviour and the use of condoms appear as preventive measures against HSV-2.

Small Ruminant Research (1)

Travassos, C. E., C. Benoit, et al. (1999). "Caprine arthritis-encephalitis virus in semen of naturally infected bucks." Small Ruminant Research **32**(2): 101.

<http://www.sciencedirect.com/science/article/B6TC5-3W788FY-1/2/ee1f531a559e9c3fcc9850b55ffa516>

Semen and peripheral blood mononuclear cells (PBMC) samples of 15 infected and 3 non-infected bucks were evaluated for the presence of caprine arthritis-encephalitis virus (CAEV), by double-nested polymerase chain reaction (d-n-PCR). In order to locate the presence of virus, semen samples were separated into cell-free seminal fluids (CFSF), enriched non-spermatozoal cells fraction (NSCF), and spermatozoa (SPZ). All PBMC samples from infected bucks were positive for CAEV-DNA but only 8 out of 15 semen samples were positive for CAEV-DNA. The presence of virus was not necessarily concordant in different fractions of semen from the same buck over time. In addition, the virus was isolated from CFSF in primary goat synovial membrane (GSM) cell culture, in which productive infection was demonstrated by syncytia formation. This is the first report describing the presence of CAEV-DNA and also of replication-competent virus in semen from CAEV seropositive bucks. Such results suggest the possibility of sexual transmission of CAEV.

Stem Cells (6)

Greco, B., H. P. Low, et al. (2004). "Differentiation Prevents Assessment of Neural Stem Cell Pluripotency after Blastocyst Injection." Stem Cells **22**(4): 600-608.

<http://stemcells.alphamedpress.org/cgi/content/abstract/22/4/600>

Earlier studies reported that neural stem (NS) cells injected into blastocysts appeared to be pluripotent, differentiating into cells of all three germ layers. In this study, we followed in vitro green fluorescent protein (GFP)-labeled NS and embryonic stem (ES) cells injected into blastocysts. Forty-eight hours after injection, significantly fewer blastocysts contained GFP-NS cells than GFP-ES cells. By 96 hours, very few GFP-NS cells remained in blastocysts compared with ES cells. Moreover, 48 hours after injection, GFP-NS cells in blastocysts extended long cellular processes, ceased expressing the NS cell marker nestin, and instead expressed the astrocytic marker glial fibrillary acidic protein. GFP-ES cells in blastocysts remained morphologically undifferentiated, continuing to express the pluripotent marker stage-specific embryonic antigen-1. Selecting cells from the NS cell population that preferentially formed neurospheres for injection into blastocysts resulted in identical results. Consistent with this in vitro behavior, none of almost 80 mice resulting from NS cell-injected blastocysts replaced into recipient mothers were chimeric. These results strongly support the idea that NS cells cannot participate in chimera formation because of their rapid differentiation into glia-like cells. Thus, these results raise doubts concerning the pluripotency properties of NS cells.

Hay, D. C., L. Sutherland, et al. (2004). "Oct-4 Knockdown Induces Similar Patterns of Endoderm and Trophoblast Differentiation Markers in Human and Mouse Embryonic Stem Cells." Stem Cells **22**(2): 225-235.

<http://stemcells.alphamedpress.org/cgi/content/abstract/22/2/225>

The transcription factor Oct-4 is a marker of pluripotency in mouse and human embryonic stem (ES) cells. Previous studies using a tetracycline-regulated Oct-4 transgene in the ZHBTc4 cell line demonstrated that downregulation of Oct-4 expression induced dedifferentiation into trophoblast, a lineage mouse ES cells do not normally generate. We found that transfection of Oct-4-specific short interfering RNA significantly reduced expression and functional activity of

Oct-4 in mouse and human ES cells, enabling its role to be compared in both cell types. In mouse ES cells, Oct-4 knockdown produced a pattern of morphological differentiation and increase in expression of the trophoblast-associated transcription factor Cdx2, similar to that triggered by suppressing the Oct-4 transgene in the ZHBTc4 cell line. In addition, downregulation of Oct-4 was accompanied by increased expression of the endoderm-associated genes Gata6 and {alpha}-fetoprotein, and a gene trap associated with primitive liver/yolk sac differentiation. In human ES cells, Oct-4 knockdown also induced morphological differentiation coincident with the upregulation of Gata6. The induction of Cdx2 and other trophoblast-associated genes, however, was dependent on the culture conditions. These results establish the general requirement for Oct-4 in maintaining pluripotency in ES cells. Moreover, the upregulation of endoderm-associated markers in both mouse and human ES cells points to overlap between development of trophoblast and endoderm differentiation.

Kim, J.-A., Y.-J. Jung, et al. (2002). "Gene Expression Profile of Megakaryocytes from Human Cord Blood CD34+ Cells Ex Vivo Expanded by Thrombopoietin." Stem Cells **20**(5): 402-416.

<http://stemcells.alphamedpress.org/cgi/content/abstract/20/5/402>

Previously, we investigated the process of megakaryocytopoiesis during ex vivo expansion of human cord blood (CB) CD34+ cells using thrombopoietin (TPO) and found that megakaryocytopoiesis was closely associated with apoptosis. To understand megakaryocytopoiesis at the molecular level, we performed a microserial analysis of gene expression (microSAGE) in megakaryocytes (MKs) and nonmegakaryocytes (non-MKs) derived from human CB CD34+ cells by ex vivo expansion using TPO, and a total of 38,909 tags, representing 8,976 unique genes, were identified. In MKs, many of the known genes, including coagulation factor VII, P-selectin (CD62P), pim-1, azurocidin, defensin, and CD48 were highly expressed; meanwhile, those genes encoding some small G proteins of the Ras family (Rab 7 and Rab 11A) and glutathione S transferase family (1, 4, A2, omega, and pi) showed lower expression levels in MKs. These gene expression profiles will be useful to understand megakaryocytopoiesis at the molecular level, including apoptosis and related signal transduction pathways.

Tuli, R., S. Tuli, et al. (2003). "Characterization of Multipotential Mesenchymal Progenitor Cells Derived from Human Trabecular Bone." Stem Cells **21**(6): 681-693.

<http://stemcells.alphamedpress.org/cgi/content/abstract/21/6/681>

The in vitro culture of human trabecular bone-derived cells has served as a useful system for the investigation of the biology of osteoblasts. The recent discovery in our laboratory of the multilineage mesenchymal differentiation potential of cells derived from collagenase-treated human trabecular bone fragments has prompted further interest in view of the potential application of mesenchymal progenitor cells (MPCs) in the repair and regeneration of tissue damaged by disease or trauma. Similar to human MPCs derived from bone marrow, a clearer understanding of the variability associated with obtaining these bone-derived cells is required in order to optimize the design and execution of applicable studies. In this study, we have identified the presence of a CD73+, STRO-1+, CD105+, CD34-, CD45-, CD144- cell population resident within collagenase-treated, culture-processed bone fragments, which upon migration established a homogeneous population of MPCs. Additionally, we have introduced a system of culturing these MPCs that best supports and maintains their optimal differentiation potential during long-term culture expansion. When cultured as described, the trabecular bone-derived cells display stem cell-like capabilities, characterized by a stable undifferentiated phenotype as well as the ability to proliferate extensively while retaining the potential to differentiate along the osteoblastic,

adipocytic, and chondrocytic lineages, even when maintained in long-term in vitro culture.

Xu, C., E. Rosler, et al. (2005). "Basic Fibroblast Growth Factor Supports Undifferentiated Human Embryonic Stem Cell Growth Without Conditioned Medium." Stem Cells **23**(3): 315-323.

<http://stemcells.alphamedpress.org/cgi/content/abstract/23/3/315>

Previous studies have shown that prolonged propagation of undifferentiated human embryonic stem cells (hESCs) requires conditioned medium from mouse embryonic feeders (MEF-CM) as well as matrix components. Because hESCs express growth factor receptors, including those for basic fibroblast growth factor (bFGF), stem cell factor (SCF), and fetal liver tyrosine kinase-3 ligand (Flt3L), we evaluated these and other growth factors for their ability to maintain undifferentiated hESCs in the absence of conditioned medium. We found cultures maintained in bFGF alone or in combination with other factors showed characteristics similar to MEF-CM control cultures, including morphology, surface marker and transcription factor expression, telomerase activity, differentiation, and karyotypic stability. In contrast, cells in media containing Flt-3L, thrombopoietin, and SCF, individually or in combination, showed almost complete differentiation after 6 weeks in culture. These data demonstrate that hESCs can be maintained in nonconditioned medium using growth factors.

Yoshimoto, M., H. Chang, et al. (2005). "Two Different Roles of Purified CD45+ c-Kit+ Sca-1+ Lin- Cells After Transplantation in Muscles." Stem Cells **23**(5): 610-618.

<http://stemcells.alphamedpress.org/cgi/content/abstract/23/5/610>

Recent studies have indicated that bone marrow cells can regenerate damaged muscles and that they can adopt phenotypes of other cells by cell fusion. Our direct visualization system gave evidence of massive muscle regeneration by green fluorescent protein (GFP)-labeled CD45+c-Kit+Sca-1+Lin- cells (KSL cells), and we investigated the role of KSL cells in muscle regeneration after transplantation with or without lethal irradiation. In the early phase, GFP signals were clearly observed in all the muscles of only irradiated mice. Transverse cryostat sections showed GFP+ myosin+ muscle fibers, along with numerous GFP+ hematopoietic cells in damaged muscle. These phenomena were temporary, and GFP signals had dramatically reduced 30 days after transplantation. After 6 months, GFP+ fibers could hardly be detected, but GFP+ c-Met+ mononuclear cells were located beneath the basal lamina where satellite cells usually exist in both conditioned mice. Immunostaining of isolated single fibers revealed GFP+ PAX7+, GFP+ MyoD+, and GFP+ Myf5+ satellite-like cells on the fibers. Single-fiber cultures from these mice showed proliferation of GFP+ fibers. These results indicate two different roles of KSL cells: one leading to regeneration of damaged muscles in the early phase and the other to conversion into satellite cells in the late phase.

Steroids (11)

Colombe, L., A. Fostier, et al. (2000). "A mineralocorticoid-like receptor in the rainbow trout, *Oncorhynchus mykiss*: cloning and characterization of its steroid binding domain." Steroids **65**(6):

<http://www.sciencedirect.com/science/article/B6TC9-408BJK1-3/2/e6d52b0b231ccec7abab3d2b7f4d85e3>

Using reverse transcriptase polymerase chain reaction (PCR) (RT-PCR) with degenerate primers followed by 3' rapid amplification of cDNA ends PCR (3'Race-PCR) we have isolated a new fish steroid receptor cDNA sequence of 1806 bp from rainbow trout (*Oncorhynchus mykiss*) testis. This sequence has clear homology with various mineralocorticoid receptor cDNA sequences (rat, human, African toad: 68-70% amino acid identity), and encompasses the second part of DNA binding domain (C domain), the whole hinge region (D domain) and the steroid binding domain (E domain) plus 726 bp of 3'untranslated sequence. COS-1 cells transfected with a pCMV5 expression vector containing the whole E domain (pCMV5-rtMR) showed high affinity binding for cortisol ($K_a = 0.53 \pm 0.03$ nM, $K_d = 1.9$ nM) in the cytosol, which could not be detected in untransfected cells. Aldosterone displaced 3H-cortisol binding, though was less effective by than unlabeled cortisol (P 3H-cortisol from the receptor-ligand complex: cortisol = corticosterone = 11-deoxycortisol = 21-deoxycortisol > 11-deoxycorticosterone = 11[beta]-hydroxyprogesterone = 17-hydroxyprogesterone > dexamethasone, whereas 17,20[beta]-dihydroxy-4-pregnen-3-one and 17,20[beta],21[beta]-trihydroxy-4 pregnen-3-one (two fish specific progestins) did not show any specific binding. These results strongly suggest that this cDNA sequence encodes a rainbow trout mineralocorticoid-like receptor, and represent the first description of such a receptor in teleost fish where aldosterone, the classic mineralocorticoid, is believed to be absent.

Furuyama, N. and Y. Fujisawa (2000). "Regulation of collagenolytic cysteine protease synthesis by estrogen in osteoclasts." *Steroids* **65**(7): 371.

<http://www.sciencedirect.com/science/article/B6TC9-40PRGST-2/2/e05b558424e4ecfeed9a40bf577eef55>

In ovariectomized (Ovx) mice, collagenolytic cysteine protease (CCP) activity in calvaria significantly increased 7 days after ovariectomy and was about 50% of that observed in sham-operated (Sham) mice 3 weeks later. In Oxv mice, subcutaneously (s.c.) administered estradiol-17[beta] (E2) (10 [mu]g/kg) for 2 weeks led to a decrease in CCP activity in calvaria to the level observed in Sham mice. In Oxv mice, though the amount of cathepsin L increased more than that of cathepsin K, cathepsin K and cathepsin L content increased by 200-400% compared with the Sham mice; cathepsin K was detected in larger amounts than cathepsin L in calvaria from both Sham and Oxv mice. The amounts of cathepsin K and cathepsin L in Oxv mice were reduced to the values seen with Sham mice after administration (s.c.) of E2 (10 [mu]g/kg) for 2 weeks. In mouse calvarial organ culture, the increase of CCP activity and release of hydroxyproline, an indicator of degradation of type-I collagen, in the presence of 1[alpha],25-(OH)2D3, parathyroid hormone, interleukin (IL)-1[alpha], IL-6, or tumor necrosis factor-[alpha] was suppressed by E2 (10⁻⁹-10⁻⁷ M). In all cases, secretion of both cathepsin K and cathepsin L were suppressed by E2. In osteoclasts, expression of cathepsin K and cathepsin L was suppressed by E2 at the mRNA level. Cathepsin B was detected faintly or not at all. These results suggest that synthesis of cathepsin K and cathepsin L was negatively regulated by E2 at the mRNA level. In Oxv mice, deficiency of E2 resulted in an augmentation of cathepsin K and cathepsin L synthesis, and the cathepsins might share roles in bone resorption in vivo.

Kobayashi, N., M. Ohtoyo, et al. (2005). "Generation of a single-chain Fv fragment for the monitoring of deoxycholic acid residues anchored on endogenous proteins." *Steroids* **70**(4): 285.

<http://www.sciencedirect.com/science/article/B6TC9-4FM01J9-1/2/e34eda6b080db206ec6aaa0a5c92d9af>

A subset of lipophilic bile acids, including deoxycholic acid (DCA) and lithocholic acid (LCA), are thought to be biologically transformed into reactive intermediates forming covalently modified, "tissue-bound" bile acids that can exert several toxic effects. We have generated a single-chain Fv fragment (scFv) as a probe to monitor DCA residues anchored on proteins. DNA fragments encoding the variable heavy (VH) and light (VL) domains of a mouse antibody raised against a DCA hapten (Ab #88) were cloned by rapid amplification of cDNA 5'-ends. These sequences were combined via a common linker sequence coding (Gly4Ser)₃ to construct a single scFv gene with the gene segments in the following order: 5'-VH-linker-VL-3'. This construct was subcloned into an antibody-expression vector, pEXmid 5; soluble scFv protein was then expressed in the bacterial periplasm of the XL0LR Escherichia coli strain. In a competitive enzyme-linked immunosorbent assay using DCA-coated microtiter plates, the scFv provided a dose-response curve for free DCA ranging between 2 and 5000 pg/assay. The scFv reacts similarly with the L-lysine adduct of DCA (cross-reactivity, 72%), while bile acids having a modified DCA steroid skeleton were well-discriminated (cross-reactivity, <1%). This scFv could also monitor trace amounts of DCA residues anchored on a protein through DCA acyl adenylate reactions, the likely reactive intermediate. The present scFv may be a useful tool for trace characterization of tissue-bound bile acids; this usefulness may be significantly enhanced by fusion with signal-generating proteins, such as alkaline phosphatase or green fluorescent protein.

Kobayashi, N., K. Shibahara, et al. (2002). "Single-chain Fv fragments derived from an anti-11-deoxycortisol antibody: Affinity, specificity, and idiotype analysis." *Steroids* **67**(8): 733.

<http://www.sciencedirect.com/science/article/B6TC9-45V71TJ-1/2/ab91ec8ff35dc6c7b90a23957325a3a0>

Single-chain Fv fragments (scFvs) against a corticosteroid, 11-deoxycortisol (11-DC), have been generated as a template antibody fragment from which a comprehensive mutated antibody library containing various anti-steroid antibodies could be constructed. The cDNAs encoding variable heavy (VH) and light (VL) domains of a mouse anti-11-DC antibody (CET-M8), were amplified by RT-PCR, combined via a common linker to construct the sequence of 5'-VH-(Gly4Ser)₃-VL-3', and cloned into a phagemid vector, pEXmid 5. The phage clones exhibiting binding activity to 11-DC were isolated after single panning against a hapten-immobilizing immunotube. The scFv gene in one of these clones was reamplified to introduce the ochre codons, and then expressed in the bacterial periplasm as the soluble antibody fragment. Two different scFvs (#6 and #12) were cloned, whose binding characteristics were examined by a radioimmunoassay using a tritium-labeled 11-DC. Both of them showed high affinity ($K_a=1.3 \times 10^{10} \text{ M}^{-1}$) and practical specificity (cross-reactivity: cortisol, <0.2%; cortisone, <0.3%) to 11-DC, and furthermore, strong reactivity with an anti-idiotypic antibody which recognizes the paratope of CET-M8. These results suggest that the present scFvs retain the three-dimensional structure of the paratope of the original monoclonal antibody.

Koritnik, D. R., A. Koshy, et al. (1995). "17[β]-Estradiol treatment increases the levels of estrogen receptor and its mRNA in male rat liver." *Steroids* **60**(8): 519.

<http://www.sciencedirect.com/science/article/B6TC9-3YYV2WJ-1S/2/6857e3b1077c23d9bb8eefcc904f3c32>

This study examined estrogen receptor dynamics in the livers of male obese rats (SHHF/Mcc-cp)

treated for two weeks with a continuous, low dose of 17[beta]-estradiol compared with untreated controls. An increased binding capacity for tritiated 17[beta]-estradiol in the cytosol, consistent with binding to the estrogen receptor, was demonstrated in treated males relative to control males ($P < 0.01$). These observations were confirmed using curve-peeling techniques with saturation analysis, ammonium sulfate precipitation/fractionation of cytosol protein, and chromatographic techniques to isolate the high-affinity binding from other interfering factors. Increased hepatic nuclear estrogen receptor levels in treated males (112.3 ± 8.3 fmol/g liver) compared with controls (64.1 ± 6.8 fmol/g liver) suggested that the liver was under estrogenic influences. This interpretation was supported by an increase in serum triglyceride levels, reflecting increased very low density lipoprotein secretion by the liver. Reductions in testosterone levels and in the weights of seminal vesicles and the testes in treated males indicated detrimental effects on reproduction. An interpretation of increased synthesis of estrogen receptor with 17[beta]-estradiol treatment was supported by the observation of an increase in the mRNA for estrogen receptor. Taken together, these observations indicate that continuous, low-dose 17[beta]-estradiol treatment induces estrogenic action in the livers of male rats and also increases hepatic estrogen receptor, probably indirectly, via an increase in its mRNA.

Melner, M. H. and S. L. Young (1991). "Intracellular mechanisms of gonadotropin-stimulated gene expression in granulosa cells." *Steroids* **56**(5): 232.

<http://www.sciencedirect.com/science/article/B6TC9-47NVJXY-BX/2/f5d1e6c20bb05d412cd9a9e9f22600b6>

Previous studies have shown that the gonadotropins follicle-stimulating hormone and luteinizing hormone stimulate proopiomelanocortin (POMC) promoter activity and mRNA levels in ovarian granulosa cells. The objective of these studies was to determine the role of cAMP-dependent protein kinases (pKA) in gonadotropin-stimulated gene expression. Primary cultures of rat granulosa cells were transfected with a gene construct consisting of the POMC promoter (-150 to + 63; designated pOMC-CAT) fused to the chloramphenicol acetyltransferase (CAT) reporter gene either alone or cotransfected with an expression plasmid (designated mutant RI), which overexpresses a mutant form of the murine RI subunit incapable of binding cAMP and serving as an irreversible inhibitor of the catalytic subunit of pKA. Follicle-stimulating hormone or isoproterenol caused a significant stimulation of pOMC-CAT activity in transfected cells. Cotransfection of pOMC-CAT with mutant RI caused a significant inhibition of basal pOMC-CAT activity and abolished the gonadotropin stimulation. As a control, transfection of the SV-40 viral enhancer-promoter fused to CAT (pSV2-CAT) was unresponsive to follicle-stimulating hormone stimulation and cotransfection with mutant RI had no significant effect on pSV2-CAT activity. These studies suggest that gonadotropin regulation of the POMC promoter is mediated by pKA and that promoter activity is stringently controlled by pKA.

Misao, R., M. Hori, et al. (1994). "Corticosteroid-binding globulin mRNA levels in human uterine endometrium." *Steroids* **59**(10): 603.

<http://www.sciencedirect.com/science/article/B6TC9-47P835S-51/2/21f67397471816a510306938c857d03f>

Corticosteroid-binding globulin (CBG or transcortin) is a specific plasma glycoprotein, which binds steroid hormones (cortisol, corticosterone, and progesterone), and plays a role in transporting these steroids, altering their concentrations in blood, and influencing their biological actions. CBG has been previously shown to be synthesized in the liver, but recently it has been reported that immunoreactive CBG is localized in target tissues. In the present work, CBG mRNA was detected in normal human endometrial tissues by Northern blot analysis and reverse transcription-

polymerase chain reaction. Its level was higher (P P P 59:603-607, 1994)

Misao, R., Y. Nakanishi, et al. (1995). "Expression of corticosteroid-binding globulin mRNA in human uterine endometrial cancers." Steroids **60**(10): 720.

<http://www.sciencedirect.com/science/article/B6TC9-3YYV2TY-X/2/de9ba34021ac5971d5f1b3622b102a1b>

Since it has been demonstrated that corticosteroid-binding globulin (CBG) plays a role in intracellular steroidal actions in target cells, the expression of CBG mRNA as the measure of CBG expression was investigated in human endometrial cancers in order to assess the biological implications of CBG. The level of CBG mRNA was analyzed using competitive reverse transcription-polymerase chain reaction-Southern blot analysis. While the level of CBG mRNA was significantly ($P < 0.01$) higher in secretory phase endometrium than in early and late proliferative phase endometrium, the level of CBG mRNA tended to decrease with advanced dedifferentiation of endometrial cancers as compared to normal endometrium. These results suggest that dedifferentiation of endometrial cancers induces a reduction in intracellular CBG synthesis.

Roy, D., G. Calaf, et al. (2003). "Role of Vitamin D receptor gene in radiation-induced neoplastic transformation of human breast epithelial cell." Steroids **68**(7-8): 621.

<http://www.sciencedirect.com/science/article/B6TC9-493PDSB-5/2/df6fead2daeb37876879ae7985c10bf0>

1[alpha],25-(OH)₂-Vitamin D₃, the physiologically active metabolite of Vitamin D is known for its pro-differentiating and antiproliferative activity on various cancer cell lines. It exerts its growth-regulatory effects through binding to the Vitamin D receptor (VDR), a member of the steroid/thyroid/retinoic acid receptor family, which functions as a ligand-dependent transcription factor. There is accumulating evidence that Vitamin D may be an important determinant of both the occurrence and progression of breast cancer. Since radiation is an important etiological factor for breast cancer progression, it is important to study the role of VDR gene in radiation-induced breast carcinogenesis. This study is focused on a human breast tumor model developed by irradiating the spontaneously immortalized MCF-10F cell line with graded doses of high-linear energy transfer (LET) radiation followed by treatment with estrogen. Study of VDR gene by restriction digestion with Apal, BsmI and TaqI detected no polymorphism but direct sequencing analyses identified few single-base mutations within intron 8 and exon 9 of the gene. Over-expression of the VDR gene was noticed in irradiated and tumorigenic cell lines compared with control. Likewise, immunohistochemical data indicated a significant increase in VDR intensity in irradiated and tumorigenic cell lines. Considering all these evidence, it is likely that VDR can be used as a prognostic marker of tumor progression in radiation- and estrogen-induced breast carcinogenesis.

Schaaf, M. J. M. and J. A. Cidlowski (2002). "AUUUA motifs in the 3'UTR of human glucocorticoid receptor [alpha] and [beta] mRNA destabilize mRNA and decrease receptor protein expression." Steroids **67**(7): 627.

<http://www.sciencedirect.com/science/article/B6TC9-45PS0KP-D/2/b7dea4d695d0a0f3fdd5d263bbe96724>

An association between a gene polymorphism of the human glucocorticoid receptor (hGR) gene and rheumatoid arthritis has recently been suggested. This polymorphism contains an A to G mutation in the 3'UTR of exon 9[beta], which encodes the 3'UTR of the mRNA of the hGR[beta] isoform. The hGR[beta] isoform can act as a dominant negative inhibitor of hGR[alpha], and therefore may contribute to glucocorticoid resistance. The A to G mutation is located in an AUUUA motif, which is known to destabilize mRNA. In the present study, the importance of the mutation in this AUUUA motif was further characterized and mutations in other AUUUA motifs in the 3'UTR of hGR[beta] and hGR[alpha] mRNA were studied. hGR[beta] and hGR[alpha] expression vectors, carrying mutations in one AUUUA motif or all AUUUA motifs were transiently transfected into COS-1 cells. Each transfected vector was analyzed for the mRNA expression level, the mRNA turnover rate and the protein expression level. The naturally occurring mutation in the 3'UTR of hGR[beta] mRNA increased mRNA stability and protein expression. Mutation of two other AUUUA motifs in the 3'UTR of hGR[beta], or mutation of all four AUUUA motifs resulted in a similar effect. Mutation of the most 5' AUUUA motif did not alter hGR[beta] mRNA expression or mRNA stability. Mutation of all 10 AUUUA motifs in the 3'UTR of hGR[alpha] mRNA increased hGR[alpha] mRNA expression and mRNA stability as well as expression of the receptor protein level. Thus, the naturally occurring mutation in an AUUUA motif in the 3'UTR of hGR[beta] mRNA results not only in increased mRNA stability, but also in increased receptor protein expression, which may contribute to glucocorticoid resistance. A similar role is suggested for two other AUUUA motifs in the 3'UTR of hGR[beta] mRNA and for the 10 AUUUA motifs that are present in the 3'UTR of hGR[alpha].

Yoshimura, S., S. Sakamoto, et al. (2003). "Sex-differences in adrenocortical responsiveness during development in rats." *Steroids* **68**(5): 439.

<http://www.sciencedirect.com/science/article/B6TC9-48NC76Y-1/2/14bdc013cff2f5757d99968db52952cb>

It is known that the stress hyporesponsive period (SHRP), which seems to be related to an immature hypothalamo-pituitary-adrenal (HPA) regulatory system, occurs during the first 2 weeks after birth in rats. In the present study, we investigated the effects of sex-steroid hormones on adrenocortical responsiveness to adrenocorticotrophic hormone (ACTH) in neonatal rats. The levels of cyclic adenosine 3',5'-monophosphate (cAMP), corticosterone, and adenylate cyclase activity increased with the dose of ACTH in adrenal cells of males and females in vitro. The ACTH responsiveness in adrenal cells increased with age (7-35 days of age), that is, the loss in responsiveness to ACTH just after birth began to recover in 14-35-day-old rats, but the responsiveness in 14-day-old rats was attenuated in males compared with females. Although castration markedly augmented the responsiveness in male rats, testosterone-replacement in the castrated male rats inhibited the enhancement. Furthermore, the responsiveness in 14-day-intact female rats was suppressed by treatment with testosterone. Expression levels of ACTH receptor mRNA in adrenals increased with age in the female rat, but not in the male. Castration enhanced the level of ACTH receptor mRNA to three-fold of that in intact male rats at 14 days of age, but replacement treatment with testosterone in castrated male rats lowered the elevated levels. Testicular androgens are thought to evoke a gender-specific response in neonates, and the temporal decrease of adrenal ACTH-responsiveness might be due to the topically immature adrenal system as well as the central nervous system in mammals.

Hofer, A., M. Hermans, et al. (2003). "Elastin Polymorphism Haplotype and Intracranial Aneurysms Are Not Associated in Central Europe." *Stroke* **34**(5): 1207-1211.

<http://stroke.ahajournals.org/cgi/content/abstract/34/5/1207>

Background and Purpose-- The occurrence of intracranial aneurysms and of aneurysmal subarachnoid hemorrhage are influenced by genetic factors. Recent genomic studies in Japan have defined 3 chromosomal loci and 1 haplotype of elastin polymorphisms as important risk factors, both for affected sib pairs and sporadic patients. **Methods--** We have genotyped 2 single nucleotide polymorphisms in the elastin gene and evaluated their allelic association with intracranial aneurysm in a Central European sample of 30 familial and 175 sporadic patients and 235 population controls. **Results--** We found no allelic association between this elastin polymorphism haplotype and intracranial aneurysm. **Conclusion--** Our data probably reflect increased genetic heterogeneity of intracranial aneurysm in Europe compared with Japan.

Khurana, V. G., Y. R. Sohni, et al. (2003). "Endothelial Nitric Oxide Synthase T-786C Single Nucleotide Polymorphism: A Putative Genetic Marker Differentiating Small Versus Large Ruptured Intracranial Aneurysms." *Stroke* **34**(11): 2555-2559.

<http://stroke.ahajournals.org/cgi/content/abstract/34/11/2555>

Background and Purpose-- Anecdotal evidence exists for at least 2 subpopulations of intracranial saccular aneurysms, namely, those that may form rapidly and rupture when small versus those that enlarge slowly and may rupture particularly when [IMG]=" BORDER="0">10 mm in diameter. We sought to determine whether the endothelial nitric oxide synthase (eNOS) T-786C single nucleotide polymorphism (SNP), implicated in cardiovascular disease susceptibility, could facilitate differentiation between small ([<=]5 mm) versus large ([IMG]=" BORDER="0">10 mm) ruptured aneurysms. **Methods--** In accordance with institutional guidelines, clinical data were recorded prospectively and genomic DNA was isolated from blood samples obtained from 52 aneurysmal subarachnoid hemorrhage (SAH) patients (cases) and 90 randomly selected controls. Samples were assayed for eNOS gene promoter T-786C SNP with the use of gene microarray technology. Statistical analyses included multiple logistic regression. **Results--** Although there was no difference in genotype distributions between cases and controls, all 13 patients with large aneurysms were (T/C) heterozygous for the polymorphism, while 9 of 22 patients (41%) with small aneurysms were (T/T or C/C) homozygous ($P=0.01$). The mean ($\pm$ SD) ruptured aneurysm diameter among all heterozygotes (8.5 $\pm$ 5.2 mm) was significantly greater than that for C/C (6.0 $\pm$ 2.3 mm) or T/T (4.7 $\pm$ 1.8 mm) homozygotes ($P=0.04$). With the use of multivariate analysis, heterozygosity remained significantly associated with aneurysm size [IMG]=" BORDER="0">10 mm ($P=0.03$). **Conclusions--** The eNOS T-786C SNP distinguishes genetically between small and large ruptured aneurysms. Although not predictive of SAH in the population at large, our data suggest that among persons with known intracranial aneurysms, eNOS T-786C genotype may be a factor influencing the size at which an aneurysm ruptures, a finding that should be taken into consideration along with other anatomic features of the aneurysm.

Onda, H., H. Kasuya, et al. (2003). "Endoglin Is Not a Major Susceptibility Gene for Intracranial Aneurysm Among Japanese." *Stroke* **34**(7): 1640-1644.

<http://stroke.ahajournals.org/cgi/content/abstract/34/7/1640>

Background and Purpose-- A 6-base insertion (6bINS) polymorphism in intron 7 of the endoglin

gene (ENG), which codes for a component of the transforming growth factor- β receptor complex, was reported to be associated with intracranial aneurysm (IA) in a Japanese population. A recent report using a white population could not replicate the association. We tested for this association with high statistical power in our independent Japanese subjects and evaluated the linkage between markers on chromosome 9, which contains ENG, and IA. Methods-- The sample for the linkage study comprised 179 individuals with IA in 85 nuclear families, with 104 possible affected sibpairs. For the association study of the 6bINS polymorphism and 4 single nucleotide polymorphisms (SNPs) in ENG, 172 Japanese patients with IA and 192 control subjects were examined. Results-- There was no evidence of linkage in the vicinity of ENG by analysis of affected sibpairs. The allele frequency of the 6bINS polymorphism was 104 of 344 (30.2%) in the total IA group and 122 of 382 (31.9%) in the control group. The statistical difference in allele frequency between the 2 groups was not significant ($\chi^2=0.245$, $df=1$, $P=0.620$). The power of the present association study was 98.3% at a significance level of 0.05 on the basis of the allele frequencies in the previous study. In addition, no associations between the 4 SNPs in ENG and IA were detected. Conclusions-- We provide evidence that there is no association between the 6bINS polymorphism or 4 SNPs in ENG and IA and that there is no linkage between the ENG locus and IA, indicating that ENG is not a major susceptibility gene for IA in Japanese.

Vemuganti, R., R. J. Dempsey, et al. (2004). "Inhibition of Intercellular Adhesion Molecule-1 Protein Expression by Antisense Oligonucleotides Is Neuroprotective After Transient Middle Cerebral Artery Occlusion in Rat." *Stroke* **35**(1): 179-184.

<http://stroke.ahajournals.org/cgi/content/abstract/35/1/179>

Background and Purpose-- The present study was performed to determine whether antisense inhibition of intercellular adhesion molecule-1 (ICAM-1) protein expression decreases focal ischemic brain damage. Methods-- Male spontaneously hypertensive rats underwent 1-hour middle cerebral artery occlusion (MCAO) and 24-hour reperfusion. Rats were infused with ICAM-1 antisense or control oligodeoxynucleotides (ODNs) (48 nmol/d ICV) or vehicle, starting 24 hours before MCAO and continuing until the time of death. ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) mRNA levels were measured by real-time polymerase chain reaction. ICAM-1 protein knockdown was confirmed by Western blotting. Infarct volume was quantified by the use of cresyl violet-stained brain sections. Neurological deficits were evaluated. Mean arterial blood pressure was recorded by laser Doppler. Tissue penetration of antisense was confirmed by the use of fluorescent ODNs. Results-- Transient MCAO upregulated ICAM-1, but not VCAM-1, mRNA expression in the ipsilateral cortex between 3 and 72 hours of reperfusion. ICAM-1 antisense infusion prevented ischemia-induced ICAM-1 protein expression and reduced total infarct volume (by 53%; $P<0.05$; 226 ± 35 mm³ in control ODN group and 104 ± 27 mm³ in antisense ODN group; $n=8$ each) and mean neurological deficit score (by 44%; $P<0.05$; 2.4 in control ODN group and 1.3 in antisense ODN group; $n=8$ each). Neither control nor antisense ODN had any effect on mean arterial blood pressure and the physiological parameters monitored during MCAO. Compared with noninfused control, intracerebroventricular infusion of artificial cerebrospinal fluid or antisense or sense ODN had no significant effect on the regional cerebral blood flow changes that accompanied ischemia and reperfusion. Conclusions-- Increased ICAM-1 expression is implicated in the pathogenesis of focal ischemia since ICAM-1 protein knockdown decreased ischemic brain damage. The mechanism by which ICAM-1 inhibition offers neuroprotection is independent of blood pressure modulation.

Wu, C., H. Fujihara, et al. (2003). "Different Expression Patterns of Bcl-2, Bcl-xl, and Bax Proteins After Sublethal Forebrain Ischemia in C57Black/Crj6 Mouse Striatum." *Stroke* **34**(7): 1803-1808.

<http://stroke.ahajournals.org/cgi/content/abstract/34/7/1803>

Background and Purpose-- Ischemic injury in neurons can be strongly reduced by a preceding sublethal ischemic episode, of which the mechanism is poorly understood. Although changes in the expression of apoptosis-related proteins (Bcl-2, Bcl-xl, and Bax) have been considered to be crucially important in ischemic injury, the roles these proteins play in ischemic preconditioning induced by sublethal forebrain ischemia have not been elucidated. Therefore, we investigated the transcription and expression of Bcl-2, Bcl-xl, and Bax in striatum of mice subjected to sublethal forebrain ischemia and lethal ischemia, with or without ischemic preconditioning. **Methods--** Sublethal forebrain ischemia was induced in C57Black/Crj6 (C57BL/6) mice by 6 minutes of bilateral common carotid artery occlusion. The transcription and expression of Bcl-2 family genes were detected by reverse transcription-polymerase chain reaction, Western blot, and immunofluorescent staining. **Results--** No detectable neuronal loss was induced in striatum by 6 minutes of bilateral common carotid artery occlusion. Transcription and expression of Bcl-2 and Bcl-xl were increased after sublethal forebrain ischemia, which attenuated the DNA fragmentation induced by lethal ischemia. The transcription and expression of Bax remained unchanged. **Conclusions--** Upregulation of Bcl-2 and Bcl-xl but not Bax may have a role in protective ischemic preconditioning. This result indicates a potential strategy for further ischemic neuronal injury therapies.

Yamada, S., M. Utsunomiya, et al. (2003). "Absence of Linkage of Familial Intracranial Aneurysms to 7q11 in Highly Aggregated Japanese Families." *Stroke* **34**(4): 892-900.

<http://stroke.ahajournals.org/cgi/content/abstract/34/4/892>

Background and Purpose-- We sought to test the linkage of familial intracranial aneurysms (FIAs) to the ELN (elastin) locus in chromosome 7q11 reported previously. **Methods--** Intracranial aneurysm (IA) probands were searched from patient records or neurosurgeons' recalls in collaborating hospitals. Members of the participating probands' families who had unknown affection status were screened by MR angiography and diagnosed by digital subtraction angiography. Inclusion criteria of families for genetic analyses were as follows: at least 3 alive affected members or 2 alive affected members with at least 1 unaffected member (≥ 60 years). Linkage to the ELN locus was tested with the use of GENEHUNTER by parametric and nonparametric methods. To exclude false-negatives in the linkage analysis, the lowest 5% limits of logarithms of the odds (LOD) and nonparametric LOD (NPL) scores for individual families and for the total set of families were simulated on assumption that the ELN locus is linked to FIAs. **Results--** Questionnaires were sent to 885 patients, and 563 responded. Seventy-nine probands were positive for family history. One hundred thirty-four family members of unknown affection status were screened. A total of 14 families with 64 members met the criteria. Linkage to the ELN locus was discarded in 11 families and was inconclusive for 3 families. The total LOD and total NPL scores for 14 families were -8.04 and -0.643, respectively. Our conclusion did not change even when the values of penetrance were changed or only affected members were analyzed. **Conclusions--** The majority of aggregated IA Japanese families may not have a genetic linkage to chromosome 7q11.

Yoneyama, T., H. Kasuya, et al. (2004). "Collagen Type I $\alpha 2$ (COL1A2) Is the Susceptible Gene for Intracranial Aneurysms." *Stroke* **35**(2): 443-448.

<http://stroke.ahajournals.org/cgi/content/abstract/35/2/443>

Background and Purpose-- The collagen $\alpha 2(I)$ gene (COL1A2) on chromosome 7q22.1, a positional and functional candidate for intracranial aneurysm (IA), was extensively screened for susceptibility in Japanese IA patients. **Methods--** Twenty-one single nucleotide polymorphisms (SNPs) of COL1A2 were genotyped in genomic DNA from 260 IA patients (including 115 familial

cases) (mean age, 59.9 years) and 293 controls (mean age, 61.6 years). Differences in allelic and genotypic frequencies between the patients and controls were evaluated with the χ^2 test. Circular dichroism spectrometry was monitored with collagen-related peptides that mimic triple-helical models of type I collagen with Ala-459 and Pro-459 to estimate the conformation and stability of alterations. Results-- Significant genotypic association in the dominant model was observed between an exonic SNP of COL1A2 and familial IA patients ($\chi^2=11.08$; $df=1$; $P=0.00087$; odds ratio=3.19; 95% CI, 2.22 to 6.50). This SNP induces Ala to Pro substitution at amino acid 459, located on a triple-helical domain. Circular dichroism spectra showed that the Pro-459 peptide had a higher thermal stability than the Ala-459 peptide. Conclusions-- The variant of COL1A2 could be a genetic risk factor for IA patients with family history.

Structure (2)

Jamal-Talabani, S., A. B. Boraston, et al. (2004). "Ab Initio Structure Determination and Functional Characterization Of CBM36: A New Family of Calcium-Dependent Carbohydrate Binding Modules." *Structure* **12**(7): 1177.

<http://www.sciencedirect.com/science/article/B6VSR-4CVG586-F/2/5d752b4ba30cb3d79912fe5ad262db62>

The enzymatic degradation of polysaccharides harnesses multimodular enzymes whose carbohydrate binding modules (CBM) target the catalytic domain onto the recalcitrant substrate. Here we report the ab initio structure determination and subsequent refinement, at 0.8 Å resolution, of the CBM36 domain of the *Paenibacillus polymyxa* xylanase 43A. Affinity electrophoresis, isothermal titration calorimetry, and UV difference spectroscopy demonstrate that CBM36 is a novel Ca^{2+} -dependent xylan binding domain. The 3D structure of CBM36 in complex with xylotri-ose and Ca^{2+} , at 1.5 Å resolution, displays significant conformational changes compared to the native structure and reveals the molecular basis for its unique Ca^{2+} -dependent binding of xylooligosaccharides through coordination of the O2 and O3 hydroxyls. CBM36 is one of an emerging spectrum of carbohydrate binding modules that increasingly find applications in industry and display great potential for mapping the "glyco-architecture" of plant cells.

Jia, J., S. Tarabykina, et al. (2001). "Structure of Apoptosis-Linked Protein ALG-2: Insights into Ca^{2+} -Induced Changes in Penta-EF-Hand Proteins." *Structure* **9**(4): 267.

<http://www.sciencedirect.com/science/article/B6VSR-43VBWR2-2/2/11711003aeb16fc37fa22c7979cd0059>

Background: The Ca^{2+} binding apoptosis-linked gene-2 (ALG-2) protein acts as a proapoptotic factor in a variety of cell lines and is required either downstream or independently of caspases for apoptosis to occur. ALG-2 belongs to the penta-EF-hand (PEF) protein family and has two high-affinity and one low-affinity Ca^{2+} binding sites. Like other PEF proteins, its N terminus contains a Gly/Pro-rich segment. Ca^{2+} binding is required for the interaction with the target protein, ALG-2 interacting protein 1 (AIP1). Results: We present the 2.3 Å resolution crystal structure of Ca^{2+} -loaded des1-20ALG-2 (aa 21-191), which was obtained by limited proteolysis of recombinant ALG-2 with elastase. The molecule contains eight $[\alpha]$ helices that fold into five EF-hands, and, similar to other members of this protein family, the molecule forms dimers. Ca^{2+} ions bind to

EF1, EF3, and, surprisingly, to EF5. In the related proteins calpain and grancalcin, the EF5 does not bind Ca^{2+} and is thought to primarily facilitate dimerization. Most importantly, the conformation of des1-20ALG-2 is significantly different from that of calpain and grancalcin. This difference can be described as a rigid body rotation of EF1-2 relative to EF4-5 and the dimer interface, with a hinge within the EF3 loop. An electron density, which is interpreted as a hydrophobic Gly/Pro-rich decapeptide that is possibly derived from the cleaved N terminus, was found in a hydrophobic cleft between these two halves of the molecule. Conclusions: A different relative orientation of the N- and C-terminal halves of des1-20ALG-2 in the presence of Ca^{2+} and the peptide as compared to other Ca^{2+} -loaded PEF proteins changes substantially the shape of the molecule, exposing a hydrophobic patch on the surface for peptide binding and a large cleft near the dimer interface. We postulate that the binding of a Gly/Pro-rich peptide in the presence of Ca^{2+} induces a conformational rearrangement in ALG-2, and that this mechanism is common to other PEF proteins.

Surgery (10)

Bandipalliam, P., J. Balmana, et al. (2004). "Comprehensive genetic and endoscopic evaluation may be necessary to distinguish sporadic versus familial adenomatous polyposis-associated abdominal desmoid tumors." *Surgery* **135**(6): 683.

<http://www.sciencedirect.com/science/article/B6WXC-4CHGDDJ-S/2/c5be7e7b08a17055c75874d14cf2b006>

Background There are limited data regarding how many patients with desmoid tumors actually represent cases with underlying familial adenomatous polyposis. **Methods** A proband presenting with desmoid tumors and several of the family members underwent a detailed family history, genetic (adenomatous polyposis coli [APC] gene sequencing), and upper and lower endoscopic evaluation. **Results** The proband's initial diagnosis was of a sporadic desmoid tumor. Colonoscopy was entirely normal. However, on subsequent esophagogastroduodenoscopy, several gastric polyps were found. The proband's mother subsequently underwent colonoscopy and was found to have multiple colon adenomas. On genetic analysis, a deletion of "T" was identified at codon 2645 of the APC gene in the proband. The proband's mother had a normal APC protein truncation test result. However, on full gene sequencing, the mother was found to harbor the same APC gene mutation. **Conclusion** A detailed family history and endoscopic and genetic evaluations for patients with desmoid tumors are vital because they may be the sentinel presentation of familial adenomatous polyposis. If confirmed in larger studies, APC full gene sequencing and upper and lower gastrointestinal tract evaluation may need to be part of standard evaluation of patients with abdominal desmoid tumors.

Barksdale, A. R., A. C. Bernard, et al. (2004). "Regulation of arginase expression by T-helper II cytokines and isoproterenol." *Surgery* **135**(5): 527.

<http://www.sciencedirect.com/science/article/B6WXC-4C89FNM-K/2/66bb05751911d5533a5aa1c321853822>

Background Trauma causes a release of catecholamines, transforming growth factor- β (TGF- β), and T-helper II cytokines (TH2). Individually, these substances also induce arginase in

macrophages. The purpose of this study was to determine the synergistic interactions between isoproterenol, TGF- β , and TH2 cytokines on arginase expression in macrophages. Methods Confluent RAW 264.7 macrophages were incubated with various combinations of interleukins 4, 10, and 13 (IL-4, IL-10, IL-13), and TGF- β with isoproterenol over 48 hours. Arginase activity, as well as arginase I expression by Western blot and reverse transcriptase-polymerase chain reaction, were measured. Results Although isoproterenol, IL-4, IL-10, and IL-13 individually induced arginase, significant synergy between the combination of isoproterenol with either TGF- β or the TH2 cytokines was observed. All cytokines except IL-10 also induced arginase I protein and mRNA. Arginase II protein was detected in cells exposed to IL-10. Conclusions We conclude that isoproterenol synergizes with IL-4, IL-13, and TGF- β to increase arginase I mRNA and protein, as well as arginase activity in RAW 264.7 macrophages. Further, IL-10 synergizes with isoproterenol to increase arginase activity and arginase II protein. These synergistic mechanisms may compete with nitric oxide synthase for - arginine substrate, thus shunting away available arginine from nitric oxide production and contributing to cellular immunosuppression observed after trauma.

Harada, N., Y. Iimuro, et al. (2003). "Inactivation of the small GTPase Rac1 protects the liver from ischemia/reperfusion injury in the rat." *Surgery* **134**(3): 480.

<http://www.sciencedirect.com/science/article/B6WXC-49NPKWT-T/2/248ff07e20e0c93e51077f7a48ff09c8>

Background In ischemia/reperfusion (I/R) injury, a massive generation of reactive oxygen species (ROS) after reperfusion is a critical factor. Rac, a member of the Rho GTPase superfamily, plays important roles in the production of ROS and activation of nuclear factor- κ B (NF- κ B) in vitro. However, the exact role of Rac in the ROS production and NF- κ B activation in vivo after I/R is still obscure. Methods We blocked Rac1 activity in the rat liver using adenovirus encoding a dominant negative rac1 mutant (Ad5N17Rac1) and examined whether inactivation of Rac1 could prevent ROS generation in the hepatic I/R injury. Seventy-two hours after the adenoviral infection, hepatic I/R was induced by Pringle's maneuver for 20 minutes, followed by reperfusion in the rats. Results Ad5N17Rac1 infection significantly attenuated ROS production after reperfusion and suppressed the hepatic injury. Furthermore, N17Rac1 suppressed NF- κ B activation and messenger RNA expression of tumor necrosis factor- α (TNF- α) and inducible nitric oxide synthetase (iNOS). Ad5LacZ, a control adenovirus, had no effect on the induced hepatic I/R injury, nor did it affect NF- κ B activation. Immunohistochemical analysis of NF- κ B (p65) revealed that translocation of p65 to the nucleus after reperfusion was blocked in many of non-parenchymal cells (NPCs) and in hepatocytes in the Ad5N17Rac1-infected liver. Conclusion We conclude that Rac1 is required in ROS generation and NF- κ B activation after hepatic I/R in vivo, and that inactivation of NF- κ B in NPCs and suppression of ROS generation in NPCs and hepatocytes possibly account for the protective effect of N17Rac1 in this study.

Kato, M., R. Doi, et al. (1997). "Calcium-evoked insulin release from insulinoma cells is mediated via calcium-sensing receptor." *Surgery* **122**(6): 1203.

<http://www.sciencedirect.com/science/article/B6WXC-4CT11G1-2S/2/3b981e2fc357c056c87b82b316d3a24a>

Background. We have previously reported that high extracellular calcium ($[Ca^{2+}]_o$) levels elicited rapid increases in the cytosolic free calcium ($[Ca^{2+}]_i$) and insulin release from human insulinoma cells. In this study we further investigated the mechanism for stimulus-secretion coupling of insulinoma cells exposed to high levels of $[Ca^{2+}]_o$. Methods. Insulinoma tissues were surgically

obtained for primary culture. The changes of $[Ca^{2+}]_i$ level in response to various agents were monitored by fluorometry. Total RNA was extracted from tissues and subjected to reverse transcription-polymerase chain reaction (RT-PCR) with calcium-sensing receptor (CaR)--specific primers. PCR products were subcloned and sequenced. Results. When $[Ca^{2+}]_o$ level was elevated, $[Ca^{2+}]_i$ in insulinoma cells was immediately increased. Application of neomycin abolished the increase in $[Ca^{2+}]_i$ level, although extracellular nifedipine and lanthanum chloride did not affect it. The depletion of intracellular calcium stores with thapsigargin or carbachol eliminated the increase in $[Ca^{2+}]_i$ level. RT-PCR analysis identified the 682 bp product, of which the sequence was identical to the corresponding regions of human parathyroid CaR. Conclusions. Intracellular Ca^{2+} release might be important in insulin release from insulinoma cells after exposing to high level of $[Ca^{2+}]_o$. CaR could be involved in this mechanism.

Kebebew, E., M. Peng, et al. (2004). "GCMB gene, a master regulator of parathyroid gland development, expression, and regulation in hyperparathyroidism." *Surgery* **136**(6): 1261.

<http://www.sciencedirect.com/science/article/B6WXC-4F1J2N7-13/2/6281c56585d95ff13cda81d9488ad884>

Background The glial cell missing gene, GCMB, encodes a transcription factor, which is a master regulator of parathyroid development. We postulated that the GCMB gene might play a role in parathyroid tumorigenesis in hyperparathyroidism. **Methods** We used real-time quantitative reverse transcriptase polymerase chain reaction to study GCMB mRNA expression in parathyroid tissue: normal (n = 3), hyperplastic (n = 16), adenomas (n = 19), and cancers (n = 8). In primary parathyroid culture, the effect of $CaCl_2$ on parathyroid hormone secretion and GCMB mRNA expression was studied by using enzyme-linked immunosorbent assay and reverse transcriptase polymerase chain reaction, respectively. **Results** GCMB mRNA expression was lower in normal (0.4 [plus-or-minus sign] 0.1, mean [plus-or-minus sign] standard error of mean) parathyroid glands as compared to adenoma (3.5 [plus-or-minus sign] 1.7), hyperplasia (3.2 [plus-or-minus sign] 1.3 primary hyperparathyroidism [n = 11] and 7.6 [plus-or-minus sign] 4.8 secondary hyperparathyroidism [n = 5]), and cancer (3.6 [plus-or-minus sign] 1.3) (P = .001). There was no difference in the level of GCMB mRNA expression between parathyroid adenoma, hyperplasia, and cancer. In primary culture of parathyroid adenoma (n = 9) and hyperplasia (n = 2), parathyroid hormone secretion was increased 2- to 15-fold with low calcium concentration (0.5 to 4.0 mmol/L $CaCl_2$ from 2 to 6 hours, P GCMB mRNA expression was down-regulated with lower extracellular $CaCl_2$ concentration (P **Conclusions** GCMB expression is upregulated in abnormal parathyroid glands of hyperparathyroidism and decreases in response to hypocalcemia. The GCMB transcription factor might mediate the effect of calcium on parathyroid cell parathyroid hormone expression/secretion.

Musholt, P. B., T. J. Musholt, et al. (1997). ""Cold" single-strand conformational variants for mutation analysis of the RET protooncogene." *Surgery* **122**(2): 363.

<http://www.sciencedirect.com/science/article/B6WXC-4CRGF5F-11/2/3ec5c5d2a40ab744ca575ed832303d1d>

Background. RET protooncogene mutation analysis is a routinely performed predictive DNA test in kindreds affected by multiple endocrine neoplasia (MEN) types 2A and 2B and familial medullary thyroid carcinoma (FMTTC), and is a valuable diagnostic tool in newly diagnosed cases of medullary thyroid carcinoma (MTC). **Methods.** We tested the suitability of the recently introduced "cold" single-strand conformational variant (SSCV) technique, which promises rapid, simple, nonradioactive detection of sequence variants in the identification of germline and somatic RET mutations. A total of 11 different mutations in exon 10 (codons 609, 611, 618, and

620) and 6 mutations in exon 11 (codon 634) were studied. Results. Conditions were optimized so that conformational variants were demonstrated for all mutations examined in a single setting for exons 10 and 11. A novel six base pair (bp) inframe deletion between cysteines 630 and 634 was detected in a sporadic MTC. This adds to the evidence that not only cysteine deletions and substitutions but also changes in the spacing between cysteine residues have a pathogenic effect. Conclusions. Our results indicate that the cold SSCV method offers the advantages of simplicity, time savings, and nonradioactive detection for screening for RET sequence variants in hereditary and sporadic MTCs.

Sablinski, T., P. R. Gianello, et al. (1997). "Pig to monkey bone marrow and kidney xenotransplantation." Surgery **121**(4): 381.

<http://www.sciencedirect.com/science/article/B6WXC-4CTDHKF-1M/2/0757dda3c7545865b9ca76e04128f51e>

Background. The intensity of discordant xenograft cellular rejection makes it unlikely that safe doses of immunosuppressive drugs will alone be sufficient to permit long-term survival. We have therefore concentrated our efforts on establishing tolerance to xenogeneic organs through lymphohematopoietic chimerism and the elimination of preformed natural antibodies (nAbs). **Methods.** Here we report the most recent series of 11 technically successful porcine to nonhuman primate transplantation procedures. In eight experimental animals induction therapy consisted of (1) 3 x 100 cGy nonlethal whole body irradiation (day -6 and day -5) to all animals, (2) horse anti-human thymocyte globulin (day -2, day -1, and day 0) to seven of the animals, (3) 700 cGy thymic irradiation (day -1) to five of the animals, and (4) pig bone marrow infused on day 0 (2-9 x 10⁸/cells/kg). On day 0, just before the renal xenograft, the recipient was splenectomized, and antipig nAbs were removed by means of perfusion of the monkey's blood through either a pig liver (n = 6) or a Gal-[alpha](1,3)-Gal adsorption column (n = 5). Three control animals did not receive this pretransplantation induction therapy but did undergo hemoperfusion and posttransplantation immunosuppression identical to the experimental animals. All 11 recipients were treated after transplantation with cyclosporin A and 15-deoxyspergualin. Recombinant pig-specific growth factors (interleukin-3 and stem cell factor) were given to six experimental animals from day 0 until the termination of the experiment. **Results.** Analysis of recipients' sera by means of flow cytometry indicated the effective removal of immunoglobulin M and immunoglobulin G nAbs by either liver perfusion or column adsorption. In the eight experimental animals, nAb titers remained low until death (up to 15 days), but in the three control animals nAb titers increased substantially with time. The longest surviving recipient maintained excellent kidney function with creatinine levels at 0.8 to 1.3 mg/dl throughout its course. Death occurred at day 15 from complications caused by a urinary leak and pancytopenia. Histologic examination of the xenograft revealed only focal tubular necrosis and cytoplasmic vacuolization, with trace amounts of fibrin and C3 in peritubular capillaries. In this animal a fraction of the peripheral blood cells (3%) at day 7 were of pig origin as detected by pig-specific monoclonal antibodies. In addition, colony-forming assays performed on a bone marrow biopsy specimen taken at day 14 indicated that approximately 30% of the relatively few myeloid progenitors detected were of swine origin. **Conclusions.** We have demonstrated that our protocol is effective in the prevention of hyperacute rejection and in the maintenance of excellent function of the renal xenograft for up to 15 days. These results also indicate that at least short-term engraftment of the xenogeneic donor bone marrow cells is possible to achieve in this discordant large animal combination. Longer survivals will be required to assess the possible effect of this engraftment on induction of tolerance.

Takabayashi, T., S. Shimizu, et al. (2004). "Interleukin-1 upregulates anaphylatoxin receptors on mononuclear cells." Surgery **135**(5): 544.

<http://www.sciencedirect.com/science/article/B6WXC-4C89FNM-N/2/c92919e9ee75cfa1ab6a7d14d8aa1ef4>

Background The anaphylatoxins, C3a and C5a, that are generated during trauma, major surgery, or infection are potent proinflammatory mediators that increase interleukin (IL-1) cytokine synthesis. We investigated the effects of IL-1 on anaphylatoxin receptor expression in monocytes. **Methods** A human monocytic cell line, MONO-MAC-6, was used. C3a and C5a binding sites were assayed by competitive binding. Levels of messenger RNA for the C3a and C5a receptors were analyzed by reverse transcriptase-polymerase chain reaction. Changes of free cytosolic Ca²⁺ concentration ([Ca²⁺]_i) in response to C3a and C5a were measured. **Results** Basal MONO-MAC-6 cell sites for C3a and C5a binding were 10,900 C3aR/cell (K_d = 2.0 nmol/L), 8700 C5aR/cell (K_d = 0.9 nmol/L). IL-1[α] increased sites for both C3a (61% increase; P < .05) and C5a (41% increase; P < .05). IL-1 receptor antagonist blocked the effects of IL-1[α] upregulation of anaphylatoxin receptors. **Conclusion** These results suggest that there is an additional link between IL-1 and anaphylatoxins to amplify proinflammatory effects through monocytes and macrophages. Although C3a and C5a can increase the monocyte production of IL-1, IL-1 increases monocyte expression of receptors for these anaphylatoxins, which further amplifies inflammation.

Witzigmann, H., F. Geiler, et al. (2002). "Prospective evaluation of circulating hepatocytes by [α]-fetoprotein messenger RNA in patients with hepatocellular carcinoma." *Surgery* **131**(1): 34.

<http://www.sciencedirect.com/science/article/B6WXC-45SJCX3-B3/2/df6de600af64fdbd451ddc836d6bd364>

Background. The significance of [α]-fetoprotein (AFP) messenger RNA as a surrogate marker for isolated tumor cells in the blood of patients with hepatocellular carcinoma (HCC) is controversial. Our goals were to correlate AFP mRNA with tumor recurrence and overall survival after patients with HCC received curative operations and to analyze AFP mRNA findings in control patients. **Methods.** In this prospective controlled study, RNA was purified from the blood of 85 patients with HCC before, during, and after therapy and from 116 control patients. Complementary DNA synthesis by reverse transcriptase and polymerase chain reaction amplification was performed with primers specifically for the AFP gene. Patients with HCC were divided into 4 subgroups depending on the therapy performed: (1) orthotopic liver transplantation (OLT), (2) resection, (3) transarterial chemoembolization, and (4) no therapy. **Results.** AFP mRNA was detected in 28% of the patients with HCC and 3% of the control patients (P = .21 and P = .94, respectively). After the tumor resection, no difference in survival at 2 years was evident in patients who were AFP mRNA positive versus those who were AFP mRNA negative. In the HCC patients who had curative operations (OLT and resection) the sensitivity and specificity of this test for tumor recurrence were 73% and 53%, respectively, excluding surgical mortality. The International Union Against Cancer tumor stages in the subgroups of OLT and resection showed no differences between patients with positive and negative findings (P = .76 and P = .15, respectively). AFP mRNA results and serum AFP levels revealed no correlation (P = .45). **Conclusions.** The qualitative measurement of AFP mRNA in the blood of patients with HCC is not a clinically relevant method for determining therapy and prognosis, especially if AFP mRNA is detected during the surgical procedure or any other liver manipulation. (*Surgery* 2002;131:34-43.)

Zarnegar, R., L. Brunaud, et al. (2002). "Increasing the effectiveness of radioactive iodine therapy in the treatment of thyroid cancer using Trichostatin A, a histone deacetylase inhibitor." *Surgery* **132**(6): 984.

<http://www.sciencedirect.com/science/article/B6WXC-47HXS82-C/2/8b0aad1ec47ce7738c4aad79ac6d3029>

Background. Radioactive iodine is used to identify and treat recurrent and metastatic thyroid cancer of follicular cell origin. Between 30% and 40% of thyroid cancers are either resistant or become resistant to radioactive iodine. Increased sodium-iodide symporter (NIS) and decreased Pendrin (PDS) activity may be associated with increased radioactive iodine effectiveness. In this investigation the effects of Trichostatin A (TSA), a histone deacetylating inhibitor, on human thyroid NIS and PDS gene expression was investigated. Method. Cell lines from papillary, Hurthle, and follicular cell carcinomas were treated with TSA for 72 hours at concentrations up to 100 ng/mL. NIS and PDS gene expression was determined using quantitative RT-polymerase chain reaction. Results. NIS messenger RNA expression in cell carcinomas was increased 107- (1.8-307) and 217- (5.7-408) fold in papillary, 39- (20-63) and 58- (37-80) fold in Hurthle, and 459- (178-810) and 781- (412-1229) fold in follicular after treatment with 50 and 100 ng/mL of TSA, respectively. PDS messenger RNA expression in cell carcinomas was decreased 0.22- (0.05-0.45) and 0.27- (0.09-0.47) fold in papillary, 0.53- (0.46-0.60) and 0.54- (0.44-0.64) fold in Hurthle, and 0.32- (0.26-0.39) and 0.56- (0.47-0.64) fold in follicular, after the same treatment. Conclusions. In thyroid cancer cell lines, TSA dramatically increased NIS gene expression and reduced PDS expression. The increased NIS expression and reduced PDS expression may make radioiodine therapy more effective in patients with thyroid cancer, especially when the tumors have no or low uptake of radioiodine. (Surgery 2002;132:984-90.)

Systematic and Applied Microbiology (1)

Ivanova, E. P., O. M. Onyshchenko, et al. (2005). "Oceanimonas smirnovii sp. nov., a novel organism isolated from the Black Sea." Systematic and Applied Microbiology 28(2): 131.

<http://www.sciencedirect.com/science/article/B7GVX-4F01192-1/2/3ce5fbe22622d5013bdf3d94eeaa5b6>

A slightly creamy, melanogenic, Gram-negative, aerobic bacterium was isolated from seawater sample collected in the Karadag Natural Reserve of the Eastern Crimea, the Black Sea. The novel organism was chemoorganotrophic, had no obligate requirement in NaCl, tolerated to 12% NaCl, grew between 10 and 45 [deg]C, was slightly alkaliphilic, and was not able to degrade starch, gelatin, agar, and Tween 80. 16S rRNA gene sequence-based analyses of the new organism revealed that *Oceanimonas doudoroffii* ATCC 27123T, *Oceanimonas baumannii* ATCC 700832T, and *Oceanisphaera litoralis* DSM 15406T were the closest relatives (similarity around 97%-96%). The G+C content of the DNA of the strain 31-13T was 55.5 mol%. Phosphatidylethanolamine (49.0%), phosphatidylglycerol (41.8%), and diphosphatidylglycerol (9.2%) were the predominant phospholipids. The major fatty acids were 16:0 (24.1%), 16:1[omega]7 (40.3%), and 18:1[omega]7 (29.2%). On the basis of the significant differences demonstrated in the phenotypic and chemotaxonomic characteristics, it is suggested that the bacterium be classified as a novel species; the name *Oceanimonas smirnovii* sp. nov. is proposed. The type strain is 31-13T (UCM B-11076T=LMG 22147T=ATCC BAA-899T).

The American Journal of Cardiology (4)

Arbustini Md, E., P. M. Grasso Bd, et al. (1997). "Enteroviral RNA and Virus-Like Particles in the Skeletal Muscle of Patients With Idiopathic Dilated Cardiomyopathy." The American Journal of Cardiology **80**(9): 1188.

<http://www.sciencedirect.com/science/article/B6T10-3V5N1K8-F/2/34e82cdbba7f17cd855f3ac210b9eb62>

The role of chronic viral infection in the etiopathogenesis of idiopathic dilated cardiomyopathy (IDC) has generated considerable research. Enteroviruses were the favorite candidates as etiologic agents of IDC. However, enteroviruses were rarely demonstrated in affected hearts. We investigated whether enteroviral infection persists in the heart and in extracardiac sites, particularly in skeletal muscle, in patients with IDC. Blood and myocardial and skeletal muscle samples were collected at cardiac transplantation from 31 IDC patients, 24 non-IDC heart disease patients, and 3 heart donors. Samples underwent ultrastructural studies and ribonucleic acid (RNA) extraction. RNA was reverse-transcribed, and 2 nested fragments (bps 179 and 126) were amplified in the highly conserved 5' noncoding region of enteroviral genomic RNA. Enteroviral RNA was found in the skeletal muscle of 12 cases, whereas only 4 hearts (2 of which with positive skeletal muscle) were positive. Of the 24 controls, 2 were positive (1 muscle and heart, 1 muscle only). Automated sequencing confirmed the enteroviral nature of the amplified products. Ultrastructural study showed enterovirus-like particles in 4 of the enterovirus-positive muscles, and myopathic changes in all enterovirus-positive cases. Skeletal muscle hosts chronic enteroviral infection in more than one third of patients with sporadic IDC. Two hypotheses may explain this link. Myocardial damage may derive directly from recurrent subclinical heart infections caused by enteroviruses harbored in skeletal muscle. Alternatively, enterovirus-related myopathy may trigger an autoimmune response to antigens shared by muscle and myocardium. Further studies are needed to assess the importance of these, non-mutually exclusive mechanisms in IDC pathogenesis.

Date, M.-o., K. Otsu, et al. (2000). "Single-strand conformation polymorphism analysis on the [delta]-sarcoglycan gene in Japanese patients with hypertrophic cardiomyopathy." The American Journal of Cardiology **85**(11): 1315.

<http://www.sciencedirect.com/science/article/B6T10-40BG48G-7/2/9a5000a8d749e278d25990dffce55c84>

To elucidate the etiology of hypertrophic cardiomyopathy (HC) in humans, we analyzed the [delta]-sarcoglycan gene (SG), which is reported to be the causal gene for HC in the Syrian hamster BIO14.6. We performed polymerase chain reaction (PCR) single-strand conformation polymorphism (SSCP) and nucleotide sequence analyses on the [delta]-SG in 102 patients with HC. SSCP was detected in exon 2 of the gene, but not in the other exons. The direct sequencing analysis of exon 2 revealed a C->T substitution at nucleotide residue 84 (TAC->TAT) with no amino acid alteration (Tyr->Tyr). There were no significant differences in allele frequencies of C/T between the patients with HC and the control group. Patients with HC were classified into 4 subgroups: obstructive HC, nonobstructive HC, apical HC, and familial HC. The allele frequency of C/T polymorphism in each of these groups was compared with that of the control group. The obstructive HC group showed a significantly greater frequency of the allele T than in the control group (31.6% vs 15.1%, RR = 2.6, p = 0.023). No other significant differences were observed. Thus, amino acid alteration in [delta]-SG may not be a common cause of HC in Japanese patients.

Grasso, M., E. Arbustini, et al. (1992). "Search for Coxsackievirus B3 RNA in idiopathic dilated cardiomyopathy using gene amplification by polymerase chain reaction." The American Journal of

Cardiology **69**(6): 658.

<http://www.sciencedirect.com/science/article/B6T10-4C6TPFK-T0/2/a54bcae3f8f26290ad7022f77d2fad9a>

A polymerase chain reaction (PCR) amplification assay was developed to detect Coxsackievirus B3 ribonucleic acid (RNA) in blood and myocardial tissue of explanted hearts from 40 patients who underwent cardiac transplantation and in 1 normal heart. Twenty-one patients were affected by idiopathic dilated cardiomyopathy of different duration and 19 by coronary artery disease. Coxsackievirus B3 in vitro infected Vero cells and cells infected by related human enteroviruses (Coxsackievirus B2, B4, and poliovirus 1) were used as reaction controls. PCR was performed using 4 pairs of primers homologous to Coxsackievirus B3 sequences. Three sets were located in regions of the genome conserved at nucleotide level between several enterovirus species (replicase gene, 5' noncoding region), while one was located in a Coxsackievirus B3-specific region (VP1 gene). Total RNA was prepared by acid guanidinium isothiocyanate extraction from tissue stored frozen at -80 [deg]C. One microgram of total RNA was retrotranscribed with either antisense primer or with random hexanucleotide primers and then subjected to 40 cycles of amplification. PCR products were separated by electrophoresis on a 10% polyacrilamide gel, electrotransferred to a nylon membrane and then hybridized to oligonucleotide probes specific for the coxsackievirus B3 genome radiolabeled with radioactive isotope of phosphorous. All pairs of primers yielded specific amplification products when tested on Coxsackievirus B3-infected Vero cells, with a sensitivity of 1 infected cell out of 105 to 106 cells starting from 1 [mu]g total RNA. Primer sets for regions of Coxsackievirus B3 genome highly conserved between related enteroviral species gave positive amplification also when challenged with RNA from cells infected by Coxsackievirus B2, B4 and poliovirus 1. The VP1 gene primer set produced positive amplification only with RNA of Coxsackievirus B3-infected cells. Coxsackievirus B3-specific amplification products were distinguished from those of related enteroviruses by hybridization with specific oligoprobes. However, Coxsackievirus B2, B4 and poliovirus 1-specific PCR products showed positive hybridization if probed with Coxsackievirus B3 genomic probes. All total RNAs from blood and myocardial samples examined by our PCR assay failed to reveal any amplification product that could be related to Coxsackievirus B3 or to enteroviruses in general, after gel electrophoresis and low stringency Southern blot hybridization with the Coxsackievirus B3 specific oligoprobes or the Coxsackievirus B3 genomic probes. The negative results obtained in our series question the hypothesized widespread persistence of enteroviral RNA in hearts with idiopathic dilated cardiomyopathy.

Rodriguez, E. R., S. Nasim, et al. (1991). "Cardiac myocytes and dendritic cells harbor human immunodeficiency virus in infected patients with and without cardiac dysfunction: Detection by multiplex, nested, polymerase chain reaction in individually microdissected cells from right ventricular endomyocardial biopsy tissue." The American Journal of Cardiology **68**(15): 1511.

<http://www.sciencedirect.com/science/article/B6T10-4C76CCK-RX/2/23bbea00d8a4726de538af0b9811bc3f>

Two hundred fifteen patients infected with human immunodeficiency virus (HIV) participated in a prospective longitudinal study of HIV-related heart disease. Evaluation included signal-averaged electrocardiography and echocardiography. Fifteen patients underwent endomyocardial biopsy, 5 had cardiovascular symptoms and 10 did not. Cardiac myocytes or dendritic cells were prepared by individual cell microdissection to sort them from other cell types such as interstitial cells or circulating blood elements. HIV proviral sequences were amplified in samples of 15 to 20 cells of each type by multiplex, nested, polymerase chain reaction and hybridized to ³²P-labeled probes specific for regions within the gag and pol genes of HIV-1. The results showed the presence of HIV sequences in myocytes of 2 of 5 patients with cardiac symptoms and in 6 of 10 without. Thus, symptomatic HIV cardiomyopathy did not appear to be a direct consequence of the virus on

myocardial cells. In dendritic cells, HIV sequences were detected in 5 of 5 patients with cardiac symptoms and in 8 of 10 with apparently normal ventricular function. Furthermore, dendritic cells were somewhat more numerous in the myocardium of symptomatic than asymptomatic patients. Our studies are the first to directly detect the HIV genome in purified cardiac myocytes from patients with and without cardiac dysfunction. Our findings do not support a direct role of the virus in myocardial dysfunction. However, the results do suggest that the interstitial dendritic cells may be involved in some manner in the development of cardiac dysfunction observed in HIV-infected patients.

The American Journal of Gastroenterology (17)

Aktas, D., A. Ayhan, et al. (1998). "No evidence for overexpression of the p53 protein and mutations in exons 4-9 of the p53 gene in a large family with adenomatous polyposis." The American Journal of Gastroenterology **93**(9): 1524.

<http://www.sciencedirect.com/science/article/B6VHV-3V3MPJB-Y/2/3c509239f04a78ce54aa1b9b710b0340>

Objective: Familial adenomatous polyposis coli (FAP) is an autosomal dominant disease characterized by an early onset of numerous adenomatous polyps of the colon and a high risk of colon carcinoma. The role of the p53 gene in the multistage process of FAP is as yet poorly defined. In the present study, a large family with evidence of polyposis and colon cancer was screened for the mutations of the p53 gene and protein overexpression. **Methods:** We examined p53 protein expression from individuals with immunohistochemical techniques using monoclonal antibody PAb1801. Polymerase chain reaction products of exons 4-9 of the p53 were examined from individuals by single strand, conformational polymorphism analysis. **Results:** We could find no evidence of overexpression and mutations of the p53 in any lesion including adenomas and carcinomas. **Conclusion:** We found that p53 gene alterations do not contribute to the genesis of adenoma or carcinoma of FAP patients for this large family examined.

Cleavinger, P. J., D. H. Persing, et al. (2000). "Prevalence of TT virus infection in blood donors with elevated ALT in the absence of known hepatitis markers." The American Journal of Gastroenterology **95**(3): 772.

<http://www.sciencedirect.com/science/article/B6VHV-3YMW3JM-17/2/0cd317c89b86788a396026b4c783dea0>

OBJECTIVE: Recently a novel DNA virus (TT virus) has been identified in Japan and shown to be associated with elevated aminotransferase levels after blood transfusion. The exact role of TTV in the pathogenesis of liver disease is yet to be established. Our aim was to determine the prevalence and role of TTV in the pathogenesis of elevated transaminases in healthy blood donors in the absence of markers for viral hepatitis A-C. **METHODS:** Stored sera were collected from 99 healthy blood donors with elevated alanine amino transferase (ALT) values that were discovered at the time of blood donation. A total of 146 samples were obtained from healthy donors with normal ALT values who were used as controls. None of the patients or controls had a history of blood transfusion or had clinical signs of acute or chronic hepatitis. Serological markers for hepatitis A, hepatitis B, and hepatitis C viruses were negative. TTV DNA was amplified and

detected using polymerase chain reaction followed by gel electrophoresis. RESULTS: Five of 99 (5%) samples obtained from donors with elevated ALT had TTV DNA detected by PCR, as compared to one of 146 (0.7%) of those with normal ALT ($p = 0.006$). Among those with elevated ALT, mean ALT values in patients with TTV (296 ± 305 U/L) were higher than in patients without TTV (95 ± 37 U/L), but the difference was not statistically significant ($p = 0.08$). The two samples with highest ALT values (both >450 U/L) were among the five samples with detectable TTV DNA in serum. CONCLUSIONS: Although TTV is not likely to explain the majority of elevated ALT cases in otherwise healthy blood donors, TTV infection may potentially be associated with some cases. Based on these findings, we propose that the role of TTV in the pathogenesis of acute and chronic liver diseases merits further investigation.

Fan, X., H. Solomon, et al. (1999). "Comparison of genetic heterogeneity of hepatitis C viral RNA in liver tissue and serum." The American Journal of Gastroenterology **94**(5): 1347.

<http://www.sciencedirect.com/science/article/B6VHV-3WDKFDS-1G/2/10bb3579a45fa4077559289927f43cbf>

OBJECTIVE: Hepatitis C virus (HCV) is known to be heterogeneous and to circulate as a group of closely related quasispecies in individual patients, although hepatic viral genetic characteristics have not been well documented. METHODS: Matched serum and liver samples were tested by reverse transcription polymerase chain reaction amplification and single stranded conformation polymorphism analysis of the hypervariable portion of the E2/NS1 region of the HCV genome. The number of quasispecies was compared with the amount of HCV RNA, HCV genotyping, and infection with the hepatitis G virus. RESULTS: Sixteen of 40 patients had HCV RNA detectable in serum and liver. The HCV genotype was identical in serum and liver of all but one case. HCV RNA levels were approximately 10-fold higher in liver than serum. The number of HCV quasispecies in serum ranged between two and six (median 3.0) and in the liver between 2 and 19 (median 3.5, mean liver/serum ratio 1 to 6.3, median 1.8). The number of quasispecies in liver was equal to or greater than that in serum in all cases. HGV infection was found in 14 cases and did not influence serum or hepatic levels of HCV RNA. CONCLUSIONS: The number of hepatic HCV quasispecies usually exceeds that in serum, independent of the amount of HCV RNA and HCV genotype. This finding is compatible with clearance of some quasispecies from serum, but not liver, by putative neutralizing antibodies.

Lee, D. K., P. I. Tarr, et al. (1999). "Bacterial DNA in mixed cholesterol gallstones." The American Journal of Gastroenterology **94**(12): 3502.

<http://www.sciencedirect.com/science/article/B6VHV-3Y2N7PH-X/2/beceb2d28bcd5a6d168977aba9dd8d7f>

OBJECTIVE: Numerous investigators have proposed a role for bacteria in biliary lithogenesis. We hypothesized that bacterial DNA is present in gallstones, and that categorical differences exist between gallstone type and the frequency of bacterial sequences. METHODS: Polymerase chain reaction (PCR) was used to amplify bacterial 16S rRNA and uidA (encoding *Escherichia coli* [E. coli] [beta]-glucuronidase) genes in different types of gallstones. PCR products were sequenced. RESULTS: Bacterial 16S rRNA and uidA DNA sequences in *E. coli* were detected in all brown pigment, common bile duct, and mixed cholesterol gallstones ($n = 14$). In contrast, only one (14%) of seven pure cholesterol gallstones yielded a PCR product. Most (88%) mixed cholesterol gallstones yielded PCR amplification products from their central, as well as their outer, portions. Sequenced products possessed 88-98% identity to 16S rRNA genes of *E. coli* and *Pseudomonas* species. CONCLUSIONS: Bacterial DNA sequences are usually present in mixed cholesterol (to 95% cholesterol content), brown pigment, and common bile duct, but rarely in pure

cholesterol gallstones. The presence of bacterial [beta]-glucuronidase is also suggested. The role of bacteria and their products in the formation of mixed cholesterol gallstones, which comprise the majority of cholesterol gallstones, warrants further study.

Li, L., L. K. Kelly, et al. (1999). "Genotypes of *Helicobacter pylori* obtained from gastric ulcer patients taking or not taking NSAIDs." The American Journal of Gastroenterology **94**(6): 1502.

<http://www.sciencedirect.com/science/article/B6VHV-3WM59KY-N/2/d9739ce7cbd5a942f47613b70816f241>

OBJECTIVE: Whether *Helicobacter pylori* infection and use of nonsteroidal antiinflammatory drugs (NSAIDs) are independent risk factors for ulcerogenesis remains unclear. We undertook this study to evaluate *H. pylori* isolates from gastric ulcer patients to determine whether the genotype of the infecting isolate could be correlated with the use or nonuse of NSAIDs. **METHODS:** Fifty-two patients presenting with gastric ulcer and infected with *H. pylori* were included; 26 patients were taking NSAIDs or aspirin (ASA) regularly at the time of ulcer diagnosis. Polymerase chain reaction (PCR) was employed to assess the presence and mosaicism of the following *H. pylori* genes: *cagA*, *vacA*, *iceA*, and *picB*. **RESULTS:** We found no statistical differences in the presence of these genes in *H. pylori* isolates from gastric ulcer patients taking or not taking prescription NSAIDs or ASA. A 297-bp fragment of the *cagA* gene was detected in 96% of the isolates from the NSAID and ASA users and 100% from the non-NSAID users ($p = 1.0$). A larger and more variable region of the *cagA* gene was detected more frequently among the isolates from non-NSAID users than those from NSAID users ($p = 0.05$). Ninety-two percent of the isolates were identified as *vacA* genotype s1. The dominant *vacA* subtype was s1b, 76.9% and 65.4% in isolates from non-NSAID-taking or NSAID-taking patients, respectively ($p = 0.4$). *iceA1* genotype was not correlated with gastric ulcer as this allele was only detected in 17.3% of all isolates. **CONCLUSIONS:** No significant differences in the presence of the candidate virulence genes *vacA*, *cagA*, *picB*, or *iceA* were detected in isolates from gastric ulcer patients taking prescription NSAIDs or ASA, compared with those not taking these drugs, indicating that single gene presence does not allow discrimination of isolates that may be important in NSAID-induced ulcerogenesis. A variable region of the *cagA* gene was more frequently detected in isolates from patients not taking NSAIDs or ASA, suggesting that this gene may be modified by NSAID- or ASA-related factors or that certain strains may be selected for in patients taking these medications.

Miehlke, S., R. M. Genta, et al. (1999). "Molecular relationships of *Helicobacter pylori* strains in a family with gastroduodenal disease." The American Journal of Gastroenterology **94**(2): 364.

<http://www.sciencedirect.com/science/article/B6VHV-3VS1R98-M/2/b1b44137982511aac4a0f83d1c3fdb38>

Objective: Few studies have examined the genetic relationships of *Helicobacter pylori* strains affecting family members. Our aim was to do so. **Methods:** We characterized *H. pylori* isolates obtained from members of a single family presenting with various gastroduodenal diseases to examine *H. pylori* bacterial genetic similarity. Endoscopic evaluation with gastric mapping was performed on each individual to establish clinical and histological disease. Genomic DNA extracted from each *H. pylori* isolate was used to generate DNA fingerprints for each strain by REP-PCR. *vacA* genotypes and *cagA* presence were established by PCR. **Results:** Gastrointestinal diseases among the five members of this family included gastric adenocarcinoma in a 52-yr-old man (index patient), gastric MALT-lymphoma in the 73-yr-old mother; intestinal metaplasia (IV) and atrophic gastritis in the 48-yr-old brother; intestinal metaplasia (I-III) in the 47-yr-old brother, and a duodenal ulcer scar in the 42-yr-old sister. REP-PCR DNA fingerprints of *H.*

pylori isolates from the index patient, his mother, and both of his brothers were identical or highly similar. By contrast, the *H. pylori* DNA fingerprint from the sister was markedly different from the *H. pylori* DNA fingerprints from the other family members. All isolates had the genotype *cagA*-positive and *vacA* s1b/ml mosaic genotype. Conclusions: The DNA fingerprints of *H. pylori* strains obtained from members of this family with malignancy or premalignant histological disease were identical or highly similar and markedly different from the *H. pylori* DNA fingerprint from the sibling with duodenal ulcer disease. All *H. pylori* isolates within the family possessed genetic markers of enhanced virulence (presence of the *cagA* gene and *vacA* s1/ml mosaicism). In addition to host genetics and environmental factors, these findings suggest that infection with genetically similar *H. pylori* strains is a significant factor in determining the clinical outcome of an infection with *H. pylori*.

Miehlke, S., J. Yu, et al. (2001). "Helicobacter pylori *vacA*, *iceA*, and *cagA* status and pattern of gastritis in patients with malignant and benign gastroduodenal disease." The American Journal of Gastroenterology **96**(4): 1008.

<http://www.sciencedirect.com/science/article/B6VHV-42SPMRX-K/2/2e838906af31fee3f1f94d28d783a5e8>

OBJECTIVE:Both bacterial virulence factors and the pattern of *Helicobacter pylori* (*H. pylori*) gastritis may contribute to the development of clinically relevant gastroduodenal disease. The aim of our study was to investigate the frequency of *H. pylori vacA* alleles, *iceA*, and *cagA*, and the pattern of gastritis in patients with gastric cancer (GC), gastric lymphoma (MALT), duodenal ulcer (DU), and functional dyspepsia (FD). **METHODS:***H. pylori* was cultured from 141 patients (34 GC, 26 MALT, 49 DU, 32 FD). Allelic variants of *vacA* and *iceA*, and *cagA* were identified by polymerase chain reaction. Antrum and corpus biopsies were obtained for assessment of gastritis according to the updated Sydney System. **RESULTS:**The *vacA* s1,m1 genotype was more frequently detected in *H. pylori* from GC patients (70.6%) than from MALT, DU, and FD patients (*p* *iceA*1 and *cagA* did not differ among the groups. The proportion of patients with severe gastritis in the corpus was significantly higher in patients with GC and MALT compared with patients with DU (*p* *CONCLUSIONS:*In a German patient population, only the *vacA* s1,m1 genotype of *H. pylori* is associated with GC, and therefore may be useful to identify infected patients being at an increased risk for GC.

Niiyama, H., K. Mizumoto, et al. (2001). "Quantitative analysis of hTERT mRNA expression in colorectal cancer." The American Journal of Gastroenterology **96**(6): 1895.

<http://www.sciencedirect.com/science/article/B6VHV-436FB4H-1F/2/fcbbdc38fc55708e7c093603423c751c>

OBJECTIVES:Telomerase is highly activated in a variety of malignant neoplasms including colon cancer. Among the major components of telomerase, human telomerase reverse transcriptase (hTERT) is thought to regulate telomerase activity. To assess the importance of telomerase for the diagnosis of colorectal cancer, we measured the expression of hTERT mRNA and telomerase activity in a large series of 140 colorectal cancers, 140 adjacent normal tissues, and 20 adenomas. **METHODS:**The expression level of hTERT was measured quantitatively by competitive reverse transcriptase-polymerase chain reaction (RT-PCR), and telomerase activity was examined by telomeric repeat amplification protocol (TRAP) assay in the same samples. **RESULTS:**The median expression level of hTERT mRNA in carcinomas was significantly higher than that in either adenomas or normal tissues. The median level of hTERT in adenomas was significantly higher than that in normal tissues. Telomerase activities in carcinomas were significantly higher than those in either adenomas or normal tissues.

Telomerase activities in adenomas were also significantly higher than those in normal tissues. Furthermore, the relative expression levels of hTERT mRNA in adenomas and carcinomas were significantly correlated with the relative telomerase activities; the Spearman rank correlation was 0.53 ($p = 0.021$) and 0.18 ($p = 0.031$), respectively. **CONCLUSIONS:** Our data suggest that determination of hTERT mRNA by competitive RT-PCR is superior in quantitative accuracy and sensitivity and would support the importance of telomerase activity for the diagnosis of colorectal cancer.

Rosen, H. R., J. G. McHutchison, et al. (2002). "Tumor necrosis factor genetic polymorphisms and response to antiviral therapy in patients with chronic hepatitis C." The American Journal of Gastroenterology **97**(3): 714.

<http://www.sciencedirect.com/science/article/B6VHV-45BV9DH-1B/2/97137211543b79d3fa10d7996a44ac8b>

OBJECTIVE: Hepatitis C virus (HCV) is the major causal agent of non-A, non-B hepatitis and the leading indication for liver transplantation worldwide. The emerging field of immunogenetics has confirmed the significant role of heritability in host immune responses to infectious pathogens. Both the major and non-major histocompatibility complex genes are increasingly identified as candidate genes hypothesized to influence the susceptibility to, or the course of, a particular disease. We hypothesized that polymorphisms within the major histocompatibility complex class III region that encode for tumor necrosis factors (TNF)-[alpha] and TNF-[beta] might be predictive of response to antiviral therapy in patients with chronic hepatitis C. **METHODS:** A total of 155 subjects, including 110 HCV-seropositive individuals undergoing antiviral therapy and 45 ethnically similar HCV-negative controls, were studied. The HCV-positive patients had undergone antiviral treatment with either interferon monotherapy ($n = 73$) or in combination with ribavirin ($n = 37$) and were categorized as either nonresponders, sustained responders, or relapsers. Sixty (55%) patients had genotype 1 (1a or 1b). Genomic DNA was extracted, followed by polymerase chain reaction amplification and sequencing for two promoter TNF-[alpha] variants (at positions -238 and -308), as well as restriction fragment length analysis for four polymorphic loci within the TNF-[beta] gene (NcoI, TNFc, aa13, aa26). **RESULTS:** Although there was a trend toward higher frequency of the A allele in the TNF 238 promoter among HCV-infected patients (12% vs 4%), there were no significant differences in the distribution of the genotypic polymorphisms between patients and controls. Patients with the TNF 238 A allele had higher pretreatment viral loads as compared with patients homozygous for the wild type allele ($7.2 \times 10^6 \pm 4.2 \times 10^6$ copies/ml vs $3.8 \times 10^6 \pm 0.34 \times 10^6$ copies/ml, $p = 0.03$). However, there was no association between TNF genetic markers, including multiple haplotypic combinations, and response to therapy. In addition, there was no correlation with these polymorphic loci and histological severity of liver disease. **CONCLUSIONS:** Although previous work has suggested potential roles for TNF in the pathogenesis of HCV infection, we were unable to identify any link between TNF genetic polymorphisms and histological severity or response to antiviral therapy.

Sapone, A., D. Vaira, et al. (2003). "The clinical role of cytochrome p450 genotypes in Helicobacter pylori management." The American Journal of Gastroenterology **98**(5): 1010.

<http://www.sciencedirect.com/science/article/B6VHV-48THNFX-H/2/2c9f71f6309e57ad589cad0cd28bfc12>

Objective The aim of this pharmacogenomics study was to investigate the influence of different cytochrome P450 (CYP) genotypes in Helicobacter pylori eradication therapy. **Methods** The study involved 143 consecutive Italian Caucasian patients with H. pylori infection diagnosed and treated with 1-wk triple therapy according to European Helicobacter Pylori Study Group guidelines. Using

human genomic DNA, CYP2C19 (*2 and *3) and CYP3A4 alleles (*1B, *2, and *3) were evaluated by polymerase chain reaction-restriction fragment length polymorphism assays and confirmed by sequencing the amplicons. Results According to the endoscopy-based gold standard, 93 patients achieved H. pylori eradication. Regarding CYP2C19 genotype, the 50 patients who remained infected were all homozygous or heterozygous extensive metabolizers (homEM or hetEM). Carriers of homEM fared significantly less well than those of hetEM; homEM genotype was also predictive of failure at univariate/multivariate analysis. Carriers of CYP3A4 polymorphisms achieved favorable eradication rates similar to patients bearing CYP2C19. All four patients with single CYP3A4*2 polymorphism achieved eradication, and only 29% (5/17) of all CYP3A4*1B carriers did not achieve eradication. All nine patients carrying CYP3A4 polymorphisms in the CYP2C19 hetEM subgroup were cured, suggesting the possibility of a positive synergism between CYP3A4 and CYP2C19. Conclusions This first pharmacogenomics study on the influence of different CYP genotypes on H. pylori therapy suggests that, as in Asian populations, CYP2C19 genotype patterns are probably also relevant in Caucasians receiving H. pylori eradication regimens that include omeprazole. The possibility of a favorable drug interaction mediated by CYP2C19 and CYP3A4 requires investigation.

Tomer, G., C. Ceballos, et al. (2003). "NOD2/CARD15 variants are associated with lower weight at diagnosis in children with Crohn's disease." The American Journal of Gastroenterology **98**(11): 2479.

<http://www.sciencedirect.com/science/article/B6VHV-4B1S6DM-12/2/c3c80e0c9815b9fe65c1ce814c8f34fe>

Objectives NOD2/CARD15 variants have recently been shown to be associated with Crohn's disease (CD). No analysis of NOD2/CARD15 gene variants has so far been reported in pediatric patients. Therefore, our aim was to analyze NOD2/CARD15 gene variants in children with CD and to perform genotype-phenotype analyses. Methods We studied 101 children with CD and 136 healthy controls. Detailed phenotypic information was obtained from each patient. Patients were genotyped for the three NOD2/CARD15 variants R702W (single nucleotide polymorphism 8 [SNP8]), G908R (SNP12), and L1007fs (SNP13), and genotype-phenotype correlations were performed. Results We found 33 NOD2/CARD15 mutations in 29 of 101 patients (29%). The frequency of NOD2 variation was 31% in white (n = 87) compared with 11% in controls ([chi]² = 14; p = 0.0001; OR = 3.7; 95% CI = 1.7-7.8). Four white patients but not control subjects were compound heterozygotes. NOD2/CARD15 variants were significantly associated with ileal disease ([chi]² = 4.5; p = 0.03; OR = 5; 95% CI = 0.9-35.9). Of the children with NOD2/CARD15 variants, 44% were 2 = 8.7; p = 0.003; OR = 4.5; 95% CI = 1.4-14.4). Similar trends were observed for height but they did not reach statistical significance. Conclusions Our results demonstrate that: 1) the three NOD2/CARD15 variants confer risk to CD in children; 2) NOD2/CARD15 variants are associated with ileal disease in children as in adults; and 3) NOD2/CARD15 variants are associated with lower weight percentiles at diagnosis in children and a tendency toward lower height percentile, suggesting an association between growth in children with CD.

Truninger, K., H. Witt, et al. (2002). "Mutations of the serine protease inhibitor, Kazal type 1 gene, in patients with idiopathic chronic pancreatitis." The American Journal of Gastroenterology **97**(5): 1133.

<http://www.sciencedirect.com/science/article/B6VHV-45R57F5-K/2/0f407e48dc8f24dc69e4af18d8e0177b>

OBJECTIVE: The pathogenesis of chronic pancreatitis (CP) is poorly understood. Genetic studies

revealed mutations in the cationic trypsinogen gene and an increased frequency of cystic fibrosis gene mutations in patients with CP. Recently, a point mutation (N34S) in the gene encoding the serine protease inhibitor, Kazal type 1 (SPINK1), was found in approximately 20% of patients with CP. The aim of our study was to determine the frequency of the N34S SPINK1 gene mutation in a well-defined patient cohort with idiopathic CP (ICP) and to compare the incidence with healthy controls. In addition, we investigated the impact of this mutation on the long-term course of CP. **METHODS:** Fourteen patients with early-onset and four patients with late-onset CP of our well-defined pancreatitis cohort were enrolled in the present study, and 397 healthy individuals served as a control population. Coding exonic and the flanking intronic sequences of SPINK1 were investigated by direct DNA sequencing. The mutations found were confirmed by melting curve analysis. In addition, the N34S mutation was detected by analyzing the DNA fragments generated by digestion with restriction enzyme TspR I. Clinical data of patients with the N34S mutation were compared with those without mutations. **RESULTS:** The N34S mutation was detected in six of 14 (43%) patients with early-onset ICP. One patient was homozygous, and five patients were heterozygous for this mutation. The N34S mutation in a heterozygous state was found in four of 397 healthy controls (1.0%). The different allele frequency observed (seven of 28 vs four of 794) was significant (odds RATIO = 66, 95% CI = 18-242, p < 0.05). **CONCLUSIONS:** Our results indicate that the N34S mutation in the SPINK1 gene is strongly associated with ICP, especially with the early-onset type. The natural course is similar in patients with mutations compared with SPINK1 mutation-negative patients. The N34S mutation may easily be screened for by restriction digestion with TspR I.

Utsunomiya, S., K. Yoshioka, et al. (1999). "TT virus infection in hemodialysis patients." The American Journal of Gastroenterology **94**(12): 3567.

<http://www.sciencedirect.com/science/article/B6VHV-3Y2N7PH-17/2/bac4101909ec6c734dce78590c06014b>

OBJECTIVE: Recently, TT virus (TTV), associated with posttransfusion hepatitis, was discovered. Prevalence of TTV infection in maintenance hemodialysis (HD) units and its pathogenicity to liver was investigated. **METHODS:** A total of 115 patients on HD were assessed for presence of serum TTV. DNA was purified from sera, and nested polymerase chain reaction was done for the detection of TTV DNA. **RESULTS:** TTV was detected in 59 patients on HD (51.3%), as compared with healthy blood donors (15 of 91 [16.5%], p = 15 IU/L in TTV-positive patients (14 of 18) than in TTV-negative patients (five of 15) (p < 0.05). **CONCLUSIONS:** TTV infection is remarkably prevalent in patients on HD and in healthy blood donors. It is suggested that TTV generally does not cause liver disease by itself, but there remains the possibility that TTV may aggravate liver disease caused by HCV.

Watanabe, H., T. Saito, et al. (2000). "Clinical implications of TT virus superinfection in patients with chronic hepatitis C." The American Journal of Gastroenterology **95**(7): 1776.

<http://www.sciencedirect.com/science/article/B6VHV-40PXN7S-10/2/a7327a3d7e7e213b378c914ca17356cf>

OBJECTIVE: TT virus (TTV) has been identified as a candidate agent of non-A-E hepatitis virus. We investigated superinfection of TTV in patients with chronic hepatitis C and studied the susceptibility to interferon (IFN) treatment and its association with liver disease caused by hepatitis C virus (HCV). **METHODS:** TTV DNA was examined using the seminested polymerase chain reaction (PCR), and its virus level was measured by the real-time fluorometric PCR. **RESULTS:** TTV DNA was detected in 20 of 102 (19.6%) patients examined. There was no significant difference in the alanine aminotransferase (ALT) level between patients with or without

TTV DNA. Quantitative analysis of HCV RNA and TTV DNA revealed no correlation between virus levels in HCV/TTV-coinfected patients. Both TTV and HCV were sensitive to IFN therapy. Complete response to IFN with a sustained loss of viremia for 24 wk after completion of IFN treatment was found in 11 of 20 (55%) patients with respect to TTV DNA and in five of 20 (25%) patients with respect to HCV RNA. The mean pretreatment HCV RNA level was significantly lower in the complete-response cases than in the no-response cases, but there was no significant difference in the pretreatment TTV DNA levels between them. ALT normalization resulting from IFN therapy was not attributable to the eradication of TTV DNA but was attributable to that of HCV RNA. Superinfection by TTV did not influence the effect of IFN against HCV. No specific TTV genotype correlating with IFN sensitivity was found. CONCLUSIONS: These results suggest that TTV infection stands independent of HCV infection, with no influence on liver injury as a result of HCV infection.

Yamaguchi, K., K. Chijiwa, et al. (2000). "Ki-ras codon 12 point and P53 mutations: a molecular examination of the main tumor, liver, portal vein, peripheral arterial blood and para-aortic lymph node in pancreatic cancer." The American Journal of Gastroenterology **95**(8): 1939.

<http://www.sciencedirect.com/science/article/B6VHV-40YYFTJ-J/2/068a20f648c4209f45000d87dc2940f>

OBJECTIVE: Frequent P53 mutations and Ki-ras codon 12 point mutations have been reported in pancreatic cancer. Pancreatic cancer often recurs in the liver and/or lymph nodes shortly after a surgical resection. The purpose of this study is to elucidate the occurrence of microcirculating cancer cells and micrometastasis in pancreatic cancer. METHODS: P53 mutations and Ki-ras codon 12 point mutations were examined in the main tumor, liver, portal vein, and peripheral arterial blood, and para-aortic lymph nodes of patients with pancreatic cancer using molecular examinations. RESULTS: P53 mutations in the main tumor were present in nine (29%) of 31 patients with pancreatic cancer, whereas a Ki-ras codon 12 point mutation was evident in 18 (62%) of 29 examined patients. The peripheral arterial and portal vein blood and liver were positive for gene abnormalities in one (5%) of 21, in none (0%) of 19, and in one (1%) of 20, respectively. A P53 mutation in the main tumor was evident in none (0%) of seven stage I or II carcinomas and in nine (38%) of 24 stage III or IV cases, whereas a Ki-ras codon 12 point mutation was present in four (67%) of six stage I or II cases and in 14 (61%) of 23 stage III or IV cases. In addition, 15 (71%) of 21 patients with gene abnormalities (Ki-ras codon 12 point and/or p53 mutation) in the main tumor showed lymph node metastasis at surgery, whereas five (42%) of 12 without gene abnormalities did not demonstrate lymph node metastasis. Two (29%) of six patients with gene abnormalities in the main tumor and without metastatic disease at surgery developed liver metastasis within 6 months after surgery, whereas all five (100%) without the gene abnormalities and metastatic disease at surgery did not develop the metastasis, with the sensitivity being 100%, specificity 44%, the predictive value of the positive test 36%, and the predictive value of the negative test 100%. Two patients who had gene abnormalities in the para-aortic lymph node were free from histopathological metastasis and these two patients developed para-aortic lymph node metastasis within 6 months after surgery. CONCLUSIONS: A molecular examination of Ki-ras codon 12 and p53 mutations therefore enables us to predict, to some degree, the occurrence of liver and lymph node metastasis in pancreatic carcinoma.

Zein, N. N., M. Arslan, et al. (1999). "Clinical significance of TT virus infection in patients with chronic hepatitis C." The American Journal of Gastroenterology **94**(10): 3020.

<http://www.sciencedirect.com/science/article/B6VHV-3XJTJDC-1P/2/b3307d268d21cfca9c7cf3bf60ee2059>

OBJECTIVE: The TT virus (TTV) is a novel DNA virus that has recently been identified. The clinical significance of TTV infection in patients with chronic hepatitis C has not been determined. The aim of this study was to determine the prevalence and possible role of TTV in a well characterized population with chronic hepatitis C infection. **METHODS:** Ninety patients with chronic HCV and known time of HCV acquisition were selected from approximately 250 patients followed at our institution. Characteristics including age, sex, histology, and length of disease were recorded. Direct sequencing of the NS5 region was used for HCV genotyping. TTV DNA detection was based on PCR. **RESULTS:** TTV infection was present in 24 of 90 (27%) HCV patients. Patients were divided into four groups based on stage of disease; chronic hepatitis (CH, 29 patients), compensated cirrhosis (CC, 17 patients), decompensated cirrhosis (DC, 28 patients), and hepatocellular carcinoma (HCC, 16 patients). TTV was present in 2/29 (7%), 2/17 (12%), 11/28 (39%), and 9/16 (56%) in those with CH, CC, DC, and HCC respectively. TTV was significantly more prevalent among those with advanced disease (DC and HCC) compared to those with stable disease (CH and CC; $p = 0.001$). Mean age, sex, and the time from exposure to HCV to development of complications were similar in TTV-positive and -negative patients. TTV infection was more common in patients infected with HCV genotype 1b. Univariate analysis showed that length of HCV infection, HCV genotype 1b, and TTV infection were important in predicting the stage of liver disease in HCV patients. However, after adjusting for length of HCV infection, TTV but not HCV genotype was important in predicting the stage of liver disease. **CONCLUSIONS:** We conclude that 1) TTV infection is common in patients with chronic HCV; 2) TTV infection is more prevalent among patients with advanced HCV-associated liver disease (DC and HCC) than in those with stable disease (CH and CC); and 3) TTV infection is more common in patients with HCV genotype 1b but is independent from genotype in predicting the stage of HCV-associated liver disease.

Zhou, Z., X.-y. Lin, et al. (2002). "Variation at NOD2/CARD15 in familial and sporadic cases of Crohn's disease in the Ashkenazi Jewish population." *The American Journal of Gastroenterology* **97**(12): 3095.

<http://www.sciencedirect.com/science/article/B6VHV-47C72J8-10/2/9bbcb5282aaa0764a97e0d0fa87f552>

OBJECTIVE: Recent reports indicate that allelic variants in NOD2/CARD15 are associated with Crohn's disease (CD) susceptibility, and that homozygosity or compound heterozygosity at this locus for any of three recently defined sequence variants confers a greatly increased risk of CD. These sequence changes include two missense mutations, R702W and G908R, and a frameshift insertion, 1007insC. The aim of this study was to determine the frequency of these NOD2/CARD15 variants in familial and sporadic CD patients in the Ashkenazi population and to determine their effects on disease susceptibility and age of disease onset (AOO). **METHODS:** Allele and genotype frequencies of these three variants were determined in 481 CD patients of Jewish descent and 110 Jewish controls; 169 patients had a family history of CD, and 312 were "sporadic" cases. Variants were detected by polymerase chain reaction using allele-specific primers labeled with fluorescent dye. **RESULTS:** Familial cases had a significantly higher frequency of the G908R variant than sporadic cases (0.127 vs 0.059, $p = 0.0003$) and correspondingly, a significantly higher proportion of homozygotes and compound heterozygotes (11.8% vs 4.5%, $p = 0.0027$). Homozygotes and compound heterozygotes had an OR for CD of 14.6 for familial cases and 5.1 for sporadic cases. There was no increased risk of CD for simple heterozygotes. The AOO was significantly lower for CD patients who were homozygotes and compound heterozygotes for NOD2/CARD15 (17.5 vs 22.4 yr, $p = 0.04$), but only for familial cases. **CONCLUSIONS:** NOD2/CARD15 contributes more to CD susceptibility in familial cases than in sporadic cases, and to an earlier AOO. There is no increased risk of CD for individuals carrying only a single copy of these NOD2/CARD15 variants, whereas individuals carrying two copies have a 5-15-fold increased risk. The penetrance of the NOD2/CARD15 mutations was estimated at less than 1%.

The American Journal of Medicine (1)

Goodman, J. L., J. F. Bradley, et al. (1995). "Bloodstream invasion in early Lyme disease: Results from a prospective, controlled, blinded study using the polymerase chain reaction." The American Journal of Medicine **99**(1): 6.

<http://www.sciencedirect.com/science/article/B6TDC-40SFT42-1N/2/2ecb6c8539a3cd36e11d6baa3b07fe10>

The Annals of Thoracic Surgery (2)

Koyanagi, K., S. Ozawa, et al. (2002). "Clinical significance of telomerase activity in peripheral blood of patients with esophageal squamous cell carcinoma." The Annals of Thoracic Surgery **73**(3): 927.

<http://www.sciencedirect.com/science/article/B6T11-45B56DP-21/2/423fe7ff5535af53ed1c1cbb9656f753>

Background. The presence of tumor cells in the blood stream is considered evidence of a high risk of distant organ metastasis. We examined the usefulness of telomerase activity in peripheral blood polymorphonuclear cells as an indicator of distant metastasis in patients with esophageal squamous cell carcinoma. Methods. Telomerase activity was measured in the peripheral blood mononuclear cell and polymorphonuclear cell fractions obtained from blood samples of healthy volunteers mixed with squamous cell carcinoma cell lines, and cell distribution was analyzed by flow cytometry. Then telomerase activity of forty-two polymorphonuclear cell fractions obtained from esophageal squamous cell carcinoma patients was measured. Results. Telomerase activity was detected in polymorphonuclear cell fractions and cell distribution analysis revealed the presence of esophageal squamous cell carcinoma cells. Organ metastasis was detected in 7 (78%) of the 9 patients with telomerase-positive polymorphonuclear cell fractions as opposed to only five (15%) of the 33 with telomerase-negative cases, and there was a significant positive correlation between telomerase activity and organ metastasis (p Conclusions. Measurement of telomerase activity in the polymorphonuclear cell fractions is useful for identifying a high risk group for distant organ metastasis in patients with esophageal squamous cell carcinoma.

Lee, L. Y., S. R. Patel, et al. (2000). "Focal angiogen therapy using intramyocardial delivery of an adenovirus vector coding for vascular endothelial growth factor 121." The Annals of Thoracic Surgery **69**(1): 14.

<http://www.sciencedirect.com/science/article/B6T11-3YC05G7-5/2/a0d5fc1c1472117f1b3426c4efafa3a0>

Background. Adenovirus (Ad) vector-mediated gene therapy strategies have emerged as

promising modalities for the "biological revascularization" of tissues. We hypothesized that direct intramyocardial, as opposed to intracoronary, administration of an Ad vector coding for the vascular endothelial growth factor 121 cDNA (AdGVVEGF121.10) would provide highly focal Ad genome levels, and increases in VEGF, ideal for inducing localized therapeutic angiogenesis. **Methods.** Persistence and regional distribution of the vector were assessed by TaqMan real-time quantitative polymerase chain reaction technology and enzyme-linked immunosorbent assay, after intramyocardial AdGVVEGF121.10 in the rat, and either intramyocardial or intracoronary (circumflex territory) vector in Yorkshire swine. Based on these results, we assessed the focal nature of the improved cardiac blood flow in a previously reported porcine myocardial ischemia model. **Results.** Intramyocardial delivery of AdGVVEGF121.10 in the rat resulted in local persistence of the Ad genome that decreased 1,000-fold over 3 weeks, with peak myocardial VEGF expression 24 to 72 h after vector delivery. After intramyocardial AdGVVEGF121.10 in the circumflex distribution of pigs, Ad vector genome and VEGF protein levels were more than 1,000-fold and more than 90-fold higher, respectively, in this distribution than in other myocardial regions. In comparison, intracoronary injection yielded maximum myocardial Ad genome and VEGF levels 33-fold and 9-fold lower, respectively, than that after intramyocardial delivery. Angiograms obtained 28 days after intramyocardial AdGVVEGF121.10 demonstrated rapid circumflex reconstitution via collaterals localized to the region of vector administration. **Conclusions.** These studies demonstrate that direct intramyocardial administration of AdGVVEGF121.10 results in focal genome and VEGF levels, including focal angiogenesis, sufficient to normalize blood flow to the ischemic myocardium, findings that are relevant to designing human trials of gene therapy-mediated cardiac angiogenesis.

The International Journal of Biochemistry & Cell Biology (6)

Edgar, A. J. and J. M. Polak (2001). "Flotillin-1: gene structure: cDNA cloning from human lung and the identification of alternative polyadenylation signals." The International Journal of Biochemistry & Cell Biology **33**(1): 53.

<http://www.sciencedirect.com/science/article/B6TCH-4292G8K-6/2/b5ecf3c65d6d1abbb50f65e9b300196f>

To identify changes in gene expression associated with emphysema, differential display was used to compare RNA extracted from emphysematous lung with that of unused donor tissue taken at the time of transplant. Two expressed clones with sequence homology to the 3' UTR of the murine flotillin-1 cDNA were identified. Flotillin-1 is a plasma membrane protein, which has been associated with detergent-insoluble glycolipid-rich domains and the formation of caveolae. One clone was 95 bp longer than the other. It arose from the use of a second polyadenylation signal and its existence was not due to differential expression nor to polymorphisms in the human flotillin-1 sequence. The 1839 bp human flotillin-1 sequence was completed by 5' RACE from a lung cDNA library. The human mRNA has a 1.9 kb transcript being highly expressed in brain, heart and lung. The single copy flotillin-1 gene is located at 6p21.3 in the MHC class I region and consists of 13 exons over 15 kb. The ORF encodes a 427 residue protein with a molecular mass 47355 Da, and an isoelectric point 7.08. Human flotillin-1 has a 98% identity with the murine protein and a 47% identity with human flotillin-2. Flotillin-1 belongs to the Band 7.2/stomatins protein family, possessing a hydrophobic N-terminal region, predicted to form a single, outside to inside, transmembrane domain. The long central [alpha]-helical domain may form a coiled-coil. We have isolated and characterised a cDNA encoding the human flotillin-1 gene, which may play an important role in raft formation.

Kou, S.-Y. and N. S. Cohen (1998). "Ethanol feeding produces deficiencies in left ventricle total RNA, total DNA and mitochondrial ribosomal RNA." The International Journal of Biochemistry & Cell Biology **30**(4): 475.

<http://www.sciencedirect.com/science/article/B6TCH-3SY2FGJ-8/2/adcb58a73ac71b02143a990d991384e1>

Chronic alcoholism causes a variety of ultrastructural, biochemical and functional alterations in the myocardium, but the underlying mechanisms are not well understood. Molecular changes that developed in the left ventricles of rats fed for 1 to 24 weeks on liquid diets containing ethanol as 36% of total calories were analyzed. Total tissue RNA and DNA were chemically extracted and measured by spectroscopic methods; mitochondrial DNA and mitochondrially-coded ribosomal RNA were measured at the 12s rRNA region by a quantitative polymerase chain reaction method; mitochondrial protein and enzyme activities were assayed. Ethanol-fed rats had 83.9 $\pm$ 2.9% (mean $\pm$ S.E.M.) as much DNA/g tissue and 74.7 $\pm$ 3.9% as much total left ventricle DNA as pair-fed controls (PPP<0.001). Total left ventricle 12s rRNA was <40% of normal. There was little or no change in mitochondrial DNA levels measured at the 12s location. Mitochondrial cytochrome contents were reduced 26-38% in the ethanol-fed rats, but only after 24 weeks. This study shows that experimental alcoholism produces rapid and sustained decreases in left ventricle total RNA and DNA and mitochondrial ribosomal RNA. The observed effects would be expected to have a major impact on left ventricle structural integrity and functional capacity.

Puddu, P., P. Borghi, et al. (1998). "Antiviral effect of bovine lactoferrin saturated with metal ions on early steps of human immunodeficiency virus type 1 infection." The International Journal of Biochemistry & Cell Biology **30**(9): 1055.

<http://www.sciencedirect.com/science/article/B6TCH-3TYNMH7-F/2/6ba6f6f79b1359590d28038652d3f4a4>

Lactoferrin is a mammalian iron-binding glycoprotein present in many biological secretions, such as milk, tears, semen and plasma and a major component of the specific granules of polymorphonuclear leucocytes. The effect of bovine lactoferrin (BLf) in apo-form or saturated with ferric, manganese or zinc ions, on human immunodeficiency virus type 1 (HIV-1) infection in the C8166 T-cell line was studied. Both HIV-1 replication and syncytium formation were efficiently inhibited, in a dose-dependent manner, by lactoferrins. BLf in apo and saturated forms markedly inhibited HIV-1 replication when added prior to HIV infection or during the virus adsorption step, thus suggesting a mechanism of action on the HIV binding to or entry into C8166 cells. Likewise, the addition of Fe³⁺+BLf prior to HIV infection and during the attachment step resulted in a marked reduction of the HIV-1 DNA in C8166 cells 20 h after infection. The potent antiviral effect and the high selectivity index exhibited by BLf suggest for this protein, in apo or saturated forms, an important role in inhibiting the early HIV-cell interaction, even though a post adsorption effect cannot be ruled out.

Suchanek, K. M., F. J. May, et al. (2002). "Peroxisome proliferator-activated receptor [beta] expression in human breast epithelial cell lines of tumorigenic and non-tumorigenic origin." The International Journal of Biochemistry & Cell Biology **34**(9): 1051.

<http://www.sciencedirect.com/science/article/B6TCH-45BHDN9-2/2/48e5c3994b13259ea5162950f8ce2736>

Peroxisome proliferator-activated receptor [beta] (PPAR[beta]) is a member of the nuclear hormone receptor superfamily and is a ligand activated transcription factor, although the precise genes that it regulates and its physiological and pathophysiological role remain unclear. In view of the association of PPAR[beta] with colon cancer and increased mRNA levels of PPAR[beta] in colon tumours we sought in this study to examine the expression of PPAR[beta] in human breast epithelial cells of tumorigenic (MCF-7 and MDA-MB-231) and non-tumorigenic origin (MCF-10A). Using quantitative RT-PCR we measured PPAR[beta] mRNA levels in MCF-7, MDA-MB-231 and MCF-10A cells at various stages in culture. After serum-deprivation, MDA-MB-231 and MCF-10A cells had a 4.2- and 3.8-fold statistically greater expression of PPAR[beta] compared with MCF-7 cells. The tumorigenic cell lines also exhibited a significantly greater level of PPAR[beta] mRNA after serum deprivation compared with subconfluence whereas such an effect was not observed in non-tumorigenic MCF-10A cells. The expression of PPAR[beta] was inducible upon exposure to the PPAR[beta] ligand bezafibrate. Our results suggest that unlike colon cancer, PPAR[beta] overexpression is not an inherent property of breast cancer cell lines. However, the dynamic changes in PPAR[beta] mRNA expression and the ability of PPAR[beta] in the MCF-7 cells to respond to ligand indicates that PPAR[beta] may play a role in mammary gland carcinogenesis through activation of downstream genes via endogenous fatty acid ligands or exogenous agonists.

Torres, J. M. and E. Ortega (2004). "Quantitation of mRNA levels of steroid 5[alpha]-reductase isozymes: a novel method that combines quantitative RT-PCR and capillary electrophoresis." The International Journal of Biochemistry & Cell Biology **36**(1): 78.

<http://www.sciencedirect.com/science/article/B6TCH-48NJ0G6-2/2/a7a9b75d95fd3414f4d1c2f59645e6d9>

A novel, accurate, rapid and modestly labor-intensive method has been developed to quantitate specific mRNA species by reverse transcription-polymerase chain reaction (RT-PCR). This strategy combines the high degree of specificity of competitive PCR with the sensitivity of laser-induced fluorescence capillary electrophoresis (LIF-CE). The specific target mRNA and a mimic DNA fragment, used as an internal standard (IS), were co-amplified in a single reaction in which the same primers are used. The amount of mRNA was then quantitated by extrapolation from the standard curve generated with the internal standard. PCR primers were designed to amplify both a 185 bp fragment of the target cDNA for steroid 5[alpha]-reductase 1 (5[alpha]-R1) and a 192 bp fragment of the target cDNA for steroid 5[alpha]-reductase type 2 (5[alpha]-R2). The 5' forward primers were end-labeled with 6-carboxy-fluorescein (6-FAM). Two synthetic internal standard DNAs of 300 bp were synthesized from the sequence of plasmid pEGFP-C1. The ratio of fluorescence intensity between amplified products of the target cDNA (185 or 192 bp fragments) and the competitive DNA (300 bp fragment) was determined quantitatively after separation by capillary electrophoresis and fluorescence analysis. The accurate quantitation of low-abundance mRNAs by the present method allows low-level gene expression to be characterized.

Yang, Z., A. Paterson, et al. (1999). "Overexpression of sense or antisense human gastrin mRNA does not affect proliferation of normal rat kidney fibroblasts." The International Journal of Biochemistry & Cell Biology **31**(3-4): 509.

<http://www.sciencedirect.com/science/article/B6TCH-3VYXT6G-K/2/d8028985b1a3e515d543818207d133fb>

Progastrin-derived peptides have been reported to stimulate mitogenesis in Swiss 3T3 fibroblasts [P. Singh, A. Owlia, R. Espejo, B. Dai, Novel gastrin receptors mediate mitogenic effects of gastrin and processing intermediates of gastrin on Swiss 3T3 fibroblasts: Absence of detectable

cholecystokinin (CCK)-A and CCK-B receptors. J. Biol. Chem. 270 (1995) 8429-8438]. The aim of the present study was to determine the generality of these findings, by investigating the effect of endogenous and exogenous progastrin-derived peptides on the proliferation of the normal rat kidney fibroblast cell line NRK. Levels of endogenous progastrin-derived peptides were modified by stable transfection of NRK cells with tetracycline-repressible plasmids containing sequences encoding human gastrin in either the sense or antisense orientation. Expression of sense and antisense gastrin mRNA was demonstrated by reverse transcriptase PCR and by radioimmunoassay, and cell proliferation rates were determined by the colorimetric MTT assay. Sense clones produced full length human progastrin, but significant quantities of glycine-extended or amidated gastrin17 were not detected. Concentrations of endogenous rat progastrin in antisense clones were significantly lower than concentrations in clones transfected with vector only. However no difference in proliferation rate was observed between sense, antisense and vector-transfected clones. No stimulation of proliferation was observed in synchronised untransfected NRK cells after supplementation of media with gastrin17 or gastrin17gly in the concentration range 0.3 to 100 nM. Our results do not provide evidence in support of the hypothesis that endogenous or exogenous progastrin-derived peptides act as growth factors in NRK fibroblasts.

The Journal of Heart and Lung Transplantation (4)

Baan, C. C., A. H. M. M. Balk, et al. (2000). "Renal failure after clinical heart transplantation is associated with the TGF- β 1 codon 10 gene polymorphism." The Journal of Heart and Lung Transplantation **19**(9): 866.

<http://www.sciencedirect.com/science/article/B6VSG-417WC22-8/2/4955b9ff7078ef9f4a831f21c41e495a>

Background: To determine whether genetic factors are involved in the development of renal dysfunction due to cyclosporine nephrotoxicity, we analyzed 2 polymorphisms in the signal sequence of the transforming growth factor (TGF)- β 1 gene; codon 10 (Leu10 $\rightarrow$ Pro) and codon 25 (Arg25 $\rightarrow$ Pro). **Method** Using sequence specific oligonucleotide probing, we analyzed both TGF- β 1 gene polymorphisms in cardiac allograft recipients (n = 168) who survived at least 1 year with minimal follow-up of 7 years. Patients received cyclosporine and steroids as maintenance immunosuppressive therapy. Renal dysfunction was defined as a serum creatinine $\geq$ 250 μ mol/liter. **Results** Renal dysfunction was observed in 2% (3/168) of the patients at 1 year, in 7% (11/160) at 3 years, in 12% (18/152) at 5 years, and in 20% (26/131) at 7 years post-transplantation. The genotypic distributions for TGF- β 1 codon 10 were 7% Pro/Pro, 61% Pro/Leu, and 32% Leu/Leu, and for codon 25 these percentages were 1% Pro/Pro, 12% Pro/Arg, and 87% Arg/Arg. We found an association between the TGF- β 1 genotype encoding proline at codon 10 and renal dysfunction. At 7 years post-transplantation, 26% (23/89) of the patients with the heterozygous Pro/Leu or homozygous Pro/Pro genotype had renal dysfunction vs only 7% (3/42) of the patients with the homozygous Leu/Leu genotype (p = 0.017). For the TGF- β 1 codon 25 genotypes, we found no association between TGF- β 1 genotypes and renal dysfunction. **Conclusion** Our data support the hypothesis that TGF- β 1 is involved in the process leading to renal insufficiency in cyclosporine-treated cardiac allograft recipients. In these patients the presence of TGF- β 1 Pro10 might be a risk factor.

Holweg, C. T. J., C. C. Baan, et al. (2001). "TGF-[beta]1 Gene polymorphisms in patients with end-stage heart failure." The Journal of Heart and Lung Transplantation **20**(9): 979.

<http://www.sciencedirect.com/science/article/B6VSG-43XF47H-9/2/5725333f2421e686eeb92b119e04f902>

Background: The regulatory cytokine transforming growth factor (TGF)-[beta]1 is thought to play a role in atherosclerotic heart disease as well as in idiopathic cardiomyopathy. The production of TGF-[beta]1 is genetically controlled as polymorphisms in the signaling sequence of the TGF-[beta]1 gene leucine10->proline and arginine25->proline are involved in the regulation of the protein production level. We investigated whether these polymorphisms are associated with end-stage heart failure caused by dilated cardiomyopathy (CMP) or ischemic heart disease (IHD). **Methods:** We determined polymorphisms using sequence specific oligonucleotide probing (SSOP) in genomic DNA samples from heart transplant recipients (n = 253) and controls (n = 94). Indications for transplantation were dilated CMP (n = 109) and IHD (n = 144). **Results:** We found a difference in TGF-[beta]1 codon 10 genotype distribution among patients with IHD, dilated CMP, and controls (p = 0.034; [chi]2 test). Patients with dilated CMP differed from patients with IHD (p = 0.044) and healthy controls (0.017). The genotype distribution between patients with IHD and controls was comparable. For codon 25, we found no difference in genotype distribution. **Conclusions:** The Leu10->Pro (codon 10) polymorphism in the TGF-[beta]1 gene is associated with end-stage heart failure caused by dilated CMP and not with IHD. This observation suggests that TGF-[beta]1 is involved in the pathogenesis of CMP.

Kittur, D. S., C. Wilasrusmee, et al. (2002). "Locally derived cytokines and upregulation of MHC class II genes in allografts." The Journal of Heart and Lung Transplantation **21**(8): 882.

<http://www.sciencedirect.com/science/article/B6VSG-46FJ5CV-8/2/86066d35d4d47574fef98761c3199f5d>

Background In vitro, various cytokines can modulate the level of expression of major histocompatibility complex (MHC) Class II antigens. Major histocompatibility complex Class II hyperexpression occurs in many immunologic disorders in vivo, but the cytokines that affect this are difficult to analyze because they are produced in small amounts, they act locally, and their mRNAs have short half-lives. **Methods** We studied the expression of cytokines known to up-regulate MHC Class II genes in heart allografts in mice from B10.BR donors to B10.D2 recipients by reverse transcription of mRNA and polymerase chain reaction amplification. The I-A[beta]k gene expression was also studied in the same fully MHC incompatible strain combination. **Results** Messenger RNA for interferon (INF)-[gamma], interleukin (IL)-4, and tumor necrosis factor (TNF)-[alpha], known inducers of MHC Class II expression in vitro, could be detected in allografts either 24 hours before or simultaneously with massive induction of graft specific I-A[beta] mRNA. Interleukin-6 mRNA could be detected as early as 1 day after grafting. **Conclusions** These data suggest that known cytokine up-regulators of MHC Class II genes, i.e., INF-[gamma], IL-4, and TNF-[alpha] may contribute to the upregulation of graft-specific MHC Class II antigens during an allograft reaction. Also, IL-6 expression in allografts may result from the stress of the grafting procedure, as it is evident very early after grafting.

Ross, D. J., A. Moudgil, et al. (1999). "Lung allograft dysfunction correlates with [gamma]-interferon gene expression in bronchoalveolar lavage." The Journal of Heart and Lung Transplantation **18**(7): 627.

<http://www.sciencedirect.com/science/article/B6VSG-3X1W73Y->

Background: Preceding episodes of acute cellular rejection (ACR) may predispose lung allografts to the subsequent development of irreversible dysfunction or bronchiolitis obliterans syndrome (BOS). Other histologic patterns such as bronchiolitis obliterans with organizing pneumonia (BOOP), organizing pneumonia, lymphocytic bronchiolitis and diffuse alveolar damage (DAD) may also adversely affect allograft function. We have previously reported the predominant expression of Th1 cytokines (IL-2 and interferon [gamma]) in rejecting and Th2 (IL-10) in a tolerant model of rat lung transplantation. Here we correlate the "Th1/Th2 paradigm" in clinical lung transplantation with histologic findings and assess the effect on serial spirometric function. Methods We examined the mRNA expression of IL-2, interferon [gamma], IL-10 and ICAM-1 in 53 bronchoalveolar lavage (BAL) specimens from 23 lung transplant (LT) recipients utilizing qualitative "nested" reverse transcriptase polymerase chain reaction (RT-PCR). We also measured IgG1 and IgG2 levels in 44 BAL specimens by ELISA. The mRNA expression for cytokines, ICAM-1 and the IgG2/IgG1 ratios were correlated with the presence or absence of ACR and alternate "histologic patterns." Serial spirometry were analyzed for the 2-3 month interval before bronchoscopic (FOB) assessment to derive "baseline" forced expiratory volume-one second (FEV1) values. The change in FEV1 coincident with ([Delta]FEV1 pre) and for the 2-3 month interval subsequent to ([Delta]FEV1 post) FOB were expressed relative to "baseline" spirometric indexes. Results Detection of mRNA for interferon [gamma] and ICAM-1 correlated significantly with ACR, whereas IL-2 and IL-10 expression did not correlate. IL-10 was virtually "ubiquitous" in most BAL samples irrespective of the presence or absence of ACR. The highest correlation was observed with interferon [gamma] for acute cellular rejection whereupon the sensitivity was 77.7%, specificity 87.7%, positive predictive value 73.6% and negative predictive value 88.2%, although for ICAM-1 these values were 75%, 65.7%, 50.0% and 85.0%, respectively. Nevertheless, 4 of 5 episodes of respiratory tract infection (bacterial, CMV, *Aspergillus* spp.) were similarly associated with cytokine mRNA. The ratios of IgG2 to IgG1, a reflection of Th1/Th2 influence, were not statistically different when analyzed for the presence or absence of ACR (0.91 +/- 0.53 vs. 1.02 +/- 0.70, respectively; p = NS). By analysis of FEV1 trends, expression of interferon [gamma] was associated with a greater and persistent decrement ([Delta]FEV1 pre: -0.265 +/- 0.78 liters, and post: -0.236 +/- 0.1161; mean +/- SE) than ACR in the absence of interferon [gamma] expression (+0.158 +/- 0.065 and +0.236 +/- 0.007 liters, respectively) (Student-Newman-Keuls, p Conclusion Our findings suggest that interferon [gamma] mRNA expression and ICAM-1 may be valuable in both the diagnosis and prognosis for lung allograft ACR. IL-10, a Th2 cytokine, was locally expressed both in the presence and absence of ACR. Expression of mRNA for interferon [gamma] in BAL and, to a lesser extent ICAM-1, were associated with increased lung allograft dysfunction. Whether BAL cytokine "immunosurveillance" would complement or possibly supplant a specific "histologic pattern" and thereby direct different therapies after lung transplantation, may be potentially rewarding areas of further investigation.

The Journal of Nutritional Biochemistry (2)

Kong, S.-E., J. C. Hall, et al. (2000). "Starvation alters the activity and mRNA level of glutaminase and glutamine synthetase in the rat intestine." *The Journal of Nutritional Biochemistry* **11**(7-8): 393.

<http://www.sciencedirect.com/science/article/B6T8P-41FTT2P-5/2/110e9197ad6c6a08e73b979b0245dc5d>

The metabolism of glutamine, the main respiratory fuel of enterocytes, is governed by the activity of glutaminase and glutamine synthetase. Because starvation induces intestinal atrophy, it might

alter the rate of intestinal glutamine utilization. This study examined the effect of starvation on the activity, level of mRNA, and distribution of mRNA of glutaminase and glutamine synthetase in the rat intestine. Rats were randomized into groups and were either: (1) fed for 2 days with rat food ad libitum or (2) starved for 2 days. Standardized segments of jejunum and ileum were removed for the estimation of enzyme activity, level of mRNA, and in situ hybridization analysis. The jejunum of the fed rats had a greater activity of both enzymes per centimeter of intestine ($P < 0.01$), a lower level of glutaminase mRNA, and a greater level of glutamine synthetase mRNA. In situ hybridization analysis showed that starvation does not alter the distribution of glutaminase and glutamine synthetase mRNA in the intestinal mucosa. This study confirms that starvation decreases the total intestinal activity per centimeter of both glutaminase and glutamine synthetase. More importantly, the results indicate that the intestine adapts to starvation by accumulating glutaminase mRNA. This process prepares the intestine for a restoration of intake.

Pogribny, I. P. and S. J. James (1997). "A method to estimate the percent loss of cytosine methyl groups at defined CpG sites in liver DNA from methyl-deficient rats." The Journal of Nutritional Biochemistry **8**(6): 355.

<http://www.sciencedirect.com/science/article/B6T8P-3S06DYB-B/2/79d610204eb2462ab5eedff6f4cf2575>

Dietary methyl deficiency provides an ideal in vivo model system in which to study progressive alterations in DNA methylation patterns as they occur during multistage hepatocarcinogenesis. Weanling male F344 rats were given a semipurified diet deficient in the methyl-donors choline, methionine, and folic acid for a 36-week period with sampling intervals at 3, 9, 24, and 36 weeks. Using a genomic sequencing procedure based on the PCR amplification of bisulfite-modified DNA, the methylation status of individual CpG sites within exons 6 and 7 of the p53 gene in liver samples from control and deficient rats was assessed. Treatment of denatured nuclear DNA with sodium bisulfite converts unmethylated cytosine residues to uracil, which are then amplified as thymine in the PCR reaction. In contrast, methylated cytosines are resistant to bisulfite deamination under these reaction conditions and are amplified as cytosine. In this report, we describe a novel application of automated sequencing technology to estimate the proportion of methylated cytosines present at defined CpG sites within the total population of DNA molecules extracted. Using the bisulfite conversion-PCR genomic sequencing method, we demonstrate the validity of peak height analysis of co-eluting peaks in the autosequencer electrophoregram to estimate the percent methylation at a defined CpG site. The sensitivity of this method is demonstrated by the progressive loss of methyl groups at a defined CpG site in the methyl-deficient rats after 9, 24, and 36 weeks. The application of this sequence-specific technology will allow site-specific definition of the methylation status of each CpG site within a coding sequence or promoter region and should provide new insights into mechanisms and consequences of methylation dysregulation as a result of dietary deprivation of methyl donors.

The Journal of Pediatrics (1)

Sen, P., N. Thakur, et al. (2004). "Expanding the phenotype of alveolar capillary dysplasia (ACD)." The Journal of Pediatrics **145**(5): 646.

<http://www.sciencedirect.com/science/article/B6WKR-4DP2SVY->

1B/2/2455a541172253fb6ab6491d3bbc95c6

ObjectivesTo define the phenotype of congenital alveolar capillary dysplasia (ACD) as a first step toward mapping the responsible gene(s).
Study designAnalysis of pathology reports and microscopic slides of 23 subjects with ACD and sequence analysis of two candidate genes.
ResultsOur review of the pre- and postmortem records delineates both the natural history of this condition and the associated anomalies. Our collection of families corroborates the likely autosomal recessive nature of this condition in some families and provides additional data for genetic and prenatal counseling. Anomalies of many organ systems were detected either in the prenatal period or during the hospital course. However, some major anomalies were not detected until postmortem examination. Left-right asymmetry and gastrointestinal malrotation emerge as important, previously recognized but underappreciated phenotypic features of ACD. Finally, we used sequence analysis to exclude mutations in the coding region of two candidate genes, bone morphogenetic protein type II receptor (BMPRII) and endothelial monocyte-activating polypeptide II (EMAP II), as candidates for ACD.
ConclusionsUnderstanding the clinical spectrum of ACD and the cloning of an "ACD gene" both have implications for counseling, for prenatal testing, and for understanding the molecular pathophysiology of ACD and other organ malformations that are associated with this condition.

The Journal of Steroid Biochemistry and Molecular Biology (39)

Arko, B., J. Prezelj, et al. (2002). "No major effect of estrogen receptor beta gene RsaI polymorphism on bone mineral density and response to alendronate therapy in postmenopausal osteoporosis." The Journal of Steroid Biochemistry and Molecular Biology **81**(2): 147.

<http://www.sciencedirect.com/science/article/B6T8X-45VCGN6-3/2/ff22c9c64d988ae6c422c45a74de2352>

Genetic factors play an important role in the pathogenesis of osteoporosis. The genes involved are, however, still largely unknown. In the present study, we have investigated whether sequence variations in the estrogen receptor beta (ER[beta]) gene are associated with bone mineral density (BMD) and biochemical markers of bone turnover in 79 Slovenian postmenopausal women with osteoporosis. We also assessed the response by BMD and bone markers to antiresorptive therapy with bisphosphonate alendronate. All eight exons of ER[beta] gene were amplified by polymerase chain reaction and screened for mutations by single-strand conformation polymorphism analysis. Potentially mutated samples were found only in exon 5 and sequence analysis identified the presence of a silent mutation in codon 328 with a nucleotide substitution GTG to GTA. For easier detection of this silent mutation, the RsaI restriction fragment length polymorphism analysis was developed. The frequencies of genotypes were as follows: Rr 5.1% and RR 94.9%. Between both genotypes, no significant differences in baseline lumbar spine and femoral neck BMD or in bone markers osteocalcin and deoxypyridinoline were observed. Similarly, no significant difference between RR and Rr genotypes in BMD or bone markers after 1 year of therapy was found. The increase in lumbar spine BMD after therapy was the only parameter that approached statistical significance ($P=0.099$). Patients with genotype Rr showed a smaller increase compared to those with RR. Our results suggest that RsaI polymorphism of ER[beta] gene is probably not an important genetic determinant of BMD and does not significantly influence the responsiveness to alendronate therapy.

Banwell, C. M., L. P. O'Neill, et al. (2004). "Targeting 1[alpha],25-dihydroxyvitamin D3 antiproliferative insensitivity in breast cancer cells by co-treatment with histone deacetylation inhibitors." The Journal of Steroid Biochemistry and Molecular Biology **89-90**: 245.

<http://www.sciencedirect.com/science/article/B6T8X-4CDJGH7-4/2/3a97ff884a65e57f99c7aea7a4554ee1>

Proliferation of the non-malignant breast epithelial cell line, MCF-12A, is sensitively and completely inhibited by 1[alpha],25-dihydroxyvitamin D3 (1[alpha],25(OH)2D3) (ED90=70 nM). We used real time RT-PCR to demonstrate that the relative resistance to 1[alpha],25(OH)2D3 of MDA-MB-231 cells (ED50>100 nM) correlated with significantly reduced Vitamin D receptor (VDR) and increased NCoR1 nuclear receptor co-repressor mRNA (0.1-fold reduction in VDR and 1.7-fold increase in NCoR1 relative to MCF-12A (P2D3 or potent analogs and the histone deacetylation inhibitor trichostatin A (TSA). For example, the co-treatment of 1,25-dihydroxy-16,23,Z-diene-26,27-hexafluoro-19-nor Vitamin D3 (RO-26-2198) (100 nM) plus TSA results in strong additive antiproliferative effects in MDA-MB-231 cells. This may represent novel chemotherapeutic regime for hormone insensitive breast cancer.

Bilimoria, M. M., V. J. Assikis, et al. (1996). "An analysis of tamoxifen-stimulated human carcinomas for mutations in the AF-2 region of the estrogen receptor." The Journal of Steroid Biochemistry and Molecular Biology **58**(5-6): 479.

<http://www.sciencedirect.com/science/article/B6T8X-3Y2N07Y-1F/2/43b70a5709cd41550108ed379f10b280>

The estrogen receptor (ER) contains two transcriptional activation domains: AF-1 and AF-2. AF-2 is dependent on a highly species-conserved region of the ER. It has been shown that site-directed point mutations of conserved hydrophobic amino acids within this region reduce estrogen-dependent transcriptional activation. In addition, when these mutated ERs are transfected into HeLa cells, both tamoxifen and ICI 164,384 become strong agonists. The implication is that mutations in this region could account for the tamoxifen-stimulated tumors seen clinically. We performed single stranded conformational polymorphism (SSCP) analysis spanning the entire ER along with DNA sequencing of the AF-2 region of the ER isolated from two different tamoxifen-stimulated breast cancers, MCF-7/TAM and MCF-7/MT2, and a tamoxifen-stimulated endometrial cancer, EnCa 101. In addition, a tamoxifen-stimulated endometrial carcinoma cell line, the Ishikawa cell line, was also studied. There were no mutations found by SSCP analysis and sequencing of all four AF-2 regions also revealed no mutations. Mutations within the AF-2 region of the human ER do not appear to account for the growth of human breast and endometrial carcinomas that are used as reproducible laboratory models of tamoxifen-stimulated growth observed clinically.

Bruggenwirth, H. T., A. L. M. Boehmer, et al. (1996). "Molecular basis of androgen insensitivity." The Journal of Steroid Biochemistry and Molecular Biology **58**(5-6): 569.

<http://www.sciencedirect.com/science/article/B6T8X-3Y2N07Y-1V/2/41d60d585b715d628090a439d3710f36>

Mutations in the androgen receptor gene in 46,XY individuals can be associated with the androgen insensitivity syndrome, of which the phenotype can vary from a female phenotype to an undervirilized or infertile male phenotype. We have studied the androgen receptor gene of androgen insensitivity patients to get information about amino acid residues or regions involved in

DNA binding and transcription activation. Genomic DNA was analysed by PCR-SSCP under two different conditions. Three new mutations were found in exon 1 of three patients with a female phenotype. A cytosine insertion at codon 42 resulted in a frameshift and consequently in the introduction of a premature stop at codon 171. Deletion of an adenine at codon 263 gave rise to a premature stop at codon 292. In both these cases, receptor protein was not detectable and hormone binding was not measurable. In a third patient, a guanine-to-adenine transition at codon 493 converted a tryptophan codon into a stop codon. Genital skin fibroblasts from this patient were not available. In exon 2 of the androgen receptor gene of a patient with receptor-positive androgen insensitivity, a cytosine-to-adenine transition, converting alanine 564 into an aspartic acid residue, resulted in defective DNA binding and transactivation. In three other receptor-positive androgen insensitivity patients no mutations were found with PCR-SSCP.

Carballo-Jane, E., S. Pandit, et al. (2004). "Skeletal muscle: a dual system to measure glucocorticoid-dependent transactivation and transrepression of gene regulation." The Journal of Steroid Biochemistry and Molecular Biology **88**(2): 191.

<http://www.sciencedirect.com/science/article/B6T8X-4BYNMC3-1/2/6723940abedc140bf0747a536e0a9534>

The use of chronic glucocorticoid (GC) therapy for the treatment of inflammatory diseases is limited by associated metabolic side effects, including muscle atrophy. Therefore, selective glucocorticoid receptor-(GR)-binding ligands that maintain anti-inflammatory activity and demonstrate diminished side-effect profiles would have great therapeutic utility. In this work, we use Taqman PCR and ELISA methods to show that GCs can inhibit basal, and lipopolysaccharide (LPS)-stimulated levels of cytokines IL-6 and TNF[alpha], and also the chemokine MCP-1 in a non-inflammatory system such as primary human skeletal muscle cells. In the murine C2C12 skeletal muscle cell line we observe a similar effect of GCs on IL-6 and MCP-1; however, in contrast to previous reports, we observe a time-dependent repression of TNF[alpha]. Furthermore, in skeletal muscle cells, concomitant with cytokine repression, GCs transcriptionally induce glutamine synthetase (GS), a marker for muscle wasting, in an LPS independent manner. Similarly, administration of dexamethasone to mice, previously administered LPS, results in an increase in GS and an inhibition of TNF[alpha] and MCP-1 in skeletal muscle tissue. Thus, skeletal muscle cells and tissues present a novel system for the identification of selective GR-binding ligands, which simultaneously inhibit cytokine expression in the absence of GS induction.

Cates, J. M., D. A. Damassa, et al. (1995). "Hepatic expression of sex hormone-binding globulin associated with the postnatal surge of serum androgen-binding activity in the Djungarian hamster." The Journal of Steroid Biochemistry and Molecular Biology **55**(2): 147.

<http://www.sciencedirect.com/science/article/B6T8X-3YYMR78-1G/2/dc43f3901bd981fde8c87d0753c5ace6>

Serum androgen-binding capacity in Djungarian hamsters, as in many other mammals, increases within days after birth and remains elevated until puberty. This increased activity has been attributed to a hepatic glycoprotein, sex hormone-binding globulin (SHBG), but expression of SHBG by the postnatal liver has not been demonstrated. Therefore, a full-length SHBG cDNA was cloned from the liver of neonatal hamsters and the expression of SHBG during development was examined. Hepatic SHBG RNA levels, as measured by both competitive RT-PCR and Northern analysis, were very low in fetal animals but increased significantly within 24 h of birth. Maximal values were maintained for 1 week after parturition, and then declined to basal adult levels. The developmental pattern in hepatic SHBG immunoactivity, as determined by Western

analysis, mirrored that of hepatic SHBG mRNA. However, changes in serum SHBG immunoactivity and steroid-binding activity occurred approximately 1 week later. There were no sex differences in the levels of hepatic SHBG mRNA or protein during development, but serum immunoactivity tended to be higher in females at puberty. Sex- and age-related differences in the relative abundance of SHBG isoforms were also noted. Results of these studies demonstrate that Djungarian hamsters express an authentic SHBG and indicate that the postnatal surge in serum androgen-binding activity is due to perinatal up-regulation of SHBG expression.

Celius, T., J. B. Matthews, et al. (2000). "Quantification of rainbow trout (*Oncorhynchus mykiss*) zona radiata and vitellogenin mRNA levels using real-time PCR after in vivo treatment with estradiol-17[beta] or [alpha]-zearalenol." The Journal of Steroid Biochemistry and Molecular Biology **75**(2-3): 109.

<http://www.sciencedirect.com/science/article/B6T8X-42D80YH-3/2/3e7f3c2c5f29dc89ea8619d9e400b7b8>

Estrogen receptor-mediated induction of zona radiata (ZR) and vitellogenin (VTG) mRNA and protein in rainbow trout (*Oncorhynchus mykiss*) was compared to assess their utility as biomarkers for exposure to estrogenic compounds. Partial sequences of rainbow trout ZR and [beta]-actin were cloned by reverse transcriptase polymerase chain reaction (RT-PCR) using degenerate primers based on conserved regions across a number of species. A 549 bp fragment of the rainbow trout ZR-gene showed a high degree of amino acid sequence identity to that of salmon (77%), winter flounder (64%), carp ZP2 (63%) and medaka (61%) ZR-proteins. The 1020 bp [beta]-actin fragment was approximately 100% identical to sequences from several species. Real-time PCR was used to quantify the induction of ZR-gene and VTG in rainbow trout liver after in vivo exposure to estradiol-17[beta] (E2) (0.01, 0.1, 1.0 or 10 mg/kg body weight (bw) fish) or [alpha]-zearalenol ([alpha]-ZEA) (0.1, 1.0 or 10 mg/kg bw). Real-time PCR and indirect enzyme-linked immunosorbent assay (ELISA) showed that ZR and VTG were induced in both the liver and the plasma after a single injection of E2 or [alpha]-ZEA. ZR was more responsive to low levels of E2 and [alpha]-ZEA than VTG, and real-time PCR was shown to be more sensitive than the ELISA. Rainbow trout ZR-gene and proteins provide a sensitive biomarker for assessing estrogenic activity.

deGraffenried, L. A., S. G. Hilsenbeck, et al. (2002). "Sp1 is essential for estrogen receptor [alpha] gene transcription." The Journal of Steroid Biochemistry and Molecular Biology **82**(1): 7.

<http://www.sciencedirect.com/science/article/B6T8X-4757VHB-2/2/511d69b3eb04f78e0cb9554eede8b8d2>

The exact molecular mechanisms regulating estrogen receptor (ER)[alpha] expression in breast tumors are unclear, but studies suggest that the regulation is at least partly transcriptional. We therefore undertook a detailed analysis of ER[alpha] promoter activity in a number of breast cancer cell lines. We find that the majority of ER[alpha] promoter activity lies within the first 245 bp of the 5'-flanking region of the gene. Three elements essential for full ER[alpha] promoter transcriptional activity were identified within the -245 to -192 bp region in transient transactivation assays using linker-scanner mutation analysis. These three elements include two binding sites for the Sp1 family of transcription factors as well as a non-consensus E box. We show that both Sp1 and Sp3 bind to this region using electrophoretic mobility shift assays. Exogenous expression of Sp1 or Sp3 in Sp1/3-negative *Drosophila* Schneider SL2 cells results in transactivation of the -245 to +212 bp fragment of the ER[alpha] promoter. These data demonstrate that transcription of ER[alpha] is dependent upon the expression of members of the Sp1 family.

Diel, P., A. Walter, et al. (1995). "Identification of estrogen regulated genes in Fe33 rat hepatoma cells by differential display polymerase chain reaction and their hormonal regulation in rat liver and uterus." The Journal of Steroid Biochemistry and Molecular Biology **55**(3-4): 363.

<http://www.sciencedirect.com/science/article/B6T8X-3YYMR58-B/2/f8f404bf50a4de5874079d1f830621ea>

We applied the differential display RT-PCR (ddRT-PCR) technology to identify estrogen-regulated hepatic genes in the estrogen receptor expressing rat hepatoma cell line Fe33. Three genes of known sequences were detected by the ddRT-PCR approach: IGF binding protein-1 (IGFBP-1), vitamin D-dependent calcium-binding protein (CaBP9k) and major acute phase protein (MAP). Effects of ethinyl estradiol on the mRNA levels of these genes were confirmed by "Northern-blot" analysis. If given in combination with dexamethasone and glucagon, ethinyl estradiol caused 40-, 15- and 11-fold increases in the mRNA steady state level of IGFBP-1, CaBP9k and MAP, respectively, in Fe33 cells 24 h after addition of hormone. Besides ethinyl estradiol, the partial estrogen agonist OH-tamoxifen caused dose dependent effects on expression of MAP and IGFBP-1. Estrogen regulation of the respective genes and the modulatory effects of progesterone (10 mg/animal/day) were studied in ovariectomized rats treated subcutaneously for 14 days with 1 [mu]g/animal/day estradiol. "Northern-blot" analysis of liver RNA revealed a 6-fold stimulation of IGFBP-1 mRNA levels in estradiol-treated compared to vehicle-treated rats and a weak but detectable increase of MAP mRNA steady state level (1.6-fold) upon estradiol administration. No effect of estradiol treatment could be monitored for CaBP9k in rat liver. Modulatory effects of progesterone on estradiol-stimulated expression in the liver could be monitored for IGFBP-1 only. In an extension of our investigation on the expression of the three genes in rat liver, we determined their expression and hormonal regulation in the uterus of the same animals. In the uterus, estradiol caused an increase in CaBP9k mRNA. In contrast, IGFBP-1 mRNA levels increased dramatically upon progesterone administration, whereas no effect of estradiol treatment could be detected. MAP mRNA levels increased only after coadministration of estradiol and progesterone. In conclusion, the ddRT-PCR proved to be a powerful method to identify estrogen-regulated genes. The study on the hormonal regulation of three genes stimulated by estrogen in Fe33 cells revealed similarities and differences in their regulation in vivo and in vitro.

Dixon, G., D. Scanlon, et al. (1997). "A reporter gene assay for fungal sterol biosynthesis inhibitors." The Journal of Steroid Biochemistry and Molecular Biology **62**(2-3): 165.

<http://www.sciencedirect.com/science/article/B6T8X-3RJX9CF-5/2/3a2c34e9048934eaa149b1fd58ddca74>

Acetoacetyl-CoA thiolase (ACoAT) catalyses the condensation of two acetyl-CoA molecules, the first step in the sterol biosynthetic pathway. We constructed a yeast strain containing a fusion of the promoter of the *Saccharomyces cerevisiae* ACoAT gene to a reporter gene (*Escherichia coli* [beta]-galactosidase). Reporter gene activity in this strain can be induced by a variety of inhibitors of sterol biosynthesis. These results suggest that the ACoAT gene is feedback regulated at the transcriptional level by products of the sterol biosynthetic pathway. The reporter gene approach described here may be used to screen chemical collections for compounds which inhibit fungal sterol biosynthesis.

Eelen, G., L. Verlinden, et al. (2004). "Microarray analysis of 1[alpha],25-dihydroxyvitamin D3-treated MC3T3-E1 cells." The Journal of Steroid Biochemistry and Molecular Biology **89-90**: 405.

<http://www.sciencedirect.com/science/article/B6T8X-4CCNS43-2/2/9ecfae9795a5eb01af96116651786328>

The active form of Vitamin D, 1[alpha],25-dihydroxyvitamin D3 [1,25-(OH)2D3], demonstrates potent antiproliferative actions on normal as well as on malignant cell types by blocking the transition from the G1- to the S-phase of the cell cycle. Key target genes for 1,25-(OH)2D3 in this non-classic effect remain largely unknown. Therefore, this study aims to identify genes that, through changes in expression after 1,25-(OH)2D3 treatment, contribute to the observed antiproliferative effect. cDNA microarrays containing 4600 genes were used to investigate changes in gene expression in MC3T3-E1 mouse osteoblasts at 6 and at 12 h after treatment with 1,25-(OH)2D3 (10⁻⁸ M), preceding (6 h) or coinciding with (12 h) the G1/S block in these cells. Approximately one fifth of the genes that were significantly down-regulated after a 12 h incubation period with 1,25-(OH)2D3 were genes involved in the DNA replication process, a basic process for cell growth that starts at the end of G1-phase and continues in S-phase. Down-regulation of these genes by 1,25-(OH)2D3 was confirmed by quantitative RT-PCR in MC3T3-E1. In conclusion, cDNA microarrays revealed that treatment of MC3T3-E1 cells with 1,25-(OH)2D3 resulted in the down-regulation of DNA replication genes in parallel with the observed G1/S-arrest.

Fiorelli, G., U. Frediani, et al. (1998). "Aromatase expression and activity in the human leukaemic cell line FLG 29.1." *The Journal of Steroid Biochemistry and Molecular Biology* **66**(3): 105.

<http://www.sciencedirect.com/science/article/B6T8X-3VGD9CN-3/2/13fc75177869e991abf330274345f7db>

The recent observation that estrogen synthesis occurs in osteoblast-like cells has suggested the aromatase activity as a possible local modulator of bone remodeling in post-menopausal women. To provide further insights into the androstenedione conversion to estrogen in bone-derived cells, we examined the human leukaemic cell line FLG 29.1, which is induced to differentiate toward the osteoclastic phenotype by TPA and TGF- β 1. Southern blot of RT-PCR products with a 32P-labeled cDNA probe for the human aromatase demonstrated that FLG 29.1 cells express aromatase mRNA. The enzyme activity, determined by measuring [3H]H₂O release from [3H]androstenedione, obeyed Michaelis-Menten kinetic with apparent K_m and V_{max} values ranging from 5 to 10 nM and from 200 to 400 fmol/mg protein/6 h. Gene expression, enzyme activity and protein immunoreactivity, evaluated by immunocytochemistry, were stimulated in a time-dependent fashion by 5% charcoal-stripped FCS and by either 1-100 nM TPA or 0.01-0.5 ng/ml TGF- β 1, with maximal responses after 2-3 h exposure. After 24 h incubation of FLG 29.1 cells in the absence of these stimuli the aromatase mRNA and the protein were barely detectable. These findings demonstrate that cells of the osteoclastic lineage synthesize estrogen in vitro and that local cytokines, such as TGF- β 1, are able to induce androstenedione conversion.

Gardner, L., T. Anderson, et al. "Sex change strategy and the aromatase genes." *The Journal of Steroid Biochemistry and Molecular Biology* **In Press, Corrected Proof**

<http://www.sciencedirect.com/science/article/B6T8X-4FSCV69-4/2/71177f42badda82f86816e62c19dfb93>

Sequential hermaphroditism is a common reproductive strategy in many teleosts. Steroid production is known to mediate both the natural and induced sex change, yet beyond this the physiology directing this process has received little attention. Cytochrome P450 aromatase is a key enzyme in the hormonal pathway catalysing the conversion of sex steroids, androgens to oestrogens, and thus is highly relevant to the process of sex change. This study reports the

isolation of cDNA sequences for aromatase isoforms CYP19A1 and CYP19A2 from teleost species representing three forms of sexual hermaphroditism: *Lates calcarifer* (protandry), *Cromileptes altivelis* (protogyny), and *Gobiodon histrio* (bi-directional). Deduced amino acid analysis of these isoforms with other reported isoforms from gonochoristic (single sex) teleosts revealed 56-95% identity within the same isoform while only 48-65% identity between isoforms irrespective of species and sexual strategy. Phylogenetic analysis supported this result separating sequences into isoform exclusive clades in spite of species apparent evolutionary distance. Furthermore, this study isolates 5' flanking regions of all above genes and describes putative cis-acting elements therein. Elements identified include steroidogenic factor 1 binding site (SF-1), oestrogen response element (ERE), progesterone response element (PRE), androgen response element (ARE), glucocorticoid response elements (GRE), peroxisome proliferator-activated receptor [alpha]/retinoid X receptor [alpha] heterodimer responsive element (PPAR[alpha]/RXR[alpha]), nuclear factor kappa[beta] (NF-kappa[beta]), SOX 5, SOX 9, and Wilms tumor suppressor (WTI). A hypothetical in vivo model was constructed for both isoforms highlighting potential roles of these putative cis-acting elements with reference to normal function and sexual hermaphroditism.

Girault, I., F. Lerebours, et al. (2002). "Real-time reverse transcription PCR assay of CYP19 expression: application to a well-defined series of post-menopausal breast carcinomas." The Journal of Steroid Biochemistry and Molecular Biology **82**(4-5): 323.

<http://www.sciencedirect.com/science/article/B6T8X-47425F0-1/2/067b56eec5d6852cbf157df2ea424189>

Aromatase, the product of the CYP19 gene, plays a key role in androgenic steroids transformation into estrogens from various hormonal sensitive tissues. Thus, in situ expression of CYP19 has been suggested to be involved in breast tumor growth especially in post-menopausal patients. We developed a real-time quantitative RT-PCR assay based on fluorescent TaqMan(R) methodology to quantify total CYP19 gene expression at the mRNA level in breast tumors. This method, based on nucleic acid quantification in homogeneous solutions, has the potential to become a standard in terms of its sensitivity, wide dynamic range and high-throughput capacity. In a well-defined series of 107 post-menopausal breast tumor samples, relative CYP19 mRNA levels ranged from 1 to 131. Among the four major CYP19 exon I-spliced transcripts, designated I.a, I.b, I.c and I.d, mRNA levels of the latter three correlated positively with total CYP19 mRNA levels. In ER[alpha]-positive breast tumors, CYP19 and ER[alpha] mRNA levels correlated negatively with each other ($P=0.0078$, $r=-0.266$), while CYP19 and ER[beta] mRNA levels correlated positively ($P=0.00012$, $r=+0.388$). Patients with high CYP19 mRNA levels did not relapse more frequently or have shorter relapse-free survival than other patients. Finally, mRNA levels of IL6, a major CYP19 regulatory factor, were significantly higher in tumors strongly expressing CYP19 than in tumors weakly expressing CYP19 ($P=0.018$). In conclusion, CYP19 expression did not influence the outcome of post-menopausal patients with breast cancer.

Gommersall, L. M., F. L. Khamis, et al. (2004). "Epigenetic repression of transcription by the Vitamin D3 receptor in prostate cancer cells." The Journal of Steroid Biochemistry and Molecular Biology **89-90**: 251.

<http://www.sciencedirect.com/science/article/B6T8X-4CDJGF1-1/2/56f56ffbaafe864273915528130e0929>

Normal prostate epithelial cells are acutely sensitive to the antiproliferative action of 1[alpha],25-dihydroxyvitamin D3 (1[alpha],25(OH)2D3), whilst prostate cancer cell lines and primary cultures display a range of sensitivities. We hypothesised that key antiproliferative target genes of the

Vitamin D receptor (VDR) were repressed by an epigenetic mechanism in 1[alpha],25(OH)2D3-insensitive cells. Supportively, we found elevated nuclear receptor co-repressor and reduced VDR expression correlated with reduced sensitivity to the antiproliferative action of 1[alpha],25(OH)2D3. Furthermore, the growth suppressive actions of 1[alpha],25(OH)2D3 can be restored by co-treatment with low doses of histone deacetylation inhibitors, such as trichostatin A (TSA) to induce apoptosis. Examination of the regulation of VDR target genes revealed that co-treatment of 1[alpha],25(OH)2D3 plus TSA co-operatively upregulated GADD45[alpha]. Similarly in a primary cancer cell culture, the regulation of appeared GADD45[alpha] repressed. These data demonstrate that prostate cancer cells utilise a mechanism involving deacetylation to suppress the responsiveness of VDR target genes and thus ablate the antiproliferative action of 1[alpha],25(OH)2D3.

Harada, N. (1993). "Genetic analysis of human placental aromatase deficiency." The Journal of Steroid Biochemistry and Molecular Biology **44**(4-6): 331.

<http://www.sciencedirect.com/science/article/B6T8X-47PR5MP-1F1/2/9771df3e80ee71dc7828db339f9269ba>

Placental aromatase deficiency, which was characterized by maternal and fetal virilization and by a low level of estrogen excretion into urine during pregnancy, was studied by biochemical and molecular genetical techniques. Among enzymes participating in the electron transport system of the patient's placental microsomes, only aromatase activity was observed to be reduced (< 3% of normal levels). Northern and Western blotting analyses showed that the transcription of the aromatase gene and the translation of its mRNA seemed to proceed normally in the patient's tissue. However, the aromatase cDNA isolated from the patient was found to contain an extra DNA fragment of 87 base pairs (bp) which encoded 29 amino acids in frame but no termination codon. The insertion was located at the splicing point between exon 6 and intron 6 of the normal aromatase gene. The extra DNA fragment represented the first part of intron 6 except that its initial GT was altered to GC. These findings indicated that, in the patient's aromatase gene, the splicing between exon 6 and intron 6 did not occur at the normal position. This reflected the presence of one point mutation in its consensus sequence which caused the next cryptic consensus sequence 87 bp downstream, to be used according to the canonical GT/AG rule. The protein molecule thus translated contained an extra 29 amino acids. Furthermore, the patient's aromatase cDNA was observed to produce a protein molecule with a trace of activity in the transient expression system of COS-7 cells and in the high level expression system of baculovirus-insect cells. Direct DNA sequencing of aromatase genes from the patient and parents confirmed that this deficiency is a hereditary disease with an autosomal recessive inheritance pattern. The patient and parents are homozygote and heterozygotes, respectively, for this mutation.

Jensen, S. S., M. W. Madsen, et al. (2002). "Sensitivity to growth suppression by 1[alpha],25-dihydroxyvitamin D3 among MCF-7 clones correlates with Vitamin D receptor protein induction." The Journal of Steroid Biochemistry and Molecular Biology **81**(2): 123.

<http://www.sciencedirect.com/science/article/B6T8X-45X2KX9-6/2/906e80990a586772512402fb2f054813>

The antiproliferative effect of 1[alpha],25-dihydroxyvitamin D3 (1[alpha],25(OH)2D3) has been studied for a decade in diverse model systems, but the signalling pathways linking 1[alpha],25(OH)2D3 to cell cycle arrest remains unclear. In our attempt to establish a model system which would allow further identification of important players in the process of the 1[alpha],25(OH)2D3 imposed cell cycle arrest, we have isolated derivatives of the human breast

cancer cell line MCF-7 and chosen two nearly 1[alpha],25(OH)2D3 resistant and two hypersensitive sub-clones. Investigation of cell cycle proteins regulated by 1[alpha],25(OH)2D3 in these clones indicates that activation of one component/pathway is responsible for the linkage between 1[alpha],25(OH)2D3 and growth arrest. Protein levels of the Vitamin D receptor (VDR) were elevated in sensitive cells upon 1[alpha],25(OH)2D3 treatment, whereas resistant clones were unable to induce VDR upon 1[alpha],25(OH)2D3 treatment. Our data show that VDR protein levels and the ability of a cell to induce VDR upon 1[alpha],25(OH)2D3 treatment correlate with the antiproliferative effects of 1[alpha],25(OH)2D3, and suggest that the level of VDR in cancer cells might serve as a prognostic marker for treatment of cancer with 1[alpha],25(OH)2D3 analogues.

Killeen, A. A., K. S. Sane, et al. (1991). "Molecular and endocrine characterization of a mutation involving a recombination between the steroid 21-hydroxylase functional gene and pseudogene." The Journal of Steroid Biochemistry and Molecular Biology **38**(6): 677.

<http://www.sciencedirect.com/science/article/B6T8X-47PR20B-3G/2/358fe550bee6c905b2e3c4c8711dcad5>

The gene encoding steroid 21-hydroxylase activity, P450c21B, is located in the major histocompatibility complex (MHC) class III region, in close proximity to a highly homologous pseudogene, P450c21A. Recombinations between P450c21B and P450c21A have been shown to result in deficiency of 21-hydroxylase activity, the usual cause of congenital adrenal hyperplasia (CAH). A mutant P450c21 gene from a patient with simple virilizing CAH was identified and shown to be consistent with a recombination between P450c21A and P450c21B. Sequence analysis of the mutant gene showed the recombination site to be located between the first exon and the second intron. The mutant gene encodes a leucine instead of the normal proline at codon 31. This mutation resides on a chromosome bearing the HLA-B44 serotype. A comparison of mutation associated with HLA-B44 and that normally found with the HLA-Bw47 serotype suggests that the HLA-B44 mutations are of more ancient origin. The patient's homologous chromosome has a deletion of P450c21B. Endocrinological testing therefore allows for testing of the mutant gene in genetic isolation. Such testing demonstrated that the patient was capable of producing aldosterone and retaining sodium in response to a low-sodium diet, indicating that the mutant gene encodes an enzyme with partial 21-hydroxylase activity.

Lajic, S., L. Eidsmo, et al. (1995). "Steroid 21-hydroxylase in the kidney: Demonstration of levels of messenger RNA which correlate with the level of activity." The Journal of Steroid Biochemistry and Molecular Biology **52**(2): 181.

<http://www.sciencedirect.com/science/article/B6T8X-3YXC0B3-1W/2/d6d67e1306f3fc93a4c25c03ffe7c5b2>

Steroid 21-hydroxylase activity was assayed in low-speed supernatants prepared from whole cell homogenates of mouse and rat tissues. Kidney supernatants had an activity which was approximately 2-5% that of adrenal preparations while heart muscle was found to be without 21-hydroxylase activity. When the enzyme kinetics were characterized, both adrenal and kidney low-speed supernatants demonstrated saturation kinetics, but with very different Vmax and Km values. Using polymerase chain reaction amplification after reverse transcriptase synthesis of cDNA from isolated RNA (RT-PCR), we found low levels of mRNA for steroid 21-hydroxylase in mouse kidney, but none in heart muscle. Thus, extra-adrenal steroid 21-hydroxylase activity in the kidney may be mediated by the same enzyme as found in adrenals.

Listwak, S. J., P. W. Gold, et al. (1996). "The human mineralocorticoid receptor gene promoter: Its structure and expression." The Journal of Steroid Biochemistry and Molecular Biology **58**(5-6): 495.

<http://www.sciencedirect.com/science/article/B6T8X-3Y2N07Y-1H/2/139b610bfdc51aa68500a6d5cb90263d>

The structure and expression of a clone containing the promoter region, all of exon 1, and part of the first intron of the human mineralocorticoid receptor (hMR) gene is presented. The clone has three sets of CAAT and TATA elements, one located at the very 5'-end of the clone, one located just 5'- to the start of transcription, and one set located in intron A, approximately 300 bp into the intron. The major start of transcription site by primer extension analysis and ribonuclease protection assays is located 26 bp downstream of a TATA-like box (TTTAA) and 90 and 143 bp downstream, respectively, of two CCAAT boxes. Putative cis-transcription factor binding sites are as follows: two potential AP1 sites, one potential AP2 site, two ATF/CREB sites, six potential GC boxes or SP1 sites, one potential perfect half-palindromic estrogen response element, and three potential PEA3 sites. Therefore, the hMR promoter region contains elements characteristic of both regulated genes and "housekeeping" genes. CAT assays of overlapping deletions of the promoter region demonstrated tissue-specific regulation in human neuroepithelioma (SK-N-MC-IXC) and non-neuronal, peripheral choriocarcinoma cell lines (JEG-3).

Misao, R., J. Fujimoto, et al. (1997). "Expression of sex hormone-binding globulin exon VII splicing variant mRNA in human uterine endometrium." The Journal of Steroid Biochemistry and Molecular Biology **62**(5-6): 385.

<http://www.sciencedirect.com/science/article/B6T8X-3RXP7K-3/2/17a190a72d63cd9f6755bd0cc0e7b62b>

We have demonstrated the expression of sex hormone-binding globulin (SHBG) exon VII splicing variant mRNA in human uterine endometrium, using the reverse transcription-polymerase chain reaction-Southern blot and DNA sequencing analyses. Analysis of the missing base pairs corresponded to the entire exon VII, which are considered to encode a portion of the steroid-binding site. Therefore, the steroid-binding affinity of this variant might be different from that of the SHBG wild type. In uterine endometria, the wild-type and variant mRNA levels tended to increase with the advance of the menstrual phase, but the ratio of the SHBG variant mRNA to SHBG wild-type mRNA levels showed no significant difference during the menstrual cycle. So far, there are no indications that the SHBG variant has any biological or clinical implications in human uterine endometrium.

Misao, R., S. Iwagaki, et al. (1999). "Expression of sex hormone-binding globulin exon 7 splicing variant mRNA in secondary spreading lesions of gynecological cancers." The Journal of Steroid Biochemistry and Molecular Biology **68**(3-4): 103.

<http://www.sciencedirect.com/science/article/B6T8X-3WG3KG9-2/2/4280cc6417131353774db44c562c9704>

This study was designed to determine the clinical implications of intracellular expression of sex hormone-binding globulin (SHBG) wild-type and exon 7 splicing variant mRNAs in secondary spreading lesions of gynecologic cancers using the reverse transcription-polymerase chain

reaction-Southern blot and DNA sequencing analyses. Compared with primary cancers, a relative increase in SHBG variant mRNA to wild-type mRNA expression was observed (4/10 cases of uterine endometrial cancers, 5/10 cases of uterine cervical cancers, 6/10 cases of ovarian cancers) or the expression of SHBG wild-type and variant mRNAs could not be detected (5/10 cases of uterine endometrial cancers, 3/10 cases of uterine cervical cancers, 4/10 cases of ovarian cancers). On the other hand, alteration to a relative increase in SHBG wild-type mRNA expression in the metastatic lesions occurred in only 3 cases (1/10 cases of uterine endometrial cancers and 2/10 cases of uterine cervical cancers) analyzed. These results suggest that the transcription of SHBG mRNA and the regulation of its splicing might be altered with metastatic potential, and this status might be involved in a change in steroidal dependency of metastatic lesions.

Misao, R., Y. Nakanishi, et al. (1997). "Effects of danazol and progesterone on sex hormone-binding globulin mRNA expression in human endometrial cancer cell line Ishikawa." The Journal of Steroid Biochemistry and Molecular Biology **62**(4): 321.

<http://www.sciencedirect.com/science/article/B6T8X-3S12BV0-9/2/395bd3c3d1b50b244f424636731df568>

To ascertain one of the biological effects of danazol and progesterone on the uterine endometrial cancer cell line, Ishikawa, we investigated the effects of these steroids on sex hormone-binding globulin (SHBG) mRNA expression by competitive reverse transcription-polymerase chain reaction-Southern blot analysis (RT-PCR-SBA). Estradiol-17[β] (E2) in any concentration given did not exert any significant effect on the expression of SHBG mRNA. Danazol and progesterone significantly ($P = 10^{-6}$ and 10^{-8} M, respectively). Progesterone, in a low concentration (10^{-10} M) with E2 (10^{-8} M), significantly ($P = 10^{-6}$ to 10^{-5} M) with E2 (10^{-8} M) significantly ($P = 10^{-6}$ M) with or without E2 (10^{-8} M), except for a temporal increase by progesterone. These findings suggest that danazol and progesterone in a superphysiological milieu down-regulate the intracellular SHBG-related steroidal actions, and that progesterone in a physiological milieu with estrogen up-regulates it in a hormone-dependent cell line. A decrease of intracellular SHBG caused by high-dose danazol or progesterone might partly contribute to the abolition of the intracellular estrogen-dominant milieu, and be related to the inhibition of estrogen-dependent growth of some endometrial cancer cells.

Misao, R., Y. Nakanishi, et al. (1997). "Expression of sex hormone-binding globulin exon VII splicing variant mRNA in human uterine myometrium and leiomyoma." The Journal of Steroid Biochemistry and Molecular Biology **63**(4-6): 269.

<http://www.sciencedirect.com/science/article/B6T8X-3S3MPFK-D/2/a9003c5634beb0b247abe15737de2db9>

We have explored the mechanism of estrogen-induced growth in human uterine leiomyomas from the aspect of sex hormone-binding globulin (SHBG) exon VII splicing variant mRNA expression using the reverse transcription-polymerase chain reaction-Southern blot and DNA sequencing analyses. The results were obtained by analysis of the missing base pairs corresponding to the entire exon VII, which are considered to encode a portion of the steroid-binding site. This absence replaces 118 amino acids from the carboxy-terminus of SHBG with nine different amino acid residues due to the formation of a new stop codon at residue 334. The ratio of the SHBG variant to its wild-type mRNA levels in uterine leiomyomas was reduced, compared with that in the corresponding myometria in individual cases, while the SHBG wild-type and variant mRNA levels showed no significant difference during the menstrual phase. These studies demonstrate coexpression of SHBG exon VII splicing variant mRNA with its wild-type in human uterine

myometria and leiomyomas. The reduced expression of the SHBG variant to wild-type mRNA levels in leiomyoma might be involved in the intracellular estrogen-predominant milieu, plausibly assisting in the development and growth of the leiomyoma.

Misao, R., Y. Nakanishi, et al. (1995). "Expression of sex hormone-binding globulin mRNA in human endometrial cancers." The Journal of Steroid Biochemistry and Molecular Biology **52**(6): 517.

<http://www.sciencedirect.com/science/article/B6T8X-3YXC07S-2/2/6fe7afef91d7a0106d0443ba4cb6cd7c>

To more fully understand the role of sex hormone-binding globulin (SHBG) on the intracellular steroidal action in endometrial cancers, we investigated the expression of SHBG mRNA as the substitute of SHBG expression in human endometrial cancers. In the present study, the levels of SHBG mRNA were analyzed using competitive reverse transcription-polymerase chain reaction (RT-PCR)-Southern-bolt analysis. The higher level of SHBG mRNA tended to be expressed in the normal secretory and late proliferative phase endometrium > early proliferative phase endometrium > well differentiated adenocarcinoma of the endometrium (G1) > moderately differentiated adenocarcinoma (G2) > poorly differentiated adenocarcinoma (G3), in the order shown. These studies indicate that endometrial cancer cells might synthesize intracellular SHBG to conserve their estrogen-dependent properties. Further, it indicates that endometrial cancer cell synthesis of SHBG mRNA is lost as these cells undergo de-differentiation.

Miyashita, K., N. Shimizu, et al. (2000). "Sequence analysis and expression of the P450 aromatase and estrogen receptor genes in the Xenopus ovary." The Journal of Steroid Biochemistry and Molecular Biology **75**(2-3): 101.

<http://www.sciencedirect.com/science/article/B6T8X-42D80YH-2/2/7aeaa7cb79cddb57e664a9faf8fcfc00>

Recent studies point to a key role for the estrogen synthesizing enzyme P450 aromatase (P450 arom) in ovary determination in fish, birds and reptiles. It is unclear whether estrogen synthesis is important in sex determination of Xenopus gonad. To determine whether the aromatase gene is transcribed in the gonads of Xenopus tadpoles during the sex determination, we cloned a P450 arom cDNA and examined the level of P450 arom and estrogen receptor (ER) gene expression in association with estrogen activity. cDNA clones for P450 arom were isolated from a Xenopus ovarian cDNA library. There was an open reading frame (ORF) of 1500 bp from the ATG start to TAA stop codons encoding 500 predicted amino acids. cDNAs for P450 arom have previously been cloned from various vertebrates. The homology between the Xenopus P450 aromatase and the human P450 arom was higher. The expression of the P450 arom gene was mainly limited to reproductive organs. To determine the beginning of estrogen activity in gonads of embryos, expression of the aromatase and ER gene was also examined by RQ-RT-PCR. Both Xenopus aromatase and ER mRNA was detected at stage 51 in gonads. These observations are consistent with estrogens having a key role in ovarian development in various other vertebrates.

Nakajin, S., T. Nakajima, et al. (1995). "Direct expression of pig testicular 3[alpha]/[beta] (20[beta])-hydroxysteroid dehydrogenase in Escherichia coli." The Journal of Steroid Biochemistry and Molecular Biology **54**(5-6): 257.

<http://www.sciencedirect.com/science/article/B6T8X-3YYT600->

9/2/f2d9bd82a88a548a132d8623c38088b1

The cDNA coding for pig testicular 3[alpha]/[beta] (20[beta])-hydroxysteroid dehydrogenase was expressed in *Escherichia coli* by placing it under the control of an isopropylthiogalactoside (IPTG) inducible tac promoter. Production of 3[alpha]/[beta] (20[beta])-HSD was demonstrated by Western blotting and by catalytic activity with 5[alpha]-dihydrotestosterone as a substrate for 3[alpha]/[beta]-HSD, and progesterone and 17[alpha]-hydroxyprogesterone as substrates for 20[beta]-HSD in the presence of NADPH. The 3[alpha]/[beta] (20[beta])-HSD enzyme was detected in a soluble fraction of the lysate of *E. coli* added to IPTG to induce the synthesis of the protein. Its molecular weight was estimated to be 30.5 kDa by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Recombinant 3[alpha]/[beta] (20[beta])-HSD was purified to apparent homogeneity as determined by SDS-PAGE by column chromatography using DEAE-cellulose. The purified enzyme reduced not only steroids but also prostaglandins and other carbonyl compounds including aldehydes, ketones and quinones as demonstrated in native enzymes purified from pig testes. The amino terminus of the purified enzyme was serine which was coded next to the ATG start codon, and the sequence of the amino terminal 24 residues was identical with the coding amino acid in the cDNA; whereas, the amino terminus of the native 3[alpha]/[beta] (20[beta])-HSD was not detected suggesting that the N-terminal amino acid was blocked.

Nazarova, N., O. Golovko, et al. "Calcitriol inhibits growth response to Platelet-Derived Growth Factor-BB in human prostate cells." *The Journal of Steroid Biochemistry and Molecular Biology* **In Press**, **Corrected Proof** <http://www.sciencedirect.com/science/article/B6T8X-4FJGW45-3/2/069a6dc4d469598df25e90a7ef280af4>

Calcitriol, a hormonal form of Vitamin D, regulates growth of normal and cancer cells of various origins by modulation of peptide growth factors signaling. Platelet-Derived Growth Factor (PDGF) signaling pathway is involved in prostate cancer progression. We studied the expression of PDGF receptors in human prostate primary stromal cells and cancer epithelial cell lines and growth response to PDGF-BB isoform. We found that the expression of PDGF receptors and PDGF-BB-mediated cell growth are regulated by calcitriol in prostate cells. Quantitative RT-PCR analysis revealed a lower level of mRNA for PDGF receptors in LNCaP and PC-3 cells than in primary stromal cells. Western blotting showed a high amount of PDGFR[alpha] and [beta] proteins in primary stromal cells that could not be detected in LNCaP, which may explain the resistance of LNCaP cells to growth-promoting effect of PDGF-BB. Addition of Epidermal Growth Factor (EGF) to the culture medium induces the expression of PDGFR[beta] and restores responsiveness of LNCaP to PDGF-BB to some extent. Calcitriol down-regulates PDGFR[beta] expression and negatively regulates PDGF-mediated cell growth. Calcitriol does not affect PDGFR[alpha] and PDGF-B mRNA expression. We suggest that inhibition of PDGFR[beta] expression by calcitriol might reduce responsiveness of prostate cells to mitogenic action of PDGF-BB.

Penolazzi, L., E. Lambertini, et al. (2004). "Methylation analysis of the promoter F of estrogen receptor [alpha] gene: effects on the level of transcription on human osteoblastic cells." *The Journal of Steroid Biochemistry and Molecular Biology* **91**(1-2): 1.

<http://www.sciencedirect.com/science/article/B6T8X-4CSG31N-1/2/c9bd1e04ccbe29676a9799dd33289d75>

In this study, the methylation status of the distal promoter F of estrogen receptor alfa (ER[alpha]) gene in human osteoblastic cells was investigated. The activity of this promoter is responsible for the ER[alpha] gene transcription in bone tissue. The methylation status of promoter F was here evaluated, for the first time, by direct sequencing of bisulfite-treated genomic DNA, at 10 CpG

specific sites localized in a region of about 800 bp. An heterogeneous methylation pattern was observed. The most notable difference was found at four particular CpGs, distant from the exon F transcription start site, showing a methylation status that correlates with the expression level, being ER[alpha] mRNA transcription reduced in a partially methylated cells but preserved in demethylated cells. The other CpG sites, localized around the transcription start site, were always demethylated except for MG-63 cells showing the lowest level of ER[alpha] expression. By quantitative RT-PCR analysis we demonstrated that ER[alpha] gene expression was higher in primary osteoblasts than in bone-derived cells (MG-63 and SaOS-2) and in all cases the ER[alpha] mRNA is represented by the isoform F. The same 10 CpG sites were investigated in non-osseous cell lines and were found fully methylated in ER[alpha]-negative breast cancer cells (MDA-MB-231) and completely demethylated in ER[alpha]-positive breast cancer cells (MCF7). The overall results suggest that methylation of the CpG sites inside ER[alpha] gene promoter F here analyzed may contribute to ER[alpha] transcriptional control, directly or indirectly, influencing the tissue specific expression of the gene.

Poola, I., S. Koduri, et al. (2000). "Identification of twenty alternatively spliced estrogen receptor alpha mRNAs in breast cancer cell lines and tumors using splice targeted primer approach." The Journal of Steroid Biochemistry and Molecular Biology **72**(5): 249.

<http://www.sciencedirect.com/science/article/B6T8X-408KCH3-7/2/f9006e6d04d66f9f263e72cea05ecf46>

Estrogen receptor (ER) alpha splice variant transcript profiles were analyzed by RT PCR in six ER positive breast cancer cell lines, MCF-7, T47D, ZR-75, LCC1, LCC2 and LCC9, three ER negative cell lines, MDA-MB-435, MDA-MB-235 and LCC6, and three ER positive malignant breast tumors using targeted primers which specifically anneal to the splice junctions of exon 2[Delta], exon 3[Delta], exons 2-3[Delta], exon 4[Delta], exon 5[Delta], exon 6[Delta] and exon 7[Delta]. The partner primers were chosen such that largest possible transcripts were amplified between exons 1 and 8. The results described here show that each splice specific primer amplified not only the single exon deleted transcript but also a number of related transcripts that have deletions in various combinations of exons. The exon 2[Delta] specific primer amplified five transcripts that have deletions in exon 2, exons 2 and 7, exons 2, 5, and 7, exons 2 and 4-5, and exons 2 and 4-6. The exon 3[Delta] specific primer amplified two transcripts that have deletions in exon 3, and exons 3 and 7. The exon 2-3[Delta] specific primer amplified three products that have deletions in exons 2-3, exons 2-3 and 7 and exons 2-3, 5 and 7. The exon 4[Delta] specific primer amplified two products that have deletions in exon 4, and exons 4 and 7. The exon 5[Delta] specific primer amplified three transcripts, that have deletions in exon 5, exons 5 and 2, and exons 5, and 2-3. The 6[Delta] specific primer amplified only one transcript that has a deletion in exon 6. The 7[Delta] specific primer amplified four transcripts, that have deletions in exon 7, exons 7 and 4, exons 7 and 3-4, and exons 7 and 3-5. None of the above splice specific primers amplified the wild type ER sequences. The six ER positive cell lines differed in the patterns of the variant transcripts and among the three ER negative cell lines analyzed, only MDA-MB-435 showed the presence of exon 2[Delta] and exon 4[Delta] transcripts. Analyses in the tumor samples indicated that the above transcripts are extensively modified.

Rousseau, C., F. Pettersson, et al. (2003). "The N-terminal of the estrogen receptor (ER[alpha]) mediates transcriptional cross-talk with the retinoic acid receptor in human breast cancer cells." The Journal of Steroid Biochemistry and Molecular Biology **86**(1): 1.

<http://www.sciencedirect.com/science/article/B6T8X-49505JV-1/2/04498f1f887509bfb19b05a8d551d051>

Transcriptional cross-talk exists between the estrogen receptor (ER[alpha]) and retinoic acid receptor (RAR) pathways in human breast cancer cells. We have previously shown that re-expression of ER[alpha] in ER-negative cells stimulates the transcriptional and growth inhibitory effects of all-trans-retinoic acid (tRA) by a mechanism that is independent of the ER ligands estradiol and tamoxifen. In this study, we generated cell lines stably expressing ER[alpha]-deletion mutants to elucidate the mechanism whereby ER[alpha] modulates RAR transcriptional activity. Using RT-PCR and RNase protection assays, we observed that expression of ER[alpha] suppresses basal expression of the RA-responsive gene RAR[beta]2, while allowing it to be strongly induced by tRA. Repression of basal RAR[beta]2 transcription was confirmed by transient expression of the reporter plasmid [beta]RE-tk-CAT, containing the RAR[beta]2 promoter. In the ER[alpha]-negative cells, on the other hand, transcription was only weakly induced by RA. We further determined that this effect of ER[alpha] on RAR[beta] induction required the N-terminal AF-1-containing region, including the DNA-binding domain, but was independent of the C-terminal ligand-binding domain. Consistent with these results, the ER agonist estradiol and the AF-2 antagonist 4-hydroxytamoxifen had no significant effect on [beta]RARE activity. Conversely, the full ER antagonist ICI 182,780, which blocks ER[alpha] AF-1 activity, was able to completely relieve repression of basal [beta]RARE activity. The effect of ER[alpha] is specific for RAR-mediated transcription and does not occur on promoters containing typical response elements for the Vitamin D or thyroid hormone receptors. Moreover, the cross-talk between ER[alpha] and RAR does not seem to be mediated by sequestration of a number of common co-regulators, suggesting a novel mechanism whereby the N-terminal region of ER[alpha] modulates the transcriptional activity of RAR.

Rumsby, G., C. Skinner, et al. (1992). "Genetic analysis of the steroid 21-hydroxylase gene following in vitro amplification of genomic DNA." The Journal of Steroid Biochemistry and Molecular Biology **41**(3-8): 827.

<http://www.sciencedirect.com/science/article/B6T8X-47DTCF8-35/2/96352fc39449c7515e900f26cb5a07bc>

The 5' end of the steroid 21-hydroxylase B gene encompassing putative control regions and the first 3 exons, has been selectively amplified in vitro from a number of patients with congenital adrenal hyperplasia caused by a deficiency of this enzyme. Sequence analysis has revealed a number of isolated instances of gene conversion to the 21-hydroxylase A sequence. One mutation, a C to G transversion at the 3' end of the second intron, thought to lead to incorrect splicing of the mRNA, was found in 11 subjects all with the classical form of the disease.

Russo, J., M. Hasan Lareef, et al. (2003). "Estrogen and its metabolites are carcinogenic agents in human breast epithelial cells." The Journal of Steroid Biochemistry and Molecular Biology **87**(1): 1.

<http://www.sciencedirect.com/science/article/B6T8X-49J8SJV-7/2/de566f8c056ea8817045b31d442c9cc1>

Estrogens play a crucial role in the development and evolution of human breast cancer. However, it is still unclear whether estrogens are carcinogenic to the human breast. There are three mechanisms that have been considered to be responsible for the carcinogenicity of estrogens: receptor-mediated hormonal activity, a cytochrome P450 (CYP)-mediated metabolic activation, which elicits direct genotoxic effects by increasing mutation rates, and the induction of aneuploidy by estrogen. To fully demonstrate that estrogens are carcinogenic in the human breast through one or more of the mechanisms explained above it will require an experimental system in which, estrogens by itself or one of the metabolites would induce transformation phenotypes indicative of

neoplasia in HBEC in vitro and also induce genomic alterations similar to those observed in spontaneous malignancies. In order to mimic the intermittent exposure of HBEC to endogenous estrogens, MCF-10F cells that are ER[alpha] negative and ER[beta] positive were first treated with 0, 0.007, 70 nM and 1 [mu]M of 17[beta]-estradiol (E2), diethylstilbestrol (DES), benz(a)pyrene (BP), progesterone (P), 2-OH-E2, 4-hydroxy estradiol (4-OH-E2) and 16-[alpha]-OH-E2 at 72 h and 120 h post-plating. Treatment of HBEC with physiological doses of E2, 2-OH-E2, 4-OH-E2 induce anchorage independent growth, colony formation in agar methocel, and reduced ductulogenic capacity in collagen gel, all phenotypes whose expression are indicative of neoplastic transformation, and that are induced by BP under the same culture conditions. The presence of ER[beta] is the pathway used by E2 to induce colony formation in agar methocel and loss of ductulogenic in collagen gel. This is supported by the fact that either tamoxifen or the pure antiestrogen ICI-182,780 (ICI) abrogated these phenotypes. However, the invasion phenotype, an important marker of tumorigenesis is not modified when the cells are treated in presence of tamoxifen or ICI, suggesting that other pathways may be involved. Although we cannot rule out the possibility, that 4-OH-E2 may interact with other receptors still not identified, with the data presently available the direct effect of 4-OH-E2 support the concept that metabolic activation of estrogens mediated by various cytochrome P450 complexes, generating through this pathway reactive intermediates that elicit direct genotoxic effects leading to transformation. This assumption was confirmed when we found that all the transformation phenotypes induced by 4-OH-E2 were not abrogated when this compound was used in presence of the pure antiestrogen ICI. The novelty of these observations lies in the role of ER[beta] in transformation and that this pathway can successfully bypassed by the estrogen metabolite 4-OH-E2. Genomic DNA was analyzed for the detection of micro-satellite DNA polymorphism using 64 markers covering chromosomes (chr) 3, 11, 13 and 17. We have detected loss of heterozygosity (LOH) in ch13q12.2-12.3 (D13S893) and in ch17q21.1 (D17S800) in E2, 2-OH-E2, 4-OH-E2, E2 + ICI, E2 + tamoxifen and BP-treated cells. LOH in ch17q21.1-21.2 (D17S806) was also observed in E2, 4-OH-E2, E2+ICI, E2+tamoxifen and BP-treated cells. MCF-10F cells treated with P or P+E2 did not show LOH in the any of the markers studied. LOH was strongly associated with the invasion phenotype. Altogether our data indicate that E2 and its metabolites induce in HBEC LOH in loci of chromosomes 13 and 17, that has been reported in primary breast cancer, that the changes are similar to those induced by the chemical carcinogen (BP) and that the genomic changes were not abrogated by antiestrogens.

Russo, J., M. Hasan Lareef, et al. (2002). "17[beta]-Estradiol is carcinogenic in human breast epithelial cells." The Journal of Steroid Biochemistry and Molecular Biology **80**(2): 149.

<http://www.sciencedirect.com/science/article/B6T8X-451DKY1-1/2/cbeedc4161a8b8fae26359749fb580d9>

The association found between breast cancer development and prolonged exposure to estrogen suggests that this hormone is of etiologic importance in the causation of this disease. In order to prove this postulate, we treated the immortalized human breast epithelial cells (HBEC) MCF-10F with 17[beta]-estradiol (E2) for testing whether they express colony formation in agar methocel, or colony efficiency (CE), and loss of ductulogenesis in collagen matrix, phenotypes also induced by the carcinogen benz[a]pyrene (BP). MCF-10F cells were treated with 0.0, 0.007, 70 nM, or 0.25 mM of E2 twice a week for 2 weeks. CE increased from 0 in controls to 6.1, 9.2, and 8.7 with increasing E2 doses. Ductulogenesis was 75+/-4.9 in control cells; it decreased to 63.7+/-28.8, 41.3+/-12.4, and 17.8+/-5.0 in E2-treated cells, which also formed solid masses or spherical formations lined by a multilayer epithelium, whose numbers increased from 0 in controls to 18.5+/-6.7, 107+/-11.8 and 130+/-10.0 for each E2 dose. MCF-10F cells were also treated with 3.7 [mu]M of progesterone (P) and the CE was 3.39+/-4.05. At difference of E2, P does not impaired the ductulogenic capacity. Genomic analysis revealed that E2-treated cells exhibited loss of heterozygosity in chromosome 11, as detected using the markers D11S29 and D11S912 mapped to 11q23.3 and 11q24.2-25, respectively These results also indicate that E2, like the

chemical carcinogen BP, induces in HBEC phenotypes indicative of neoplastic transformation.

Sun, T., Y. Zhao, et al. (1995). "Cloning and expression of cytochrome P450(11[beta]) of porcine adrenal cortex." The Journal of Steroid Biochemistry and Molecular Biology **52**(3): 227.

<http://www.sciencedirect.com/science/article/B6T8X-3YXC09G-1B/2/a979ca0ab33a3bf8ad6b46dd94e1c076>

Four cDNA clones were isolated from a porcine adrenal gland library by using a bovine cytochrome P450(11[beta]) cDNA fragment as a probe. Nucleotide sequences+ of the four clones overlapped with each other. The deduced amino acid sequences indicated that these clones were derived from a porcine P450(11[beta]) cDNA. Consecutive alignment of these clones covered almost 70% of a coding region of the cDNA, but its 5'-terminus was missing. The adrenal mRNA was reverse-transcribed, and polymerase chain reaction was used to obtain a cDNA fragment including the 5'-terminus. A cDNA constructed from this fragment and the isolated four fragments covered the entire apparent open reading frame of the enzyme, which was thus concluded to comprise 503 amino acids including a putative extension peptide of 24 amino acids at the NH2-terminus. The amino acid sequence was 82% identical to that of bovine P450(11[beta])-3. The cDNA was transfected into COS-7 cells, and steroidogenic activity of the cells was measured. The cells not only converted 11-deoxycorticosterone to corticosterone and 18-hydroxycorticosterone, but also produced aldosterone. Thus we conclude that the primary sequence of porcine P450(11[beta]) which plays a role in the biosynthesis of glucocorticoids as well as mineralocorticoids was determined.

Thompson, D. A., L. A. McPherson, et al. (1997). "Identification of two estrogen receptor transcripts with novel 5' exons isolated from a MCF7 cDNA library." The Journal of Steroid Biochemistry and Molecular Biology **62**(2-3): 143.

<http://www.sciencedirect.com/science/article/B6T8X-3RJX9CF-3/2/51c617ade56865bc15af2ac523438551>

Two novel transcripts of human estrogen receptor (ER) have been identified that differ in the 5' untranslated sequence. It has previously been determined that an alternate ER transcript is generated from transcription initiated upstream of the main ER cap site (P1), and utilizes a splice acceptor site at +163. Here we report the isolation of 21 ER clones from a MCF7 cDNA library. Eleven of these clones correspond to transcripts that initiate at the P1 cap site, whereas the remaining 10 clones are derived from two previously unidentified ER transcripts (designated E and H) that both utilize the +163 splice acceptor site. A panel of breast and endometrial carcinoma cell lines were screened by reverse transcriptase-polymerase chain reaction (RT-PCR) for expression of the E and H transcripts. It was found that all ER-positive cell lines expressed both of the novel transcripts. In addition, 10 primary human breast cancers were analyzed, of which six expressed the E transcript and five abundantly expressed the H transcript. These data indicate that expression of ER in human breast cancers can be dependent upon an alternate promoter at least 20 kb upstream of the primary cap site for ER.

Tuohimaa, P., A. Lyakhovich, et al. (2001). "Vitamin D and prostate cancer." The Journal of Steroid Biochemistry and Molecular Biology **76**(1-5): 125.

<http://www.sciencedirect.com/science/article/B6T8X-439WS7B->

Our recent epidemiological study (Ahonen et al., Cancer Causes Control 11(2000) (847-852)) suggests that vitamin D deficiency may increase the risk of initiation and progression of prostate cancer. The nested case-control study was based on a 13-year follow-up of about 19000 middle-aged men free of clinically verified prostate cancer. More than one-half of the serum samples had 25OH-vitamin D (25-VD) levels below 50 nmol/l, suggesting VD deficiency. Prostate cancer risk was highest among the group of younger men (40-51 years) with low serum 25-VD, whereas low serum 25-VD appeared not to increase the risk of prostate cancer in older men (>51 years). This suggests that VD has a protective role against prostate cancer only before the andropause, when serum androgen concentrations are higher. The lowest 25-VD concentrations in the younger men were associated with more aggressive prostate cancer. Furthermore, the high 25-VD levels delayed the appearance of clinically verified prostate cancer by 1.8 years. Since these results suggest that vitamin D has a protective role against prostate cancer, we tried to determine whether full spectrum lighting (FSL) during working hours could increase serum 25-VD concentrations. After 1-month exposure, there was no significant increase in the serum 25-VD level, although there was a bias towards slightly increasing values in the test group as opposed to decreasing values in controls. There was no significant change in the skin urocanic acid production. The possibility to use FSL in cancer prevention is discussed. In order to clarify the mechanism of VD action on cell proliferation and differentiation, we performed studies with the rat and human prostates as well prostate cancer cell lines. It is possible that 25-VD may have a direct role in the host anticancer defence activity, but the metabolism of vitamin D in the prostate may also play an important role in its action. We raised antibodies against human 1[alpha]-hydroxylase and 24-hydroxylase. Our preliminary results suggest that vitamin D is actively metabolised in the prostate. Vitamin D appears to upregulate androgen receptor expression, whereas androgens seem to upregulate vitamin D receptor (VDR). This may at least partially explain the androgen dependence of VD action. VD alone or administered with androgen causes a suppression of epithelial cell proliferation. VD can activate mitogen-activated kinases, erk-1 and erk-2, within minutes and p38 within hours. Also, auto/paracrine regulation might be involved, since keratinocyte growth factor (mRNA and protein) was clearly induced by VD. Based on these studies, a putative model for VD action on cell proliferation and differentiation is presented.

Vallee, M., C. Albert, et al. (2001). "Isolation and characterization of the monkey UDP-glucuronosyltransferase cDNA clone monUGT1A01 active on bilirubin and estrogens." The Journal of Steroid Biochemistry and Molecular Biology **77**(4-5): 239.

<http://www.sciencedirect.com/science/article/B6T8X-43G2YP5-7/2/dceda5a9ab78d6b59645ca83932f8fd7>

Although enzymes that catalyze the formation of steroids are well known, less information is available about the enzymes involved in the metabolism of these hormones. Steroid glucuronidation, by UDP-glucuronosyltransferase enzymes, is one mechanism by which steroid hormones can be metabolized and eliminated from a tissue. Previous results suggest that the monkey represents the most appropriate animal model for studying the physiologic relevance of steroid glucuronidating enzymes. The monkey UGT1A01 cDNA clone was isolated by RT-PCR amplification of the liver RNA. The cDNA contains an open reading frame of 1599 bp encoding a protein of 533 residues. The primary structure of monkey UGT1A01 is 95% identical to human UGT1A1. To compare monkey and human UGT1A1 enzymes, both cDNA clones were transfected into HK293 cells and stable cell lines expressing each UGT1A1 protein were established. Western blot analysis of the monUGT1A01-HK293 and hUGT1A1-HK293 cell lines using a anti-UGT1A polyclonal antibody (RC-71) revealed expression of exogenous 55 kDa UGT1 proteins. The transferase activities of monkey and human UGT1A1 proteins were tested with over 60 compounds and were demonstrated to be active on the same compounds. For endogenous compounds only bilirubin and C18 steroids were glucuronidated by these enzymes.

Using microsome preparation (from HK293 cell expressing monkey UGT1A01), the apparent K_m values were 13, 5 and 6 $[\mu]M$ for the conjugation of estradiol, 2-hydroxyestradiol and 2-hydroxyestrone, respectively, and were very similar to the values obtained with human UGT1A1. Specific RT-PCR analysis demonstrated the expression of monkey and human UGT1A1 transcripts in several tissues including liver, kidney, intestine, prostate, testis and ovary suggesting a contribution of this isoenzyme to estrogen metabolism in the cynomolgus monkey as in human.

Villalpando, I. and V. Lopez-Olmos (2003). "Insulin-like growth factor I (IGF-I) regulates endocrine activity of the embryonic testis in the mouse." The Journal of Steroid Biochemistry and Molecular Biology **86**(2): 151.

<http://www.sciencedirect.com/science/article/B6T8X-49HXFTK-1/2/c9dd44879b2ca2ec0cd7a221ffadcc37>

Insulin-like growth factor I (IGF-I) is important for gonadal and reproductive functions in mammals, although the physiological role of this growth factor during gonadal development in rodents remains largely unknown. Here, we examined the steady-state levels of IGF-I mRNA by the reverse transcriptase polymerase chain reaction (RT-PCR). IGF-I protein expression was also detected by Western blot. The effect of IGF-I as promoter of 17 $[\alpha]$ -hydroxylase/C17-20 lyase and 17 $[\beta]$ -hydroxysteroid dehydrogenase enzyme activity in vitro was evaluated by radioimmunoassay. Onset of IGF-I gene expression was on day E10 (urogenital ridge stage). IGF-I mRNA expression was markedly reduced on days E12 and E13 (testicular differentiation stage). IGF-I transcripts increased on day E14 and their transcription levels were maintained throughout the stages analyzed. Several IGF-I protein bands of 31-100 kDa were observed. Culture experiments demonstrated that 17 $[\alpha]$ -hydroxyprogesterone and testosterone (T) secretion levels increased in the presence of IGF-I on days E11-E17. Additive effects of IGF-I plus (Bu) $_2$ cAMP were also seen during testicular development. It is proposed that IGF-I regulates the expression of key steroidogenic enzymes important for endocrine activity of the testis during prenatal development leading to establishment of the male phenotype and fertility.

The Lancet (36)

Ashshi, A. M., R. J. Cooper, et al. (2000). "Detection of human herpes virus 6 DNA in fetal hydrops." The Lancet **355**(9214): 1519.

<http://www.sciencedirect.com/science/article/B6T1B-407R3CT-K/2/9a52a09a517b53f2a0d9f1abe5238053>

Human herpes virus 6 (HHV6) DNA was detected in two of eight fetuses with hydrops and none of ten non-hydrotic dead fetuses. Both cases with HHV6 DNA had chromosomal abnormalities. Positive results were confirmed with a second PCR specific for an alternate region of the HHV6 genome. Restriction endonuclease analysis confirmed that the viral DNA was representative of HHV6 type A.

Bahn, S., M. Mimmack, et al. (2002). "Neuronal target genes of the neuron-restrictive silencer factor in neurospheres derived from fetuses with Down's syndrome: a gene expression study." The Lancet **359**(9303): 310.

<http://www.sciencedirect.com/science/article/B6T1B-451NBBT-D/2/9a3279afd73cc0d16e0edc53550f77db>

SummaryBackground Identification of genes and characterisation of their function is an essential step towards understanding complex pathophysiological abnormalities in Down's syndrome. We did a study to investigate abnormalities in gene expression in human neuronal stem cells and progenitor cells from Down's syndrome and control post-mortem human fetal tissue. **Methods** Indexing-based differential display PCR was done on neuronal precursor cells derived from the cortex of a fetus with Down's syndrome, and findings were compared with those of two control samples. Findings were validated against neurosphere preparations from three independent Down's syndrome fetuses and five independent controls by real-time quantitative PCR. **Findings** Results of differential display PCR analysis showed that SCG10--a neuron-specific growth-associated protein regulated by the neuron-restrictive silencer factor REST--was almost undetectable in the Down's syndrome sample. This finding was validated by real-time PCR. We also found that other genes regulated by the REST transcription factor were selectively repressed, whereas non-REST-regulated genes with similar functions were unaffected. Changes in expression of several key developmental genes in the Down's syndrome stem-cell and progenitor-cell pool correlated with striking changes in neuron morphology after differentiation. **Interpretation** Our findings suggest a link between dysregulation of the REST transcription factor and some of the neurological deficits seen in Down's syndrome. Experimental REST downregulation has been shown to trigger apoptosis, which could account for the striking and selective loss of neurons in the differentiated Down's syndrome cell preparations.

Baker, E. H., Y. B. Dong, et al. (1998). "Association of hypertension with T594M mutation in [beta] subunit of epithelial sodium channels in black people resident in London." The Lancet **351**(9113): 1388.

<http://www.sciencedirect.com/science/article/B6T1B-3Y9H2ND-4Y/2/ff3cc49a315132abaab11e5a750e9df0>

Background Liddle's syndrome is a rare inherited form of hypertension in which mutations of the epithelial sodium channel result in increased renal sodium reabsorption. Essential hypertension in black patients also shows clinical features of sodium retention so we screened black people for the T594M mutation, the most commonly identified sodium-channel mutation. **Methods** In a case-control study, 206 hypertensive (mean age 48.0 [SD 11.8] years, men:women 80:126) and 142 normotensive (48.7 [7.4] years; 61:81) black people who lived in London, UK, were screened for T594M. Part of the last exon of the epithelial sodium-channel [beta] subunit from genomic DNA was amplified by PCR. The T594M variant was detected by single-strand conformational polymorphism analysis of PCR products and confirmed by DNA sequencing. **Findings** 17 (8.3%) of 206 hypertensive participants compared with three (2.1%) of 142 normotensive participants possessed the T594M variant (odds ratio [OR]=4.17 [95% CI 1.12-18.25], p=0.029). A high proportion of participants with the T594M variant were women (15 of 17 hypertensive participants and all three normotensive participants), whereas women comprised a lower proportion of the individuals screened (61.2% hypertensive, 57.7% normotensive). However, the association between the T594M variant and hypertension persisted after adjustment for sex and body-mass index (Mantel-Haenszel OR=5.52 [1.40-30.61], p=0.012). Plasma renin activity was significantly lower in 13 hypertensive participants with the T594M variant (median=0.19 ng mL⁻¹ h⁻¹ than in 39 untreated hypertensive individuals without the variant (median=0.45 ng mL⁻¹ h⁻¹ p=0.009). **Interpretation** Among black London people the T594M sodium-channel [beta] subunit mutation occurs more frequently in people with hypertension than those without. The T594M variant may increase sodium-channel activity and could raise blood pressure in affected people

by increasing renal tubular sodium reabsorption. These findings suggest that the T594M mutation could be the most common secondary cause of essential hypertension in black people identified to date.

Clay, T. M., J. L. Bidwell, et al. (1991). "PCR-fingerprinting for selection of HLA matched unrelated marrow donors." The Lancet **337**(8749): 1049.

<http://www.sciencedirect.com/science/article/B6T1B-49K5B3P-32T/2/d1160bfef2f462146440a72c0a252723>

HLA typing contributes to the delays that occur in the search for HLA-matched unrelated marrow donors, and that result in poor patient survival. A new DNA technique for testing DR match between patient and unrelated marrow donors has been assessed. The technique is based on the formation of heteroduplexes between heterologous amplified coding and non-coding DNA sequences during the final annealing stage of the polymerase chain reaction (PCR), and different HLA-DR/Dw types give unique banding patterns (PCR fingerprints) on non-denaturing polyacrylamide gel electrophoresis. HLA-DR matching is by visual comparison of patients' with donors' fingerprints. Identity can be confirmed by mixing donor and recipient DNA before the final stage of the PCR (DNA crossmatching). In an assessment of the technique in 53 unrelated HLA-A and HLA-B matched patient-donor pairs, 42 pairs gave the same results with PCR fingerprinting as with DNA-RFLP analysis. In the 11 other pairs DR/Dw mismatches were detected by PCR fingerprinting but not by the standard DNA-RFLP method; PCR-SSO typing with selected sequence-specific oligonucleotides (SSO) confirmed that mismatches were due to different subtypes of DR4. PCR fingerprinting might thus accelerate the selection of unrelated marrow donors by simplifying the logistics of the donor search.

Fischereder, M., B. Luckow, et al. (2001). "CC chemokine receptor 5 and renal-transplant survival." The Lancet **357**(9270): 1758.

<http://www.sciencedirect.com/science/article/B6T1B-436W05H-H/2/9c30d00629369dcc58f86f59057c5f1c>

SummaryBackgroundAbout 1% of white populations are homozygous carriers of an allele of the gene for the CC chemokine receptor 5 (CCR5) with a 32 bp deletion (CCR5[Delta]32), which leads to an inactive receptor. During acute and chronic transplant rejection, ligands for CCR5 are upregulated, and the graft is infiltrated by CCR5-positive mononuclear cells. We therefore investigated the influence of CCR5[Delta]32 on renal-transplant survival.**Methods**Genomic DNA from peripheral-blood leucocytes of 1227 renal-transplant recipients was screened by PCR for the presence of CCR5[Delta]32. Demographic and clinical data were extracted from hospital records. Complete follow-up data were available for 576 recipients of first renal transplants. Graft survival was analysed by Fisher's exact test and Kaplan-Meier plots compared with a log-rank test.**Findings**PCR identified 21 patients homozygous for CCR5[Delta]32 (frequency 1[middle dot]7%). One patient died with a functioning graft. Only one of the remaining patients lost transplant function during follow-up (median 7[middle dot]2 years) compared with 78 of the 555 patients with a homozygous wild-type or heterozygous CCR5[delta]32 genotype. Graft survival was significantly longer in the homozygous CCR5[Delta]32 group than in the control group (log-rank $p=0\text{[middle dot]}033$; hazard ratio $0\text{[middle dot]}367$ [95% CI $0\text{[middle dot]}157\text{--}0\text{[middle dot]}859$]).**Interpretation**Patients homozygous for CCR5[Delta]32 show longer survival of renal transplants than those with other genotypes, suggesting a pathophysiological role for CCR5 in transplant loss. This receptor may be a useful target for the prevention of transplant loss.

Gilks, W. P., P. M. Abou-Sleiman, et al. (2005). "A common LRRK2 mutation in idiopathic Parkinson's disease." The Lancet **365**(9457): 415.

<http://www.sciencedirect.com/science/article/B6T1B-4FBVXJV-14/2/2f2237832e72339635c19cb0c53ebff7>

Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene have been shown to cause autosomal dominant Parkinson's disease. Few mutations in this gene have been identified. We investigated the frequency of a common heterozygous mutation, 2877510 g->A, which produces a glycine to serine aminoacid substitution at codon 2019 (Gly2019 ser), in idiopathic Parkinson's disease. We assessed 482 patients with the disorder, of whom 263 had pathologically confirmed disease, by direct sequencing for mutations in exon 41 of LRRK2. The mutation was present in eight (1.6%) patients. We have shown that a common single Mendelian mutation is implicated in sporadic Parkinson's disease. We suggest that testing for this mutation will be important in the management and genetic counselling of patients with Parkinson's disease.

Gu, M., J. M. Cooper, et al. (2000). "Oxidative-phosphorylation defects in liver of patients with Wilson's disease." The Lancet **356**(9228): 469.

<http://www.sciencedirect.com/science/article/B6T1B-411G2C5-F/2/dc49b8cf18626bde7effa50ad3143051>

Background Wilson's disease (WD) is caused by mutations in a P-type ATPase and is associated with copper deposition in liver and brain. The WD protein is present in the trans-Golgi network and may also be imported into mitochondria. The WD protein functions as a P-type copper transporting ATPase in the Golgi but any action in mitochondria is at present unknown. Methods We studied mitochondrial function and aconitase activity in WD liver tissue and compared the results with those in a series of healthy controls and patients without WD. Findings There was evidence of severe mitochondrial dysfunction in the livers of patients with WD. Enzyme activities were decreased as follows: complex I by 62%, complex II+III by 52%, complex IV by 33%, and aconitase by 71%. These defects did not seem to be secondary to penicillamine use, cholestasis, or poor hepatocellular synthetic function. Interpretation The results show that there is a defect of energy metabolism in WD. The pattern of enzyme defects suggests that free-radical formation and oxidative damage, probably mediated via mitochondrial copper accumulation, are important in WD pathogenesis. These results provide a rationale for a study of the use of antioxidants in WD.

Guy, E. and R. Farquhar (1991). "Borrelia burgdorferi in urban parks." The Lancet **338**(8761): 253.

<http://www.sciencedirect.com/science/article/B6T1B-49K0C6T-3SB/2/d484f26c8dc3e03cbb0c88e3ad058ec9>

Hatzakis, A., G. Touloumi, et al. (2000). "Effect of recent thymic emigrants on progression of HIV-1 disease." The Lancet **355**(9204): 599.

<http://www.sciencedirect.com/science/article/B6T1B-3YTB3WH-B/2/ff8ab7a1c64ccd0f063daa07e51e5b62>

Background The concentration of T-cell receptor-rearrangement excision DNA circles (TREC) in peripheral-blood T cells is a marker of recent thymic emigrant [alpha]b T cells. We studied the predictive ability of measurements of TREC for clinical outcome in HIV-1-infected individuals. **Methods** We measured TREC in peripheral-blood mononuclear cells with a real-time PCR assay. We studied 131 Greek participants in the Multicenter Hemophilia Cohort Study who had known HIV-1 seroconversion dates. The prognostic value of baseline TREC, CD4 T-cell count, and HIV-1 RNA concentration was assessed by Kaplan-Meier and Cox's regression analysis. **Findings** Four participants had progressed to AIDS by first blood sampling. Among the remaining 127 individuals, the median value of TREC per 10⁶ cells was 6900 (IQR 2370-15 604). Baseline TREC values were lower in the 53 who progressed to AIDS than in those who did not (geometric mean 2843 [95% CI 1468-5504] vs 6560 [4723-9113] TREC per 10⁶ cells; $p=0.017$). The relative hazard of AIDS, adjusted for plasma viral load, CD4 T-cell count, and age at seroconversion was 1.44 (95% CI 1.04-2.01; $p=0.031$) per ten-fold increase in TREC; that for death was 1.52 (1.12-2.06; $p=0.007$). The adjusted relative hazards of death were 2.91 (1.91-4.44; p Interpretation The concentration of TREC in the peripheral T-cell pool complements HIV-1 RNA load and CD4 T-cell count in predicting the rate of HIV-1 disease progression. Recent thymic emigrants have a role in the pathogenesis of HIV-1 disease.

Helweg-Larsen, J., T. L. Benfield, et al. (1999). "Effects of mutations in *Pneumocystis carinii* dihydropteroate synthase gene on outcome of AIDS-associated *P. carinii* pneumonia." *The Lancet* **354**(9187): 1347.

<http://www.sciencedirect.com/science/article/B6T1B-3XYFJJ5-F/2/2a6659976699f596831e59e09a3773e9>

Background Sulpha drugs are widely used for the treatment and long-term prophylaxis of *Pneumocystis carinii* pneumonia (PCP) in HIV-1-infected individuals. Sulpha resistance in many microorganisms is caused by point mutations in dihydropteroate synthase (DHPS), an enzyme that is essential for folate biosynthesis. We assessed whether mutations in the DHPS gene of *P. carinii* were associated with exposure to sulpha drugs and influenced outcome from PCP. **Methods** We studied bronchoalveolar samples collected in 1989-99 from a prospective cohort of HIV-1-infected patients who had PCP. In 144 patients with 152 episodes of PCP, we analysed portions of DHPS using PCR and direct sequencing. The relation between survival, *P. carinii* DHPS mutations, and other predictors of treatment failure was assessed by Kaplan-Meier and multivariate Cox regression analysis. **Findings** *P. carinii* DHPS mutations were found in 31 (20.4%) of 152 PCP episodes. 3-month survival was significantly lower in patients infected with mutant *P. carinii* DHPS strains than in those with wild-type strains ($p=0.002$). After adjustment for other prognostic variables, presence of DHPS mutations remained the most important predictor of mortality (hazard ratio 3.1 [95% CI 1.2-8.1]). DHPS mutations were significantly more common in patients who had previous exposure to sulpha drugs (18 of 29 [62%]) than in those who had no exposure (13 of 123 [10.5%]); p DHPS mutations ($p=0.01$ for trend) was closely correlated with the rate of previous or current use of sulpha drugs as chemoprophylaxis. **Interpretation** Mutations in DHPS are associated with impaired prognosis in PCP, and may develop as a result of exposure to sulpha drugs.

Hill, A. V. S., C. E. M. Allsopp, et al. (1991). "HLA class I typing by PCR: HLA-B27 and an African B27 subtype." *The Lancet* **337**(8742): 640.

<http://www.sciencedirect.com/science/article/B6T1B-49K2KBM-1H1/2/53214cf820778f8cbb3f5b51735ef164>

We describe a rapid method of HLA class I typing using the polymerase chain reaction and

oligonucleotide hybridisation that eliminates the requirements for viable lymphocytes and allows subtypes to be defined. We have used this to demonstrate that the predominant subtype of HLA-B*27 in the Gambia, West Africa, is HLA-B*2703, which is very rare or absent in other racial groups. This subtype differs from the common Caucasian HLA-B*27 subtypes in its recognition by cytotoxic T cells. We propose that HLA-B*2703, unlike other HLA-B*27 subtypes, may not be associated with ankylosing spondylitis, thus accounting in part for the rarity of this condition in black populations.

Holding, C. and M. Monk (1989). "DIAGNOSIS OF BETA-THALASSAEMIA BY DNA AMPLIFICATION IN SINGLE BLASTOMERES FROM MOUSE PREIMPLANTATION EMBRYOS." The Lancet **334**(8662): 532.

<http://www.sciencedirect.com/science/article/B6T1B-49HFW1P-4CR/2/35da56adf530a8c756d89df6d86d4690>

Mouse preimplantation embryos were accurately diagnosed as normal or mutant at the beta-major haemoglobin locus by amplification of specific DNA sequences in a single cell. A DNA sequence containing the whole of exon 3 and some 3' untranslated sequences within the beta-major haemoglobin gene was amplified in single blastomeres by means of the polymerase chain reaction (PCR). Blastomeres were removed from embryos of four to eight cells from normal BALB/c mice and from mutant (thalassaemic) BALB/c mice homozygous for a deletion of the whole beta-major haemoglobin gene. The sensitivity of the amplification procedure was enhanced by the sequential use of two sets of oligonucleotide primers for 30 cycles of amplification each, the second pair being located within the segment amplified by the first pair. The product (204 base-pairs) could be easily visualised in ethidium bromide-stained agarose gels. Stringent precautions to prevent contamination were taken, and with these precautions the PCR amplification procedure could be carried out under normal laboratory conditions. These procedures for diagnosis of genetic disease before

Ingrosso, D., A. Cimmino, et al. (2003). "Folate treatment and unbalanced methylation and changes of allelic expression induced by hyperhomocysteinaemia in patients with uraemia." The Lancet **361**(9370): 1693.

<http://www.sciencedirect.com/science/article/B6T1B-48KVF5R-B/2/3d77a37ffeeae4d8f7f353993dfe2401>

SummaryBackgroundHyperhomocysteinaemia occurs in several genetically determined and acquired disorders and is highly prevalent in patients with uraemia. In these disorders, homocysteine precursor S-adenosylhomocysteine, a powerful competitive inhibitor of S-adenosylmethionine-dependent methyltransferases, is increased, suggesting unbalanced methylation. We aimed to investigate whether DNA hypomethylation is present in patients with uraemia who also have hyperhomocysteinaemia and whether regulation of specific classes of genes, dependent on DNA methylation, is compromised.**Methods**We selected men with hyperhomocysteinaemia and uraemia who were having standard haemodialysis treatment, and compared them with healthy male controls. We measured the homocysteine concentration from plasma samples and obtained DNA and RNA samples from peripheral mononuclear cells. DNA methylation was assessed by cytosine extension assay and by Southern blotting. Allelic expression of pseudoautosomal and imprinted genes was investigated by analysis of suitable restriction fragment length polymorphisms.**Findings**Total DNA hypomethylation was higher in patients than in controls (z score -4[middle dot]593, p=0[middle dot]0006) and allelic expression was changed in both sex-linked and imprinted genes. The shift from monoallelic to biallelic expression was dependent on homocysteine concentrations. Folate therapy, a common method

to reduce hyperhomocysteinaemia, restored DNA methylation to normal levels and corrected the patterns of gene expression. Interpretation Our results suggest that hyperhomocysteinaemia affects epigenetic control of gene expression, which can be reverted by folate treatment. Our data support the hypothesis that the toxic action of homocysteine can be mediated by macromolecule hypomethylation.

Joynson, D. H. M., C. Verhofstede, et al. (1990). "Congenital toxoplasmosis and TORCH." The Lancet **336**(8715): 622.

<http://www.sciencedirect.com/science/article/B6T1B-49M0JHS-20R/2/a055fc745e2c578f5f4c912ed171d1b9>

Kahn, R., H. Herwald, et al. (2002). "Contact-system activation in children with vasculitis." The Lancet **360**(9332): 535.

<http://www.sciencedirect.com/science/article/B6T1B-46HNW1W-D/2/3593cd67943d813e92da0a08f435bedd>

Summary Background The contact system triggers the kallikrein-kinin cascade, liberating bradykinin from high-molecular-weight kininogen. Effectors of the contact system have proinflammatory and vasoactive properties. Vasculitis is a condition characterised by inflammation around vessel walls, leading to secondary tissue damage for which the underlying molecular mechanisms are poorly understood. Our aim was to investigate contact-system activation in children with vasculitis. **Methods** We compared 17 children, aged 4-19 years, with vasculitis, engaging the skin, joints, intestines, or kidneys, with 21 controls, aged 2-18 years. We analysed proteolysis of high-molecular-weight kininogen by immunoblotting. Plasma bradykinin concentrations were quantified by ELISA. Kidney and skin biopsies were stained in situ for kinins. Concentrations of heparin binding protein (HBP) were quantified by ELISA. **Findings** We noted extensive proteolysis of high-molecular-weight kininogen in the plasma of 13 of 17 patients, but in only one of 21 controls. **Interpretation** Activation of the contact system could play a part in the pathogenesis of vasculitis, and explain the inflammation, pain, vasodilatation, and oedema seen in patients.

Kawakami, T., K. Okamoto, et al. (2004). "XIST unmethylated DNA fragments in male-derived plasma as a tumour marker for testicular cancer." The Lancet **363**(9402): 40.

<http://www.sciencedirect.com/science/article/B6T1B-4BC1S83-J/2/12d29dfac4096e6364ae164ccf25cba3>

Summary Testicular germ-cell tumours (TGCTs) are the most common malignant diseases among young men, with peak incidence at age 20-40 years. We developed a DNA tumour marker for TGCTs based on the unmethylated DNA profile of a neoplasm. The 5' end of the XIST gene is mainly hypomethylated in TGCTs irrespective of XIST expression. Male somatic cells, however, show complete methylation through the CpG sites, including the minimum promoter and XIST-conserved repeats. Identification of a XIST unmethylated fragment in male plasma might be diagnostic for TGCTs.

Laule, M., I. Cascorbi, et al. (1999). "A1/A2 polymorphism of glycoprotein IIIa and association with excess procedural risk for coronary catheter interventions: a case-controlled study." The Lancet **353**(9154): 708.

<http://www.sciencedirect.com/science/article/B6T1B-3W9KVPB-C/2/3ccb53a874d0f2ca0139bb43f8b86ef5>

Background A five-fold increase in risk of stent thrombosis in carriers of A1/A2 (Leu33Pro) polymorphism of glycoprotein IIIa has been described. Whether this increased procedural risk applies to other coronary interventions is unknown. We investigated the role of A1/A2 polymorphism as a putative risk factor. **Methods** We genotyped 1000 consecutive patients with angiographically confirmed coronary-artery disease and 1000 controls matched for age and sex. 653 of the 1000 patients received interventions (271 coronary angioplasty, 102 directional coronary atherectomy, and 280 stenting) and were assessed for a 30-day composite endpoint of need for target-vessel revascularisation, myocardial infarction, and death. **Findings** The composite endpoint occurred in 41 (6.3%) patients. There was no evidence that the A2 allele was associated with excess procedural risk (relative risk 1.36 [95% CI 0.70-2.70], $p=0.37$). Nor, in subgroup analyses, did A2 predict events that complicated coronary angioplasty (1.17 [0.40-2.70]), directional coronary atherectomy (1.50 [0.30-8.70]), or stenting (1.45 [0.60-3.50]). Neither heterozygotes (A1/A2) nor homozygotes (A2/A2) were over-represented in any subgroup, including those with acute coronary syndromes, early disease manifestation (age Interpretation A1/A2 polymorphism is not a major risk factor for 30-day adverse events that complicate coronary angioplasty, directional coronary atherectomy, or stenting. Furthermore, A1/A2 polymorphism has no apparent impact on more chronic processes such as atherogenesis of the coronary arteries.

Lewis, D. L., M. Arens, et al. (1992). "Cross-contamination potential with dental equipment." The Lancet **340**(8830): 1252.

<http://www.sciencedirect.com/science/article/B6T1B-49K57JH-18X/2/42d0cf88fde351239acb240791367319>

Some types of reused dental equipment, especially handpieces and their attachments for drilling and cleaning teeth, might be responsible for cross-contamination if patient material were to lodge temporarily in difficult-to-disinfect internal mechanisms. This possibility is worrisome with respect to transmission of hepatitis B and human immunodeficiency viruses (HBV, HIV). Previous cross-contamination studies have relied on laboratory experiments with bacteria or dye tracers. To assess possible risks more thoroughly, we tested 30 new prophylaxis angles and 12 new high-speed handpieces to see whether they would take up and expel contaminants in laboratory and clinical trials. In treatments of three patients, including two infected with HIV, human-specific DNA ([beta]-globin, HLA DQ[alpha]) and HIV proviral DNA were detected inside or coming back from the devices. Similarly, when handpieces were operated in contact with blood pooled from HBV-infected patients, HBV DNA was detected in samples taken from inside the equipment and from their attached air/water hoses. When we used bacteriophage [phi]X174 as a model virus in laboratory tests, many infective viral particles were recovered from internal mechanisms of handpieces, their connecting air/water hoses, and from water spray expelled when the equipment was reused. We recommend that reused high-speed, air-driven handpieces and prophylaxis angles should be cleaned and heat-treated between patients. Further studies are needed to determine ways of eliminating the risks associated with exhaust hoses and air/water input lines.

Limaye, A. P., L. Corey, et al. (2000). "Emergence of ganciclovir-resistant cytomegalovirus disease among recipients of solid-organ transplants." The Lancet **356**(9230): 645.

<http://www.sciencedirect.com/science/article/B6T1B-41357RN-F/2/ea698fd2a271021da108258eaf25c646>

SummaryBackgroundConcerns have been raised about emergence of ganciclovir resistance as a result of the advent of both routine oral ganciclovir prophylaxis and highly potent immunosuppression. We retrospectively assessed the occurrence of ganciclovir-resistant cytomegalovirus disease among transplant recipients who had received oral ganciclovir prophylaxis and highly potent immunosuppression.**Methods**We studied 240 recipients of liver, kidney, or pancreas transplants. Antiviral susceptibility testing of blood cytomegaloviral isolates was done when patients failed to respond to intravenous ganciclovir treatment for symptomatic cytomegalovirus infection. Portions of the UL97 gene associated with ganciclovir resistance were sequenced in cytomegalovirus isolates with phenotypic resistance to ganciclovir.**Findings**Ganciclovir-resistant cytomegalovirus disease developed in five (7%) of 67 seronegative recipients of cytomegalovirus-seropositive organs (D+/R-) compared with none of 173 seropositive recipients ($p=0.002$). Among the 25 (10.4%) patients who developed cytomegalovirus disease within 1 year after transplantation, five had ganciclovir-resistant cytomegalovirus disease. Among D+/R- transplant recipients, ganciclovir-resistant cytomegalovirus disease was more common among the group receiving the most potent immunosuppression--ie, recipients of kidney and pancreas or pancreas alone (four of 19) compared with all other transplant recipients (one of 48, $p=0.02$). Ganciclovir-resistant cytomegalovirus disease was diagnosed at a median of 10 months after transplantation (range 7-12) after lengthened exposure to ganciclovir, was associated with previously described mutations of the UL97 gene, and led to serious clinical complications.**Interpretation**Ganciclovir-resistant cytomegalovirus is an important cause of late morbidity among D+/R- transplant recipients who have had lengthened exposure to ganciclovir and have received highly potent immunosuppression. Strategies to reduce this complication, especially among D+/R- patients, are warranted.

Lipschik, G. Y., V. A. Andrawis, et al. (1992). "Improved diagnosis of *Pneumocystis carinii* infection by polymerase chain reaction on induced sputum and blood." *The Lancet* **340**(8813): 203.

<http://www.sciencedirect.com/science/article/B6T1B-49K5G3B-7N/2/99bf161b0209ccf0359f3db8a841141b>

Detection of *Pneumocystis carinii* by the polymerase chain reaction (PCR) may facilitate non-invasive diagnosis of *P. carinii* pneumonia and study of its epidemiology. We have compared the sensitivity and specificity of two PCR methods with those of conventional staining for detection of *P. carinii* in induced sputum, bronchoalveolar lavage fluid (BAL), and blood. Of 71 sputum samples, 17 were from patients with microbiologically confirmed *P. carinii* pneumonia. A nested PCR method correctly identified the presence of *P. carinii* in all 17 (100% sensitive, 95% confidence interval [CI] 81-100%) and found no organisms in 50 of 54 microbiologically negative samples (93% specific, 95% CI 82-98%). PCR with a single primer pair was 71% sensitive (44-90%) and 94% specific (85-99%). The sensitivity of conventional staining methods (direct and indirect fluorescence antibody and toluidine-blue-O tests) was significantly less (38-53%) than that of nested PCR (p*P. carinii* was detected in BAL or sputum from 10 immunocompromised patients without microbiological evidence of *P. carinii* pneumonia, which suggests that symptom-free carriers or subclinical infection can exist. *P. carinii* was detected by nested PCR in blood from 2 of 3 patients with disseminated pneumocystosis but in only 1 of 11 patients with *P. carinii* infection restricted to the lungs. Nested PCR on induced sputum is more sensitive than conventional staining methods for the diagnosis of *P. carinii* pneumonia and provides a non-invasive method of detecting disseminated disease.

Luoma, P., A. Melberg, et al. "Parkinsonism, premature menopause, and mitochondrial DNA polymerase [gamma] mutations: clinical and molecular genetic study." The Lancet **364**(9437): 875.

<http://www.sciencedirect.com/science/article/B6T1B-4D8V8SH-19/2/97c2d16510ef0a7f6f0d4e0da9896ab5>

SummaryBackgroundMutations in the gene encoding mitochondrial DNA polymerase [gamma] (POLG), the enzyme that synthesises mitochondrial DNA (mtDNA), have been associated with a mitochondrial disease--autosomal dominant or recessive progressive external ophthalmoplegia--and multiple deletions of mtDNA. Mitochondrial dysfunction is also suspected to participate in the pathogenesis of Parkinson's disease. However, no primary gene defects affecting mitochondrial proteins causing mendelian transmission of parkinsonism have been characterised. We aimed to analyse the gene sequence of POLG in patients with progressive external ophthalmoplegia and their healthy relatives. **Methods**In seven families of various ethnic origins we assessed patients with progressive external ophthalmoplegia and unaffected individuals by clinical, biochemical, morphological, and molecular genetic characterisation and positron emission tomography (PET). **Findings**We recorded mutations in POLG in members of all seven families. Clinical assessment showed significant cosegregation of parkinsonism with POLG mutations (pPOLG gene defect resulted in secondary accumulation of mtDNA deletions in patients' tissues). **Interpretation**Dysfunction of mitochondrial POLG causes a severe progressive multisystem disorder including parkinsonism and premature menopause, which are not typical of mitochondrial disease. Cosegregation of parkinsonism and POLG mutations in our families suggests that when defective, this gene can underlie mendelian transmission of parkinsonism. **Relevance to practice**Awareness that mitochondrial POLG mutations can underlie parkinsonism is important for clinicians working in diagnosis of movement disorders, as well as for studies of the genetics of Parkinson's disease. Further, progressive external ophthalmoplegia with muscle weakness and neuropathy can mask symptoms of parkinsonism, and clinicians should pay special attention to detect and treat parkinsonism in those individuals.

Mitchell, T. J., A. J. Walley, et al. (2000). "Delta 32 deletion of CCR5 gene and association with asthma or atopy." The Lancet **356**(9240): 1491.

<http://www.sciencedirect.com/science/article/B6T1B-41PVXTP-M/2/287ce1e5dd5a24f5f33b7a7eef5ddc8b>

SummaryThe CCR5-[Delta]32 deletion polymorphism (CCR5-[Delta]32) was investigated for linkage and association to asthma and atopy using two panels of nuclear families containing 1284 individuals. No statistically significant linkage to asthma/ wheeze or atopy was observed in either of the two panels of families. Multiallelic transmission disequilibrium tests (TDT) of the combined data found no significant association for atopy (52 independent alleles transmitted, 51 non-transmitted) or asthma/wheeze (39 transmitted, 44 non-transmitted). Although functional evidence might suggest that CCR5 is a good candidate gene for atopic asthma, this study provides no genetic evidence from CCR5-[Delta]32 polymorphism to support this hypothesis.

Monk, M. and C. Holding (1990). "Amplification of a [beta]-haemoglobin sequence in individual human oocytes and polar bodies." The Lancet **335**(8696): 985.

<http://www.sciencedirect.com/science/article/B6T1B-49M0N8R-373/2/1e7485d6a509b2e1d656648127268f79>

A 680 base-pair sequence of the human [beta]-haemoglobin gene was reproducibly amplified in

individual unfertilised human oocytes and in first polar bodies isolated from them. Specificity and sensitivity of amplification were achieved by two sequential reactions with two sets of primers, amplifying first a 725 base-pair sequence and secondly a 680 base-pair sequence from within the first amplified fragment. A restriction enzyme digest of the DNA amplified from a single oocyte with the endonuclease Dde I confirmed the identity of the amplified [beta]-haemoglobin fragment; this technique provides a diagnostic test for the genetic defect responsible for sickle cell anaemia. Analysis of the DNA from the first polar body may enable detection of such defects in unfertilised eggs from carrier women. Selection of eggs without the defect for fertilisation may therefore obviate the need for diagnostic procedures on embryos.

Munroe, P. B., H. M. Mitchison, et al. (1996). "Prenatal diagnosis of Batten's disease." The Lancet **347**(9007): 1014.

<http://www.sciencedirect.com/science/article/B6T1B-4B8JK7N-169/2/02abac5f95591fd0c1d66dda15a92f07>

SummaryBackground Batten's disease is the most common progressive encephalopathy of childhood in Western countries. The major mutation is a 1 kb deletion, which is carried by 81% of Batten's disease patients. We report on the use of direct gene analysis in the prenatal diagnosis of this disease.Methods and findings A Finnish woman with a son with Batten's disease came for genetic counselling for her current pregnancy. Electron microscopy of a chorionic villus sample gave suggestive findings. We used PCR to look for the intragenic microsatellite marker D16S298; 96% of Finnish Batten's disease patients carry allele 6 at this marker. The fetus and the affected son both carried the same high-risk genotype, 6/6. Both were homozygous for the 1 kb deletion. The pregnancy was terminated. Electron microscopy of the fetus showed typical Batten's disease changes.Interpretation We have successfully used direct gene analysis in the prenatal diagnosis of Batten's disease.

Naylor, J. A., P. M. Green, et al. (1991). "Detection of three novel mutations in two haemophilia A patients by rapid screening of whole essential region of factor VIII gene." The Lancet **337**(8742): 635.

<http://www.sciencedirect.com/science/article/B6T1B-49K2KBM-1GY/2/54e17c75b8fae4185bf0bbc088fa13a1>

In an attempt to replace the existing, DNA-based, 50% effective, carrier and prenatal diagnoses of haemophilia A with the 100% successful direct detection of defective genes, a new procedure was developed to screen and identify mutations in all the essential regions of the factor VIII gene (putative promoter, coding sequence, and the cleavage and polyadenylation region). Genomic DNA and cDNA obtained by reverse transcription of the leaky mRNA found in peripheral lymphocytes were amplified by means of the polymerase chain reaction to yield a set of eight segments comprising the essential gene sequences. The segments were then screened individually for mutations by the amplification mismatch detection method, which detects and locates any type of sequence discrepancy between the test DNA and the control probe by cleavage of the probe at the site of mismatches. Two haemophilia A patients were studied. The first showed two single-base changes: one (substitution of tryptophan 2229 by cysteine in the C2 domain) is the probable cause of the disease, since it affects a conserved residue of factor VIIIa, whereas the other (the conservative substitution of aspartic acid at position 1241 by glutamic acid) occurs in a domain (B) irrelevant to factor VIII activity. The second patient showed a complete failure of pre-mRNA splicing due to a single-base substitution that changes the obligatory AG acceptor splice site of intron 5 to GG. The method characterises the gene defect in 10 days or less and should lead to the rapid accumulation of information on the molecular biology of haemophilia A.

Old, J. M., N. Y. Varawalla, et al. (1990). "Rapid detection and prenatal diagnosis of [beta]-thalassaemia: studies in Indian and Cypriot populations in the UK." The Lancet **336**(8719): 834.

<http://www.sciencedirect.com/science/article/B6T1B-49M0MHX-2WH/2/ee54c2e8ae7ce7266c4d57e02db9e134>

The application of the amplification refractory mutation system (ARMS) to the detection of individual [beta]-thalassaemia mutations in heterozygous parents and at risk fetuses has been assessed in Indian and Cypriot immigrant populations in the UK. 100 first trimester prenatal diagnoses have been done, entailing the detection of 17 different mutations. The method, which allows the determination of the mutations in both parental and fetal DNA on the same day, should have wide application to the carrier detection and prenatal diagnosis of monogenic diseases with heterogeneous molecular defects.

Price, C. G. A., F. E. Cotter, et al. (1989). "POLYMERASE CHAIN REACTION TO CONFIRM EXTRANODAL PROGRESSION OF FOLLICULAR LYMPHOMA." The Lancet **333**(8647): 1132.

<http://www.sciencedirect.com/science/article/B6T1B-49KJXD5-147/2/a76c783b8db34652886a5a633022622e>

Quillent, C., E. Oberlin, et al. (1998). "HIV-1-resistance phenotype conferred by combination of two separate inherited mutations of CCR5 gene." The Lancet **351**(9095): 14.

<http://www.sciencedirect.com/science/article/B6T1B-3TXSRB9-4/2/7ca76ab386c17ac1ef25bff5bbb5f0db>

Background Despite multiple exposures to HIV-1, some individuals remain uninfected, and their peripheral-blood mononuclear cells (PBMC) are resistant to in-vitro infection by primary HIV-1 isolates. Such resistance has been associated with a homozygous 32-base-pair deletion ([Delta]32) in the C-C chemokine receptor gene CCR5. We examined other mutations of the CCR5 gene that could be associated with resistance to HIV-1 infection. **Methods** We assessed the susceptibility of PBMC to in-vitro infection by HIV-1 isolates that use the CCR5 as the major coreceptor for viral entry in 18 men who had frequent unprotected sexual intercourse with a seropositive partner. We also did genotypic analysis of CCR5 alleles. One of the 18 exposed but uninfected men (who we refer to as ExU2) showed total resistance to in-vitro infection by CCR5-dependent viruses, and was found to carry a CCR5 [Delta]32 allele and a single point mutation (T->A) at position 303 on the other allele. To find out whether the CCR5 mutation was restricted to ExU2's family or existed in the general population, we did genetic analyses of the CCR5 genotype in ExU2's father and sister and also in 209 healthy blood donors who were not exposed to HIV-1. **Findings** The m303 mutation found in ExU2 introduced a premature stop codon and prevented the expression of a functional coreceptor. The family studies revealed that the m303 mutant allele was inherited as a single mendelian trait. Genotype analysis showed that three of the 209 healthy blood donors were heterozygous for the mutant allele. **Interpretation** We characterise a new CCR5 gene mutation, present in the general population, that prevents expression of functional coreceptors from the abnormal allele and confers resistance to HIV-1 infection when associated to the [Delta]32 CCR5 mutant gene.

Roberts, R. G., D. R. Bentley, et al. (1990). "Direct diagnosis of carriers of Duchenne and Becker muscular dystrophy by amplification of lymphocyte RNA." The Lancet **336**(8730-8731): 1523.

[http://www.sciencedirect.com/science/article/B6T1B-4B023PS-
RY/2/8fd3065f966705ba91ee5744cdf4ed3a](http://www.sciencedirect.com/science/article/B6T1B-4B023PS-RY/2/8fd3065f966705ba91ee5744cdf4ed3a)

Rapid detection of deletion and duplication mutations that cause Duchenne and Becker muscular dystrophy was achieved in patients and carriers after amplification of small amounts of mRNA from peripheral blood lymphocytes. The entire coding region of the dystrophin mRNA was amplified in 10 sections by reverse transcription and nested polymerase chain reaction, and the products were directly visualised on acrylamide minigels with ethidium staining. Major structural gene mutations were identified by the appearance of a band of different size to that of the wild type. The altered band was readily detected in all patients and heterozygous relatives. This nonradioactive test of venous blood samples can be used for unambiguous and rapid identification of virtually all carriers of deletions or insertions within the dystrophin gene.

Roth, W. K., M. Weber, et al. (1999). "Feasibility and efficacy of routine PCR screening of blood donations for hepatitis C virus, hepatitis B virus, and HIV-1 in a blood-bank setting." The Lancet **353**(9150): 359.

[http://www.sciencedirect.com/science/article/B6T1B-3W49589-
6/2/662c95d38f4c48bc31c59b324a6df469](http://www.sciencedirect.com/science/article/B6T1B-3W49589-6/2/662c95d38f4c48bc31c59b324a6df469)

Background Despite sensitive antibody-based blood-donor screening, a residual risk of transfusion-transmitted viral infections exists. Only direct monitoring by sensitive nucleic-acid tests would provide data accurately to measure the risk and to assess risk-reduction procedures. We investigated the feasibility and efficacy of routine screening of donors for hepatitis C virus (HCV), hepatitis B virus (HBV), and HIV-1 by PCR. Methods For PCR testing, individual donor plasma samples were pooled (96 x 100 [mu]L) overnight by two automatic pipetting machines. Viruses were concentrated by centrifugation and nucleic acids were extracted. HCV PCR was done on the Cobas Amplicor system (Hoffmann-La Roche, Mannheim, Germany). HBV and HIV-1 sequences were amplified by single (non-nested) in-house PCRs and detected by agarose-gel electrophoresis. Detection limits were 1000-5000 genome equivalents/mL in the donor blood. Findings PCR testing was done in parallel to antibody screening with a maximum throughput of 3000 samples in 7-8 h. Positive samples were identified 1-2 days later. 111 of 373423 donations (107 of 4500 pools) were PCR and antibody/antigen-confirmed positive. We found one HCV PCR-positive antibody-negative donation with normal alanine aminotransferase and one HCV PCR-positive donation with an elevated alanine aminotransferase (100 IU), which was negative in the AxSYM 2.0 and Matrix 1.0, but positive after control in the Abbott Prism test (Abbott GmbH, Wiesbaden, Germany). Interpretation PCR is a suitable and fast blood-donor screening procedure and contributes to a reduction in viral transmission by transfusion of blood components. In our selected donor population, the yield of detected contaminated donations from donors in the time window in which they are highly infectious but do not have any symptoms or detectable antigen and antibody concentrations (diagnostic window), confirms theoretical estimates.

Wakefield, A. E., F. J. Pixley, et al. (1990). "Detection of *Pneumocystis carinii* with DNA amplification." The Lancet **336**(8713): 451.

[http://www.sciencedirect.com/science/article/B6T1B-49M0HT4-
1K1/2/782aac00830cfaa0c559ab64dcc5ce73](http://www.sciencedirect.com/science/article/B6T1B-49M0HT4-1K1/2/782aac00830cfaa0c559ab64dcc5ce73)

Oligonucleotide primers and probes were used in the polymerase chain reaction to amplify *Pneumocystis carinii* specific DNA sequences from alveolar lavage samples from 47 diagnostic bronchoscopies. No *P. carinii* DNA was found in lavage from 10 immunocompetent patients; only low levels were found in 3 of 13 samples from immunosuppressed individuals without *P. carinii* pneumonia (PCP), and the highest levels, readily demonstrated by simple ethidium bromide staining, were found in all of 16 samples from immunosuppressed patients with PCP confirmed by means of standard silver staining and in 4 from patients with clinical PCP but negative silver staining. DNA amplification provides a highly sensitive and specific technique for the identification of *P. carinii* that should be valuable in epidemiological studies on this parasitic infection and in diagnosis.

Wenham, P., W. Price, et al. (1991). "Apolipoprotein E genotyping by one-stage PCR." The Lancet **337**(8750): 1158.

<http://www.sciencedirect.com/science/article/B6T1B-49K5BJG-3DT/2/ee69fa32cbc4832c8c462800ecb94228>

Wong, Z. Y. H., M. Stebbing, et al. (1999). "Genetic linkage of [beta] and [gamma] subunits of epithelial sodium channel to systolic blood pressure." The Lancet **353**(9160): 1222.

<http://www.sciencedirect.com/science/article/B6T1B-3WK3BSG-B/2/f186398e4702485929c6f6857d84871f>

Background Mutations in the genes on chromosome 16p12 that encode the [beta] and [gamma] subunits of the epithelial sodium channel (SCNNIB and SCNNIG, respectively) have been linked with rare sodium-dependent forms of low and high blood pressure. Other DNA variants in or around these genes may contribute to variation in blood pressure and the risk of coronary heart disease and stroke. **Methods** We studied 286 white families from the general population in Victoria, Australia. Each family comprised both parents and two natural children. All participants were genotyped at chromosome 16p12 by use of four highly polymorphic microsatellite markers. Quantitative phenotype measurements were correlated with genotype in identity-by-descent sibling-pair linkage analyses. **Findings** We found significant linkage between systolic blood pressure and chromosome 16p12 after parametric analyses ($p=0.0003$) and non-parametric analyses ($p=0.001$). The mean difference in systolic blood pressure between siblings identical-by-descent at these loci was half as large (7.1 mm Hg) as the difference between siblings non-identical at these loci (14.0 mm Hg, $p=0.001$). No linkage between chromosome 16p12 and diastolic blood pressure or body-mass index was observed. **Interpretation** Chromosome 16p12 and the SCNNIB and SCNNIG genes are implicated in the physiological variation of systolic blood pressure. Our findings are important in explaining individual cardiovascular risk within the general population.

Wright, T. L., D. Mamish, et al. (1992). "Hepatitis B virus and apparent fulminant non-A, non-B hepatitis." The Lancet **339**(8799): 952.

<http://www.sciencedirect.com/science/article/B6T1B-49K572T-XT/2/817f081654d906ee8a2d097d4205efe1>

While there is evidence that hepatitis C virus (HCV) does not cause fulminant non-A, non-B hepatitis, the causal agent remains unknown. To evaluate the role of hepatitis B virus (HBV) in

this disease, we used a two-step polymerase chain reaction (PCR) to amplify the surface and core regions of HBV DNA in serum and liver samples taken prospectively from twenty-six patients (mean age 36 years, range 1 to 64) with acute hepatic failure undergoing liver transplantation. HBV DNA was absent from the serum of all patients before transplantation. Seventeen patients were diagnosed as having non-A, non-B hepatitis because they lacked serological evidence of hepatitis A virus or HBV infection. Liver samples were taken from twelve of these patients, and six samples were positive for HBV DNA. By contrast HBV DNA was not detected in liver from three patients with acute liver failure caused by hepatitis A or toxins. HCV RNA was not found in pretransplant samples by PCR. Four of the six patients with detectable HBV DNA in liver and presumptive non-A, non-B hepatitis had detectable HBV DNA in serum after transplantation. One additional patient who did not donate pretransplant liver had HBV DNA in a post-transplant serum sample. Thus, HBV DNA was present before or after transplantation in seven of seventeen patients with apparent non-A, non-B hepatitis. Three of five patients with detectable post-transplant serum HBV DNA were serologically positive for HBV surface antigen. These findings indicate that HBV may be a common cause of fulminant hepatic failure in patients lacking serological evidence of HBV infection.

Yagi, H., Y. Furutani, et al. (2003). "Role of TBX1 in human del22q11.2 syndrome." The Lancet **362**(9393): 1366.

<http://www.sciencedirect.com/science/article/B6T1B-49V58KN-B/2/a1dfc425a7e308d963895e91bba51c7f>

SummaryBackgroundDel22q11.2 syndrome is the most frequent known chromosomal microdeletion syndrome, with an incidence of 1 in 4000-5000 livebirths. It is characterised by a 3-Mb deletion on chromosome 22q11.2, cardiac abnormalities, T-cell deficits, cleft palate facial anomalies, and hypocalcaemia. At least 30 genes have been mapped to the deleted region. However, the association of these genes with the cause of this syndrome is not clearly understood.**Methods**To test for the chromosomal deletion at 22q11.2, we did fluorescence in-situ hybridisation analysis with ten probes on 22q11.2 in 235 unrelated patients with clinically diagnosed del22q11.2 syndrome. To investigate mutations in the coding sequence of TBX1, we also did genetic analysis in 13 patients from ten families who have the 22q11.2 syndrome phenotype but no detectable deletion of 22q11.2.**Findings**96% (225 of 235) of patients had a defined 1[middle dot]5-3-Mb deletion at 22q11.2. We identified three mutations of TBX1 in two unrelated patients without the 22q11.2 deletion--one with sporadic conotruncal anomaly face syndrome/velocardiofacial syndrome and one with sporadic DiGeorge's syndrome--and in three patients from a family with conotruncal anomaly face syndrome/velocardiofacial syndrome. We did not record these three mutations in 555 healthy controls (1110 chromosomes);**Interpretation**Our results suggest that the TBX1 mutation is responsible for five major phenotypes in del22q11.2 syndrome. Therefore, we conclude that TBX1 is a major genetic determinant of the del22q11.2 syndrome.

Yerly, S., L. Kaiser, et al. (1999). "Transmission of antiretroviral-drug-resistant HIV-1 variants." The Lancet **354**(9180): 729.

<http://www.sciencedirect.com/science/article/B6T1B-3XFTGMD-D/2/8bd731cae2532a0c2241dab7b74cf73e>

Background Resistance of HIV-1 to antiretroviral drugs is the main cause of antiretroviral treatment failure. We assessed the transmission of drug-resistant variants among individuals with primary HIV-1 infection.**Methods** Population-based sequencing of the viral reverse-transcriptase and protease genes derived from plasma viral RNA was done in 82 consecutive individuals with

documented primary HIV-1 infection from January, 1996, to July, 1998. Phenotypic resistance to protease inhibitors was assessed by recombinant virus assay in individuals with two or more mutations associated with resistance to protease inhibitors. Findings Zidovudine-resistance mutations were detected in seven (9%) of 82 individuals. Mutations associated with resistance to other reverse-transcriptase inhibitors (RTIs) were detected in two individuals. Primary-resistance mutations associated with protease inhibitors (V82A, L90M) were detected in three (4%) of 70 individuals; two of these had also RTI-resistance mutations. Decreased sensitivity to three or four protease inhibitors was seen in three individuals, one of whom was infected with HIV-1 variants that harboured 12 mutations associated with resistance to multiple RTI and protease inhibitors. Interpretation To introduce the best antiretroviral treatment, resistance testing should be done in recently HIV-1-infected individuals.

Theriogenology (11)

Blocher, S., R. Behr, et al. (2003). "Different CREM-isoform gene expression between equine and human normal and impaired spermatogenesis." *Theriogenology* **60**(7): 1357.

<http://www.sciencedirect.com/science/article/B6TCM-48PDMBD-3/2/e4dcd43d4d514e2e0699077fa7f34bdc>

Histone-to-protamine exchange causes chromatin condensation ceasing gene expression in elongating spermatids. Gene expression of protamines is regulated by the transcription factor cAMP-responsive element modulator (CREM). Altered CREM expression results in male infertility, as shown by CREM-knock-out mice being sterile due to round spermatid maturation arrest and patients exhibiting round spermatid maturation arrest revealing a lack or substantial reduction of both CREM-mRNA and CREM-protein. Similar defects in histone-to-protamine exchange have been suggested in infertile stallions exhibiting enlarged sperm heads. The CREM-gene consists of 14 exons. Alternative exon splicing results in the production of both activator and repressor proteins. To further clarify the role of different CREM-isoforms for male infertility, the expression pattern of various CREM-isoforms during equine and human normal and impaired spermatogenesis was investigated by RT-PCR. Stallions with normal spermatogenesis expressed six activators and three repressors. In men three activators and seven different repressors were detected. In one stallion and patients with impaired spermatogenesis, only repressors were found. It is concluded that (i) stallion and man reveal a different CREM expression pattern, (ii) the expression of CREM activators is a prerequisite for normal spermatogenesis, and (iii) the lack of CREM activator expression results in male infertility.

Bureau, M., S. Dea, et al. "Evaluation of virus decontamination techniques for porcine embryos produced in vitro." *Theriogenology* **In Press, Corrected Proof**

<http://www.sciencedirect.com/science/article/B6TCM-4F05GD0-4/2/e911158ef64bb0539b4ab4a2b97a9233>

The objective of this study was to explore approaches to decontaminate embryos either contaminated naturally or under experimental conditions with different viruses. Embryos were obtained from in vitro maturation and fertilisation of porcine oocytes. After 7 days of development, morula and blastocyst stages were exposed for 1 h to the following viruses: encephalomyocarditis virus (EMCV), porcine circovirus type 2 (PCV2), porcine parvovirus (PPV), porcine reproductive

and respiratory syndrome virus (PRRSV), and bovine viral diarrhea virus (BVDV) at an infectivity of 100 TCID₅₀/mL. Embryos samples were treated with different washing procedures, which all included the following standard washing solutions: PBS+0.4% BSA (five times for 10 s), Hank's + 0.25% trypsin (two times for 60-90 or 120-150 s, or one time of 5 min), Hank's + 0.1 mg/mL DNase 1 + 20 U/mL RNase One (one time of 30 min) and PBS + 0.4% BSA again (five times for 10 s). Two new approaches were used to improve trypsin treatment, 0.1% hyaluronidase (one time for 5 min) instead of trypsin and a pre-incubation with oviductal cells. Therefore, in the first experiment, oocytes received standard maturation treatments and in the second, they were also co-incubated with oviductal cells for the last 3 h of maturation. The effectiveness of the different washing techniques in removing viruses was evaluated by polymerase chain reaction (PCR) analysis. In the first experiment, trypsin treatment did not eliminate PRRSV, PPV, PCV, and EMCV from contaminated embryos. Surprisingly, treatment with hyaluronidase eliminated all tested viruses. In the second experiment, all viruses tested were removed from the oocytes following the different enzymatic treatments. In conclusion, in vitro embryo decontamination was more effective following exposure to oviductal secretions and hyaluronidase eliminated more virions than trypsin in washing techniques.

Fieni, F., J. Rowe, et al. (2002). "Presence of caprine arthritis-encephalitis virus (CAEV) infected cells in flushing media following oviductal-stage embryo collection." *Theriogenology* **57**(2): 931.

<http://www.sciencedirect.com/science/article/B6TCM-44XCDXK-3/2/7d81006d231ddc375bcd93c6a0dc32e7>

To improve the knowledge on the risk of transmission of the caprine arthritis-encephalitis virus (CAEV) during embryo manipulations, we conducted a double-nested polymerase chain reaction (PCR) for CAEV proviral-DNA on flushing media recovered from the oviducts 48 h after the beginning of estrus and on blood from 89 donor does. Sixty-four does had negative blood and flushing media by PCR. Among the 25 CAEV infected goats (blood PCR positive), 11 were PCR flushing media positive (P). The mean number of embryos recovered was not significantly different between goats with flushing media PCR positive and goats with flushing media PCR negative (6.0+/-5.4 versus 7.8+/-4.4, respectively: mean+/-S.D.) nor between goats with blood PCR positive and goats with blood PCR negative (7.0+/-5.0 versus 5.9+/-5.3: mean+/-S.D.). The presence of CAEV infected cells in oviductal flushing media from infected donor does was indicated for the first time during this study. The absence of flushing media PCR positive for goat blood PCR negative seemed to allow the use of the blood PCR test to confidently predict the absence of CAEV provirus in the oviductal fluid.

Fieni, F., J. Rowe, et al. (2003). "Presence of caprine arthritis-encephalitis virus (CAEV) proviral DNA in genital tract tissues of superovulated dairy goat does." *Theriogenology* **59**(7): 1515.

<http://www.sciencedirect.com/science/article/B6TCM-4728DTG-2/2/fa35c7d8604f893259e13efaca1db8fc>

Transmission of caprine arthritis-encephalitis virus (CAEV) is not completely understood and the vertical route of infection from the goat to the embryo or to the fetus needs to be investigated. This route of infection involves the presence of CAEV in the genital tract tissues. Prior studies have detected CAEV-infected cells in genital secretions and in flushing media recovered during embryo collection from infected goats. To specify the origin of these cells, we conducted a double-nested polymerase chain reaction (PCR) test on embryo flushing media and on mammary gland, mammary lymph node, synovial membrane, pelvic lymph node, uterus and oviduct tissues from 25 CAEV-infected (blood PCR positive) embryo donor goats for the presence of CAEV proviral DNA. The presence of proviral DNA was found in 22 of 25 mammary gland samples, 14 of

25 uterus samples, and in 16 of 25 oviduct samples. Nineteen of 25 goats had at least one positive genital tract sample. Flushing media from 11 goats were PCR positive. All goats with positive-flushing media were oviduct positive. Of this group of does, except for 1 of the 11, infection of flushing media correlated with infection of almost all the other tissues examined. The frequency of positive tissues for flushing media-positive goats (61/66; 92%) was significantly higher than that for flushing media-negative goats (50/84; 60%) (P<0.05). This study demonstrated the presence of CAEV-infected cells in the goat genital tract. The presence of CAEV-infected cells in the uterus and oviducts suggests potential for vertical transmission of CAEV from doe to embryo or fetus.

Fujishiro, A., K. Kawakura, et al. (1995). "A fast, convenient diagnosis of the bovine freemartin syndrome using polymerase chain reaction." Theriogenology **43**(5): 883.

<http://www.sciencedirect.com/science/article/B6TCM-3YS90XS-14/2/45ae21f799b4939dff1acfa79a8bd05b>

To establish the polymerase chain reaction (PCR) method for detecting the XY cells in cases suspected to have the bovine freemartin syndrome, a PCR reaction test was conducted on blood from a normal bull diluted in blood from a normal cow. From the results obtained, it was shown that the Y-specific sequence was detectable down to a concentration of 0.1%. Various types of the bovine freemartin syndrome, which occurs in heterosexual twins, single-born sterile heifers, and heifers born with Acardius amorphus, were examined by the chromosome analysis and the PCR method. The Y-specific sequence was detected in all 26 cases that showed chromosome chimerism but which was absent in the 5 cases without a chimerism. The PCR method was found to be effective and convenient for quickly diagnosing the various types of bovine freemartin syndrome.

Hilgers, L. J. and C. Herr (1993). "DNA contamination of reagents used in embryo transfer and culture." Theriogenology **40**(5): 923.

<http://www.sciencedirect.com/science/article/B6TCM-49PSB4V-BX/2/ac8d75b6f6b522251357ea6aefbde64a>

Commonly used reagents in the culture and transfer of embryos are isolated from blood and tissue samples and thus have the potential for chromosomal and or mitochondrial DNA contamination. In this study, we evaluated the results obtained from PCR analysis of bovine trypsin, bovine sera, and bovine albumin precipitates. Bovine sera samples that were tested yielded minor to heavy DNA contamination signals depending on the manufacturer and specific type of sera. Bovine albumin precipitates showed very little DNA contamination or none at all. Bovine trypsin samples yielded moderate DNA contamination signals depending on the ability of the trypsin to be inactivated prior to PCR analysis.

O'Brien, J. K., T. Stojanov, et al. (2005). "Flow cytometric sorting of non-human primate sperm nuclei." Theriogenology **63**(1): 246.

<http://www.sciencedirect.com/science/article/B6TCM-4CVVC2S-1/2/3dece4b0253af601c2ee985ae6ce38b5>

Pre-determination of the sex of offspring has implications for management and conservation of

captive wildlife species, particularly those with single sex-dominated social structures. Our goal is to adapt flow cytometry technology to sort spermatozoa of non-human primate species for use with assisted reproductive technologies. The objectives of this study were to: (i) determine the difference in DNA content between X- and Y-bearing spermatozoa (ii) sort sperm nuclei into X- and Y-enriched samples; and (iii) assess the accuracy of sorting. Spermatozoa were collected from two common marmosets (*Callithrix jacchus*), seven hamadryas baboons (*Papio hamadryas*) and two common chimpanzees (*Pan troglodytes*). Human spermatozoa from one male were used as a control. Sperm nuclei were stained (Hoechst 33342), incubated and analyzed using a high-speed cell sorter. Flow cytometric reanalysis of sorted samples (sort reanalysis, 10,000 events/sample) and fluorescence in situ hybridization (FISH; 500 sperm nuclei/sample) were used to evaluate accuracy of sorting. Based on fluorescence intensity of X- and Y-bearing sperm nuclei, the difference in DNA content between X and Y populations was 4.09 $\pm$ 0.03, 4.20 $\pm$ 0.03, 3.30 $\pm$ 0.01, and 2.97 $\pm$ 0.05%, for marmoset, baboon, chimpanzee and human, respectively. Sort reanalysis and FISH results were similar; combined data revealed high levels of purity for X- and Y-enriched samples (94 $\pm$ 0.9 and 93 $\pm$ 0.8%, 94 $\pm$ 0.7 and 94 $\pm$ 0.5%, 91 $\pm$ 0.9 and 97 $\pm$ 0.6%, 94 $\pm$ 0.6 and 94 $\pm$ 0.9%, for marmoset, baboon, chimpanzee and human, respectively). These data indicate the potential for high-purity sorting of spermatozoa from non-human primates.

Oshima, K., H. Watanabe, et al. (2003). "Gene expression of leukemia inhibitory factor (LIF) and macrophage colony stimulating factor (M-CSF) in bovine endometrium during early pregnancy." *Theriogenology* **60**(7): 1217.

<http://www.sciencedirect.com/science/article/B6TCM-48R7F4N-1/2/aa6bafb79651ab7680e0244288aa5eae>

Leukemia inhibitory factor (LIF) and macrophage colony stimulating factor (M-CSF), members of the group of hemopoietic cytokines, play a primary role in the control of embryo development and implantation and in the growth of the placenta in humans and mice. Gene expressions of LIF and M-CSF were investigated using quantitative RT-PCR in bovine endometrial tissues during early and mid-pregnancy (Days 16-17, 20-21, 30-36, 48-49 and 74-140) and during the estrous cycle (Days 13-14). Leukemia inhibitory factor and M-CSF genes were expressed in all samples examined. Significant differences were found between the gene expression patterns of LIF and M-CSF. Leukemia inhibitory factor expression level at Days 48-49 was the highest in caruncular endometrium, however, the large variability negated any significant differences. Leukemia inhibitory factor expression levels in intercaruncular endometrium at Days 48-49 and 74-140 of pregnancy were greater than at Days 13-14 of the estrous cycle and at other days of pregnancy. No significant change was recognized in M-CSF expression levels in caruncular endometrium. Macrophage colony stimulating factor expression level in intercaruncular endometrium at Days 74-140 was greater than those of the other samples. These results suggest that LIF and M-CSF are produced in the endometrium and may play different roles in early and mid-pregnancy.

Rui, R., H. Shim, et al. (2004). "Attempts to enhance production of porcine chimeras from embryonic germ cells and preimplantation embryos." *Theriogenology* **61**(7-8): 1225.

<http://www.sciencedirect.com/science/article/B6TCM-4B4S5RN-7/2/2eeae34ac1b63f30a5423c563878854c>

Porcine embryonic germ (EG) cells share common features with porcine embryonic stem (ES) cells, including morphology, alkaline phosphatase activity and capacity for in vitro differentiation. Porcine EG cells are also capable of in vivo development by producing chimeras after blastocyst injection; however, the proportion of injected embryos that yield a chimera and the proportion of

cells contributed by the cultured cells in each chimera are too low for practical use in genetic manipulation. Moreover, somatic, but not germ-line chimerism, has been reported from blastocyst injection using porcine ES or EG cells. To test whether efficiency of chimera production from blastocyst injection can be improved upon by changing the host embryo, we used as host embryos four groups according to developmental stage or length in culture: fresh 4-cell and 8-cell stage embryos subsequently cultured into blastocysts, fresh morulae, fresh blastocysts, and cultured blastocysts. Injection and embryo transfer of fresh and cultured blastocysts produced similar percentages of live piglets (17% versus 19%). Four piglets were judged to have a small degree of pigmentation chimerism, but microsatellite analysis failed to confirm chimerism in these or other piglets. Polymerase chain reaction analysis for detection of the porcine SRY gene in female piglets born from embryos injected with male EG cells identified six chimeras, at least one, but not more than two, from each treatment. Chimerism was confirmed in two putative pigmentation chimeras and in four piglets without overt signs of chimerism. The low percentage of injected embryos that yielded a chimera and the small contribution by EG cells to development of each confirmed chimera indicated that procedural changes in how EG cells were combined with host embryos were unsuccessful in increasing the likelihood that porcine EG cells will participate in embryonic development. Alternatively, our results suggested that improvements are needed in EG cell isolation and culture procedures to ensure in vitro maintenance of EG cell developmental capacity.

van Rens, B. T. T. M., G. J. Evans, et al. (2003). "Components of litter size in gilts with different prolactin receptor genotypes." *Theriogenology* **59**(3-4): 915.

<http://www.sciencedirect.com/science/article/B6TCM-46SG670-5/2/d82c19ecd4b0991fdf4e42175ba07ff6>

Behavioral estrus and components of litter size at Day 35/36 of pregnancy were studied in gilts with prolactin receptor (PRLR) genotype AA (n=9), AB (n=25), and BB (n=22). This PRLR polymorphism (two alleles, A and B) has been associated with litter size, although it is not known whether the polymorphism itself causes differences in litter size or whether it is a marker for a closely linked causative gene. Estrus length in three successive estrous cycles was not affected by genotype, but estrous cycle length tended (PPPP2) than embryos of BB (42.0 $\pm$ 2.3 g, P2, PP2, P<0.05) gilts. Results of this experiment show that the PRLR gene or a very closely linked gene affects porcine ovaries, uterus, and placenta in a way that might lead to differences in litter size. Since other genes and also environmental factors, however, might change the effect within the 112 days to parturition, it is preferable to state that the PRLR gene is a candidate gene for ovulation rate rather than for litter size.

van Rens, B. T. T. M. and T. van der Lende (2002). "Litter size and piglet traits of gilts with different prolactin receptor genotypes." *Theriogenology* **57**(2): 883.

<http://www.sciencedirect.com/science/article/B6TCM-44X0BGC-9/2/2cc8807b0c87967270316dae96cf4a9a>

Seventy-seven Large White x Meishan F2 crossbred gilts with prolactin receptor (PRLR) genotype AA (n=26), AB (n=36) and BB (n=15) were compared for teat number (FTm), age at first estrus, gestation length (GL), litter size, and litter means of functional teat number (FTp), birthweight (BW), and pre-weaning growth rate (GR). Own placental information was available for 88% of 620 live-born piglets (62 gilts), since placentae were labeled during farrowing. The effect of PRLR genotype of the mother on average placenta weight (PLW) and placenta efficiency (EFF=BW/PLW), was therefore, also analyzed. PRLR genotype significantly (PP=0.056) and number of piglets born alive (NBA, P=0.072), but it did not affect (P>0.3) GL, BW or GR, neither

before nor after correction for litter size. BB gilts were significantly younger at first estrus and younger and lighter at insemination than AA gilts ($P=0.047$) and tended to have a larger NBA ($P=0.062$) than BB gilts. TNB was 11.4 ± 0.7 , 10.8 ± 0.6 , and 8.8 ± 0.9 ; NBA was 11.1 ± 0.6 , 10.5 ± 0.6 , and 8.7 ± 0.9 ; BW was 1309 ± 40 , 1277 ± 34 , and 1290 ± 53 g; and GL was 113.6 ± 0.3 , 113.8 ± 0.3 , and 113.5 ± 0.4 days for AA, AB and BB gilts, respectively. The effects on litter size and age at first estrus are independent effects. PRLR affected PLW ($P=0.050$) and EFF ($P=0.066$), resulting in a difference between AA and BB gilts. PLW was 160 ± 9 , 181 ± 7 and 196 ± 11 g and EFF was 7.6 ± 0.2 , 7.3 ± 0.2 and 6.7 ± 0.3 for AA ($n=19$), AB ($n=29$) and BB ($n=14$) gilts, respectively. After correction for TNB, the differences disappeared. Functional teat number of the AA, AB and BB gilts was 15.35 ± 0.22 , 15.53 ± 0.18 , and 15.60 ± 0.29 , respectively, and was not affected by PRLR genotype ($P=0.7$). Functional teat number of piglets from AA, AB and BB mothers was 14.20 ± 0.10 , 14.37 ± 0.08 , and 14.63 ± 0.13 , respectively. Piglets from BB mothers had on average larger numbers of functional teats compared to piglets from AA mothers ($P=0.028$). In conclusion, PRLR gene is a major gene or marker for age at first estrus, litter size, and litter average of number of functional teats in the Large White x Meishan F2 crossbred gilts studied. The favorable allele for litter size (A allele) is the unfavorable allele for age at first estrus and for litter mean of functional teat number.

Thorax (3)

Glare, E. M., M. Divjak, et al. (2002). "{beta}-Actin and GAPDH housekeeping gene expression in asthmatic airways is variable and not suitable for normalising mRNA levels." *Thorax* **57**(9): 765-770.

<http://thorax.bmjournals.com/cgi/content/abstract/57/9/765>

Background: The use of reverse transcription-polymerase chain reaction (RT-PCR) to measure mRNA levels has led to the common use of {beta}-actin and GAPDH housekeeping genes as denominators for comparison of samples. Expression of these genes is assumed to remain constant, so normalising for variations in processing and signal quantitation. However, it is well documented that {beta}-actin and GAPDH expression is upregulated with proliferation, activation, and differentiation. We hypothesised that airway samples which differ in their cellular profiles and activation status have different levels of expression of GAPDH and {beta}-actin. Methods: The mRNA for {beta}-actin, GAPDH, and interleukin (IL)-2 was measured in bronchoalveolar lavage (BAL) fluid cells and endobronchial biopsy tissue by competitive RT-PCR in a cross sectional study of 26 normal controls and 92 asthmatic subjects. Results: For both BAL fluid cells and biopsy tissue, asthmatics overall had reduced expression of GAPDH and {beta}-actin mRNA. In asthmatic subjects not using inhaled corticosteroids (ICS), GAPDH mRNA levels in both BAL fluid and biopsy tissue, and {beta}-actin mRNA in BAL fluid cells were 10 times lower than samples from both normal controls and from asthmatic subjects using ICS. {beta}-Actin mRNA in biopsy specimens showed the same pattern of expression, but asthmatic subjects not using ICS were not significantly different from those receiving ICS treatment. IL-2 mRNA levels did not differ between the subject or treatment groups but, when expressed as a ratio with {beta}-actin, significant differences were seen. Conclusions: {beta}-Actin and GAPDH used as denominators of gene expression quantitation in asthma research can cause confounding. Housekeeping genes need careful validation before their use in such quantitative mRNA assays.

Kenyon, N. J., R. W. Ward, et al. (2003). "TGF- β 1 causes airway fibrosis and increased collagen I and III mRNA in mice." *Thorax* **58**(9): 772-777.

<http://thorax.bmjjournals.com/cgi/content/abstract/58/9/772>

Background: Subepithelial collagen and extracellular matrix protein deposition are important pathophysiological components of airway remodelling in chronic asthma. Animal models based on the local reaction to antigens show structural alterations in the airway submucosal region and provide important information regarding disease pathophysiology. We describe a murine model of peribronchial fibrosis using intratracheally instilled transforming growth factor (TGF)- β 1 in BALB/C mice that facilitates a mechanistic approach to understanding the cellular and molecular pathways leading to airway fibrosis. **Methods:** BALB/C mice were intratracheally instilled with either TGF- β 1 or buffered saline. Airway fibrosis was assessed by light microscopy, hydroxyproline content, and polymerase chain reaction (PCR) for collagen I and III on microdissected airway samples. The lysyl oxidase inhibitor β -aminopropionitrile (BAPN) was administered to TGF- β 1 treated mice to block airway collagen deposition. Airway hyperresponsiveness was also measured after treatment with TGF- β 1. **Results:** During the 7 days after administration of TGF- β 1 the mice developed increased subepithelial collagen which could be blocked by BAPN. Increased mRNAs for collagen types I and III were seen in microdissected airways 1 week after TGF- β 1, and significantly increased total collagen was found in the airways 4 weeks after TGF- β 1. A detectable increase in airway hyperreactivity occurred. **Conclusions:** This new model should facilitate detailed study of airway remodelling that occurs in the absence of detectable cellular inflammation, and allow examination of the functional consequences of a major structural alteration in the conducting airways uncomplicated by inflammatory cell influx.

Tanino, M., T. Betsuyaku, et al. (2002). "Increased levels of interleukin-8 in BAL fluid from smokers susceptible to pulmonary emphysema." *Thorax* **57**(5): 405-411.

<http://thorax.bmjjournals.com/cgi/content/abstract/57/5/405>

Background: It has previously been shown that smokers with computed tomographic (CT) evidence of subclinical emphysema have signs of neutrophil activation, despite having no appreciable increase in the number of neutrophils in their bronchoalveolar lavage (BAL) fluid. **Methods:** The levels of the following chemoattractants in BAL fluid from 61 community based older volunteers classified into four groups according to current smoking status and the presence or absence of emphysema were determined: interleukin 8 (IL-8), epithelial neutrophil activating protein 78 (ENA-78) and leukotriene B4 (LTB4) which are primarily chemotactic for neutrophils; monocyte chemoattractant protein 1 (MCP-1) and macrophage inflammatory protein-1 α (MIP-1 α) which are predominantly chemotactic for mononuclear leucocytes. **Results:** Of the five chemoattractants studied, only the level of IL-8 in BAL fluid clearly distinguished between subjects with and without emphysema among current smokers (median values 34.7 and 12.2 pg/ml, respectively, $p < 0.01$). In addition, the levels of IL-8 and neutrophil elastase- α 1 protease inhibitor complex in BAL fluid were significantly correlated ($r = 0.65$, $p < 0.01$). There was no difference in either the release of IL-8 from cultured alveolar macrophages at 24 hours or the expression of IL-8 messenger RNA of alveolar macrophages in the two groups of current smokers with and without emphysema. **Conclusion:** An accelerated response of IL-8 to chronic smoking is a factor that characterises those smokers who are susceptible to pulmonary emphysema, although the cellular source of IL-8 remains to be determined.

Thrombosis Research (24)

Anvari, A., M. Janisiw, et al. (2000). "Platelet Glycoprotein Ia Gene Dimorphism [alpha]2-807 in Malignant Arrhythmia in Coronary Artery Disease." *Thrombosis Research* **98**(4): 281.

<http://www.sciencedirect.com/science/article/B6T1C-4096410-6/2/b4022aa8f8d898804f76f4de171f931b>

It has been shown recently that the variable expression of the platelet collagen receptor integrin [alpha]2[beta]1 predisposes to thrombotic risk on the one hand and hemorrhagic risk on the other hand. The level of expression of the integrin [alpha]2[beta]1 is genetically controlled and associated with the [alpha]2-807 dimorphism. The expression level of this platelet collagen receptor may play a central role in the rapidly evolving coronary artery lesions that lead to malignant arrhythmia and sudden cardiac death. We studied allelic frequencies of the [alpha]2-807 dimorphism for their relation as a risk factor for malignant arrhythmia in a well-defined subgroup of patients with coronary artery disease. We compared allelic frequencies (by sequence specific primer polymerase chain reaction) of the dimorphism that is associated with integrin [alpha]2[beta]1 levels in 94 Caucasoid survivors of sudden cardiac death with a matched group of 106 patients with coronary artery disease without sudden cardiac death. Gene frequencies in the patient groups did not differ and were similar to those in the general population represented by 217 healthy individuals. There was no overrepresentation of an allele in any group. The inherited dimorphism that is associated with the levels of platelet integrin [alpha]2[beta]1 is not associated with malignant arrhythmia in coronary artery disease patients.

Citron, B. A., I. V. Smirnova, et al. (1997). "QUANTITATIVE PCR ANALYSIS REVEALS NOVEL EXPRESSION OF PROTHROMBIN mRNA AND REGULATION OF ITS LEVELS IN DEVELOPING MOUSE MUSCLE." *Thrombosis Research* **87**(3): 303.

<http://www.sciencedirect.com/science/article/B6T1C-3RD1C85-4/2/9a614d671ed73585aef4fcfbe7152147>

Precise determination of mRNA levels is an essential element in any investigation of complex regulatory systems. Classical methodologies such as Northern hybridization suffer from requirements for significant samples of material and also a degree of non-specificity. Recently, quantitative techniques involving PCR amplification have been devised. We have developed and applied such procedures to the determination of prothrombin messages in skeletal muscle cells during development. In addition to its role in the blood coagulation cascade, the serine protease thrombin has been shown to participate in several signaling events in the neuromuscular system. The inactive precursor, prothrombin, primarily produced in the liver, has also been shown to be synthesized and developmentally-regulated in the brain. In skeletal muscle, thrombin is a mediator of activity-dependent polyneuronal synapse elimination (ADPSE) which occurs in early postnatal development. Recent experiments showing that thrombin is released from myotubes in culture under the influence of acetylcholine suggest that locally-synthesized prothrombin may be the source of this Hebbian synaptic interaction. We have determined that prothrombin is expressed in skeletal muscle, as the likely source of thrombin involved in ADPSE, and the current results show the quantitative expression of muscle prothrombin during this time of intense synapse remodeling. Published by Elsevier Science Ltd

Conroy, J. M., G. Trivedi, et al. (2000). "The Allele Frequency of Mutations in Four Genes that Confer

Enhanced Susceptibility to Venous Thromboembolism in an Unselected Group of New York State Newborns." Thrombosis Research **99**(4): 317.

<http://www.sciencedirect.com/science/article/B6T1C-41185GV-2/2/1bf20f2c49819f8e726a49b321b47d0>

The frequencies of Factor V G1691A (FVL), prothrombin (PT) G20210A, 5'10'methylenetetrahydrofolate reductase (MTHFR) C677T, and methionine synthase (MS) A2756G (four mutations associated with an increased risk of venous thromboembolism [VTE]) were determined in a sample of approximately 1500 New York State residents. Dried blood spots from approximately equal numbers of Caucasians, African-Americans and Hispanics were anonymously obtained from the New York State Department of Health Newborn Screening Program. Following PCR amplification of dried blood spot DNA, allele-specific oligonucleotide hybridization was used to detect mutant alleles. The total number of individuals at increased genetic risk for VTE was 271 (17.5%) of the 1553 persons tested. Increased genetic risk was defined as the heterozygous state for FVL or PT and the homozygous state for the MTHFR or MS polymorphisms. Sixteen individuals had more than one genetic risk factor. The MS gene variant allele frequencies for African-Americans and Hispanics are the first to be reported. This report also provides an estimate of the variant PT allele in the largest group of Hispanics studied to date.

Dogulu, C. F., T. Kansu, et al. (2003). "Evidence for genetic susceptibility to thrombosis in idiopathic intracranial hypertension." Thrombosis Research **111**(6): 389.

<http://www.sciencedirect.com/science/article/B6T1C-4B4YW7W-3/2/d5c1733691a0d2720ef4d4028561876f>

Endler, G., M. Exner, et al. (2001). "A Common C->T Polymorphism at nt 46 in the Promoter Region of Coagulation Factor XII is Associated With Decreased Factor XII Activity." Thrombosis Research **101**(4): 255.

<http://www.sciencedirect.com/science/article/B6T1C-42HFNP9-5/2/09dd3f777e37fb1de517b1efd0877113>

Coagulation factor XII (FXII) deficiency is rarely found to be associated with bleeding, but single reports demonstrated thromboembolic events in FXII-deficient patients. Currently, the biological role of FXII is still discussed controversially. It is well known that plasma levels of FXII show great interindividual variability. Recently, it has been demonstrated that a frequently occurring C->T polymorphism in the FXII promoter region at nucleotide (nt) 46 is associated with lower plasma FXII activity levels in Orientals. In our study, we evaluated the frequency of this polymorphism in a randomly selected sample of newborns and investigated whether this C->T polymorphism also contributes to the frequently observed moderate FXII deficiency in Europeans. We developed a new mutagenically separated polymerase chain reaction assay (MS PCR), which allows mutation detection without the use of restriction enzymes. Among 100 healthy newborns, we found 64% homozygous carriers of the wildtype FXII 46C allele, 29% were heterozygous for FXII C46T, and 7% homozygous for FXII 46T. Evaluation of plasma FXII activity and genotype in 80 randomly selected and unrelated individuals revealed a highly statistically significant ($P<.001$) association of the FXII 46T allele with reduced FXII plasma activity. Individuals carrying the homozygous FXII 46C genotype had a mean of 1.17 U/ml ($\pm$ 0.31 U/ml), individuals heterozygous for FXII C46T showed a mean of 0.70 U/ml ($\pm$ 0.31 U/ml), and subjects homozygous for FXII 46T had only 0.44 U/ml ($\pm$ 0.10 U/ml) plasma FXII activity.

Funk, M., G. Endler, et al. (2004). "The effect of a promoter polymorphism in the heme oxygenase-1 gene on the risk of ischaemic cerebrovascular events: The influence of other vascular risk factors." Thrombosis Research **113**(3-4): 217.

<http://www.sciencedirect.com/science/article/B6T1C-4CBVKT4-1/2/8cc57bc9bab0d3a76aaa24c7cb077a54>

Heme oxygenase-1 (HO-1) has been demonstrated to exert potent anti-oxidant and anti-inflammatory effects in the context of atherosclerotic vascular disease, and therefore was referred to as a potential vascular protective factor. A (GT)_n dinucleotide repeat polymorphism in the HO-1 promoter has been shown to modulate HO-1 gene expression. Short (We determined the number of GT repeats in the HO-1 promoter in 399 patients with ischaemic cerebrovascular events and 398 healthy controls and compared the frequencies of short (=25) repeat (class L) alleles after adjustment for potentially confounding factors. Genotype distributions of S/S, S/L and L/L in patients were 9.8% (n=39), 45.1% (n=180) and 45.1% (n=180), which was similar to the distribution in controls with 11.5% (n=46), 44.5% (n=177) and 44.0% (n=175). In the presence of vascular risk factors, the HO-1 genotype became functionally relevant: in patients without hyperlipidemia the S/S genotype exerted a protective effect on the development of ischaemic cerebrovascular events (OR 0.2; 95% CI 0.1-0.6), while this effect was no longer present in hyperlipidemic patients. Short (<25 GT) repeats in the HO-1 gene promoter confer a reduced risk for cerebrovascular events in individuals with normal plasma lipid levels. This may explain controversial findings in different populations.

Garg, U. C., D. K. Arnett, et al. (1998). "No Association between Factor V Leiden Mutation and Coronary Heart Disease or Carotid Intima Media Thickness: The NHLBI Family Heart Study." Thrombosis Research **89**(6): 289.

<http://www.sciencedirect.com/science/article/B6T1C-3V4X5FX-5/2/d5c2b2bb991aefd9b8e042948663bce7>

Garg, U. C., D. K. Arnett, et al. (1998). "Lack of Association between Platelet Glycoprotein IIb/IIIa Receptor PI Polymorphism and Coronary Artery Disease or Carotid Intima-Media Thickness." Thrombosis Research **89**(2): 85.

<http://www.sciencedirect.com/science/article/B6T1C-3VWNB97-P/2/c6fe5cbb115d2257e0504a215d562ec8>

Grubic, N., M. Stegnar, et al. (1996). "A NOVEL G/A AND THE 4G/5G POLYMORPHISM WITHIN THE PROMOTER OF THE PLASMINOGEN ACTIVATOR INHIBITOR-1 GENE IN PATIENTS WITH DEEP VEIN THROMBOSIS." Thrombosis Research **84**(6): 431.

<http://www.sciencedirect.com/science/article/B6T1C-3RH1FTJ-6/2/cfe840d9374078de01f327e804637d49>

Plasma plasminogen activator inhibitor-1 (PAI-1) level was observed to be associated with sequence variations at the PAI-1 locus. Therefore, PAI-1 gene promoter was screened for possibly new polymorphisms and to investigate the contribution of these sequence variations to

PAI-1 levels in patients with deep vein thrombosis (DVT). DNA was isolated from blood of 83 consecutive unrelated patients (42 $\pm$ 11 years old) and from 50 apparently healthy subjects of similar age and gender distribution. Six fragments covering DNA sequence - 1523 base pairs (bp) upstream from the start of PAI-1 gene transcription to +90 bp in the first exon, were amplified by polymerase chain reaction and analyzed by single-strand conformation polymorphisms. Two polymorphisms were found: a previously described 4G/5G deletion/insertion polymorphism - 675 bp upstream from the start of transcription and a novel G/A single base substitution polymorphism further upstream at -844 bp. The two polymorphisms were in strong linkage disequilibrium. Significant differences between patients and controls were observed neither for the frequencies of the 4G/5G alleles (and, respectively) nor for the frequencies of the G/A alleles (and, respectively). The distribution of both polymorphisms was similar in idiopathic and secondary DVT as well as in first and recurrent DVT. In patients association between the 4G/5G genotypes and PAI activity was observed, with the highest values in the 4G/4G genotype (13.3 U/mL), median values in the 4G/5G genotype (9.8 U/mL) and the lowest values in the 5G/5G genotype (2.0 U/mL). Despite the lack of association between the G/A genotypes and plasma PAI-1 levels, electrophoretic mobility shift assay showed specific binding of a nuclear protein from human vascular endothelial cells extracts to both the G and the A variant, suggesting functional importance of this novel G/A polymorphism in regulating the expression of PAI-1 gene. Copyright (c) 1996 Elsevier Science Ltd

Hainaut, P., C. Jaumotte, et al. (2002). "Hyperhomocysteinemia and venous thromboembolism: a risk factor more prevalent in the elderly and in idiopathic cases." *Thrombosis Research* **106**(2): 121.

<http://www.sciencedirect.com/science/article/B6T1C-46HFP67-5/2/75be73ed3a4aaef7696ee19907756a50>

Fasting plasma homocysteine level and the related clinical findings were analysed in 240 consecutive patients with venous thromboembolism. Hyperhomocysteinemia, defined as a plasma level above 20 μ mol/l (corresponding to the percentile 95th in the controls), was present in 11.2% of the patients. Plasma homocysteine level was similar in patients presenting with either deep venous thrombosis, pulmonary embolism or both conditions. It was significantly higher in patients with primary (unprovoked) VTE than in patients with secondary disease (associated with at least one risk factor): 12.3 vs. 9.55 μ mol/l (ppp=0.034). Furthermore, hyperhomocysteinemia was correlated with reduced protein C level (p=0.013). In a multivariate analysis, two factors were significantly associated with hyperhomocysteinemia: older age (pp<0.02). Since the frequency of homozygous MTHFR thermolabile variant was rather similar in patients and controls, testing for C677T mutation was not helpful in screening VTE patients. However, the homozygous mutation was significantly more prevalent among hyperhomocysteinemia patients, confirming its role in the genesis of hyperhomocysteinemia. According to its prevalence, to the putative role in venous and arterial disease and the availability of an effective and low-cost corrective therapy, hyperhomocysteinemia deserves interest, especially in the elderly and in the patients with idiopathic VTE disease.

Kim, J. A., N. D. Tran, et al. (2003). "Astrocyte regulation of human brain capillary endothelial fibrinolysis." *Thrombosis Research* **112**(3): 159.

<http://www.sciencedirect.com/science/article/B6T1C-4B8X378-2/2/8ba5a2ddf652fa8507d2ad0bb1268c30>

Introduction: Astrocytes are known to regulate a wide variety of brain endothelial cell functions. Prior work, using a mixed species co-culture system, has shown astrocyte regulation of brain capillary endothelial expression of key hemostasis factors tissue plasminogen activator (tPA) and

its inhibitor, plasminogen activator inhibitor-1 (PAI-1). The purpose of this study is to define the fibrinolytic regulatory role of human astrocytes on human brain capillary endothelial cells. Materials and methods: We used a blood-brain barrier model consisting of human astrocytes grown on transwell membrane inserts and co-cultured with human brain capillary endothelial cells. Following 48 h co-culture, we analyzed both endothelial mono-cultures and astrocyte-endothelial co-cultures for expression of tPA and PAI-1 mRNA, protein, and activity. Results and conclusions: There were significant changes for both tPA and PAI-1 mRNA: tPA mRNA levels were decreased in co-cultures (55+/-16% of mono-cultures, pppp<0.0005). TGF- β neutralizing antibody attenuated the observed changes in both tPA and PAI-1. These data indicate that human astrocytes regulate human brain capillary fibrinolysis in vitro by inhibiting tPA and enhancing PAI-1 expression. This regulation is mediated, in part, by transforming growth factor- β . Our findings provide further evidence for the role of astrocytes in brain-specific hemostasis regulation.

Kitaguchi, T., M. Murata, et al. (1997). "CHARACTERIZATION OF THE GENE ENCODING MOUSE PLATELET GLYCOPROTEIN Ib[beta]." *Thrombosis Research* **87**(2): 235.

<http://www.sciencedirect.com/science/article/B6T1C-3S9C2D5-8/2/871e4471f907f8f771fc39928e72044d>

Platelet glycoprotein (GP) Ib/IX/V complex is a major receptor for von Willebrand factor (vWF), which mediates platelet adhesion and aggregation under high shear stress conditions. It is composed of GPIb[alpha], GPIb[beta], GPIX, and GPV. All subunits for the human receptor have been cloned and characterized. However, the function of GPIb[beta] is still elusive. To obtain further information of GPIb[beta], we have determined the genomic sequence of mouse GPIb[beta] (1466 bp). The deduced amino acid sequence (206aa) was 88% identical to the human GPIb[beta] protein. All cysteine residues, putative N-linked glycosylation site (Asn41), and putative phosphorylation site (Ser166) were conserved. The promoter region contained putative GATA and ets binding motif implicated in megakaryocytic expression. Mouse GPIb[beta] also contained a leucine-rich glycoprotein (LRG) sequence of 24 amino acids same as human GPIb[beta]. (c) 1997 Elsevier Science Ltd

Larsen, T. B., J. F. Lassen, et al. (1998). "The Arg506Gln Mutation (FV Leiden) Among a Cohort of 4188 Unselected Danish Newborns." *Thrombosis Research* **89**(5): 211.

<http://www.sciencedirect.com/science/article/B6T1C-3WBFNRW-2/2/5aaf15c87525d4bb79d2df57841cf40e>

Resistance to activated protein C (APC) is the most prevalent single phenomenon associated with thromboembolic disease. It is caused by a single point mutation in the factor V gene (Arg506Gln or FV Leiden), replacing an Arg506 with a Gln at the APC-cleavage site in factor V. In this study we present a prevalence study of the Arg506Gln mutation in a large Danish cohort. By screening 4188 newborns (8376 alleles) we identified 3.4% alleles (95% CI: 3.0-3.8) of the Arg506Gln mutation, corresponding to a heterozygous prevalence of 6.6% (95% CI: 5.9-7.4) in Denmark. This is significantly lower than what has been reported from southern Sweden. The birth cohort has been selected from the entire country, providing representative and accurate estimates of the gene frequencies. Equal gender distribution was found, and the Arg506Gln mutation is probably not a considerable risk factor in fetal life in the general population.

Lu, D., D. K. Schmidel, et al. (1994). "Structure of mouse protein S as determined by PCR amplification and DNA sequencing of cDNA." Thrombosis Research **74**(2): 135.

<http://www.sciencedirect.com/science/article/B6T1C-4C35TK5-5V/2/51e29962954f852d36d3f7eb5ed40b22>

The cDNA sequence of mouse protein S was derived by conventional PCR amplification from liver mRNA, initially using primers derived from the human cDNA sequence, followed by direct DNA sequencing. Seven overlapping PCR fragments covering all of the mature protein, part of the propeptide, and the 3' noncoding region were generated and sequenced. In some cases primers based upon the human cDNA sequence were ineffective. Subsequent successful amplification with mouse-derived primers to the same regions and comparison of the mouse and human sequences in these regions suggest that the failure of the human primers was due to insufficient degree of heterospecies identity. The mouse protein S cDNA sequence of the coding region shares 82% identity to human. The 3' noncoding region of mouse protein S cDNA has several small deletions and insertions compared to human protein S cDNA. Mature mouse protein S consists of 634 amino acids in a single polypeptide chain and displays domain organization similar to that for other species. The amino acid sequence of mouse protein S is about 80% identical to that of other species. Eleven glutamic acid residues were found in the amino terminal region and are predicted to be sites of [gamma]-carboxylation. Amino acid residues #80244 are defined as four cysteine-rich repeat sequences homologous to epidermal growth factor. The remainder of the molecule is homologous to plasma sex steroid binding protein. The mouse protein S contains two potential N-glycosylation sites at positions #458 and 468 and is lacking the putative glycosylation site at #490 found in human protein S.

Malarstig, A., T. Tenno, et al. (2003). "A quantitative real-time PCR method for tissue factor mRNA." Thrombosis Research **112**(3): 175.

<http://www.sciencedirect.com/science/article/B6T1C-4BDM1XH-1/2/230d9df3b18e0940ecdabb8063cba69b9>

Background: Tissue factor (TF) is primarily known for its function to initiate blood coagulation. The range of in vivo expression of TF is wide and requires a dynamic assay for monitoring. A general method for TF mRNA quantitation that is dynamic, sensitive and applicable to a variety of experimental systems or clinical situations is therefore desirable. Objectives: To develop a method for sensitive and dynamic quantitation of TF mRNA in human blood cells. Methods: TF mRNA expression was analysed and evaluated in monocyte isolations, in whole blood (healthy volunteers and patients scheduled for percutaneous coronary intervention, PCI) and in a panel of human cell lines. RNA was extracted, reverse transcribed and subjected to real-time PCR amplification, according to the TaqMan technology. A TF plasmid was constructed as calibrator of the assay. Two housekeeping genes used as endogenous controls for cDNA quality and integrity were evaluated. Results: The assay was linear by seven orders of magnitude and detected down to 102 copies of the TF plasmid. The coefficient of variation was 4% intra-assay and 28% between the assays when using [beta]2MG as endogenous control. The [beta]-actin gene expression was induced by treatment with lipopolysaccharide (LPS) in blood leukocytes and could not be used as an endogenous control. However, [beta]2MG showed only minor variations upon treatment with LPS. The TF mRNA and antigen expression, measured in a Western blot, correlated well ($R^2=0.903$) in a panel of 11 human cell lines. Conclusions: We have established a method for sensitive and dynamic quantitation of TF mRNA in experimental systems and for clinical situations.

Moller, A.-S. W., K. B. F. Haug, et al. (2001). "Non-Mannose-Capped Lipoarabinomannan Stimulates

Human Peripheral Monocytes to Expression of the "Early Immediate Genes" Tissue Factor and Tumor Necrosis Factor-[alpha]." Thrombosis Research **102**(3): 273.

<http://www.sciencedirect.com/science/article/B6T1C-430NR3N-C/2/66140550144752a35abe142253513cc3>

In the present study, we have shown that stimulation of cryopreserved, human peripheral blood monocytes with the cell wall components from Gram-negative bacteria, lipopolysaccharide (LPS), and from rapid-growing *Mycobacterium* sp., non-mannose-capped lipoarabinomannan (AraLAM), both induce expression of the "early immediate genes" tissue factor (TF) and tumor necrosis factor-[alpha] (TNF-[alpha]). This was demonstrated both at the protein and the mRNA levels. Antibodies against the CD14 receptor could block the stimulating effects. AraLAM was a significantly weaker inducer than LPS, and we speculate that this may reside in the number of the fatty acids in the part of the molecule that interacts with the CD14/Toll-like receptors (TLR). Finally, both LPS and AraLAM activated the "early immediate genes" through translocation of the transcription factor proteins NF-[kappa]B/Rel and increasing the binding activity of AP-1.

Murakami, H., M. Okazaki, et al. (2003). "Increase in hepatic mRNA expression of coagulant factors in type 2 diabetic model mice." Thrombosis Research **111**(1-2): 81.

<http://www.sciencedirect.com/science/article/B6T1C-49NVDS7-1/2/54f35f6b16adc9b05d68507ffbc6cdb8>

Aim: The purpose of the study is to investigate whether hypercoagulation in diabetes is observed not only in increased plasma levels of the coagulative factors but also in increased hepatic mRNA levels. Materials and methods: The age-related changes of coagulation factors were determined using KK and KK-Ay mice as a model for human type 2 diabetes mellitus. Expression of the [alpha]-, [beta]- and [gamma]-chains of fibrinogen-mRNA and prothrombin-mRNA from the mouse liver was examined by reverse transcription-polymerase chain reaction (RT-PCR). Results: Hemoglobin A1c (HbA1C), plasma fibrinogen, triglyceride and insulin levels increased apparently, especially in KK-Ay mouse at 4 months old. The mRNA levels of [gamma]-chain of fibrinogen significantly increased at 3 and 4 months of age in both mice. The mRNA levels of [alpha]- and [beta]-chains of fibrinogen and prothrombin were significantly increased in 4-month-old KK-Ay mouse. Conclusions: These results suggest that the increase in hepatic mRNA expression of coagulant factors contributes to the hypercoagulable state in type 2 diabetic mice.

Simioni, P., F. Vianello, et al. (2001). "A Dysfunctional Factor X (Factor X San Giovanni Rotondo) Present at Homozygous and Double Heterozygous Level: Identification of a Novel Microdeletion (delC556) and Missense Mutation (Lys408->Asn) in the Factor X Gene: A Study of An Italian Family." Thrombosis Research **101**(4): 219.

<http://www.sciencedirect.com/science/article/B6T1C-42HFNP9-1/2/6e1209c8cd2586ab51ce811ee8208756>

Low levels of factor X (F.X) were detected in a 4-year-old boy who experienced acute lymphoblastic leukemia and bleeding manifestations. Laboratory data suggested the presence of a dysfunctional F.X molecule. Two novel F.X gene mutations were identified in the proband that was double heterozygous for both: a microdeletion (delC556) in exon VI resulting in a frameshift leading to a termination codon at position 226. This deletion was found in six family members with reduced F.X antigen and activity levels. A second mutation characterised by a G1344->C transversion in exon VIII was detected in the proband resulting in a Lys408->Asn substitution.

This latter mutation was present in several asymptomatic family members from the paternal and the maternal side. The proband's sister was homozygous for the Lys408->Asn substitution and exhibited low F.X activity with a normal antigen level. The naturally occurring F.X Lys408->Asn (F.XK408N) variant was isolated from plasma of either homozygous or double heterozygous individuals. NH2-terminal sequencing of the heavy chain of F.XK408N failed to show any sequence abnormality in patients who were also carriers of the delC556, suggesting that this latter lesion accounted for the lack of F.X synthesis. Purified F.X Lys408->Asn had an identical behaviour to normal F.X as judged by SDS-PAGE and immunoblotting. Clotting assay using purified F.XK408N and F.X-deficient plasma resulted in a laboratory phenotype similar to that observed in a homozygous subject for F.X Lys408->Asn substitution. This is the first characterisation of a naturally occurring F.X variant with a mutation at the COOH-terminal end of the molecule.

Tokunaga, F., S. Takeuchi, et al. (2000). "Secretion, [gamma]-carboxylation, and Endoplasmic Reticulum-associated Degradation of Chimeras with Mutually Exchanged Gla Domain Between Human Protein C and Prothrombin." Thrombosis Research **99**(5): 511.

<http://www.sciencedirect.com/science/article/B6T1C-4135X00-G/2/43341000563b3a9cf4f382b0ccdb6cf9>

Warfarin, an antagonist of vitamin K, causes diminution of vitamin K-dependent coagulation factors in the circulation. Although all vitamin K-dependent factors have Gla domains, the warfarin-induced decrease in their plasma concentration differs among factors. In warfarin-treated HepG2 cells, we found modest and severe intracellular degradation of prothrombin and protein C, respectively. To investigate the structural features of these proteins that contribute to their warfarin sensitivity, chimeric prothrombin containing the prepropeptide and Gla domain of protein C was expressed in baby hamster kidney (BHK) cells. This chimera showed similar secretion kinetics and warfarin sensitivity to those of wild-type prothrombin, demonstrating that the Gla domain cannot solely explain the warfarin sensitivity of protein C. In contrast, two chimeric protein Cs containing either the Gla domain alone or the prepropeptide and Gla domain of prothrombin showed impaired secretion. Even though [gamma]-carboxylation proceeded normally, both chimeras were degraded intracellularly by the proteasome. From these results, we conclude that not only the folding of the Gla domain, but the entire structure and conformation of protein C and prothrombin, contribute to their quality control and susceptibility to warfarin-induced ER (endoplasmic reticulum)-associated degradation.

Tokunaga, F., S. Wakabayashi, et al. (1992). "Identification of one base deletion in exon IX of the protein C gene that causes a type I deficiency." Thrombosis Research **68**(4-5): 417.

<http://www.sciencedirect.com/science/article/B6T1C-4C06B5X-84/2/c16a22f707a603d462ee35379cd9d52d>

Vianello, F., A. M. Lombardi, et al. (2001). "A New Factor X Defect (Factor X Padua 3): A Compound Heterozygous Between True Deficiency (Gly380->Arg) and an Abnormality (Ser334->Pro)." Thrombosis Research **104**(4): 257.

<http://www.sciencedirect.com/science/article/B6T1C-44HS961-4/2/bec46537b37365f5e481e293909b0b98>

We report a novel mutation in Factor X (FX) gene which results in a phenotype without any bleeding tendency. The proband has been found to be a compound heterozygote between a novel FX true deficiency (Gly380->Arg) and a previously reported dysfunctional mutation Ser334->Pro (FX Marsiglia). Prothrombin time (PT) and partial thromboplastin time (PTT) were moderately prolonged and were fully corrected by the addition of normal serum. Her FX activity level varied between 8% and 19% of normal according to the method used whereas the FX antigen level was 40% of the normal control value. All the exons and intron/exon junctions of the FX gene were studied using a combined approach of polymerase chain reaction and conformation sensitive gel electrophoresis. A transversion G to A in exon 8 resulting in the replacement of Gly380 by Arg was found in the proband, in the father and in a proband's brother, whereas heterozygous FX Marsiglia was present in the proband's mother and her sister. Gly380 is strictly linked to Ser379, a component of the catalytic triad. The substitution of Gly for Arg causes the introduction of a large charged amino acid which could affect the catalytic function of FX leading to secretion problem, accounting for the cross-reactive material (CRM) negative phenotype.

Vianello, F., A. M. Lombardi, et al. (2002). "Conformation sensitive gel electrophoresis for detection of factor X gene mutations." *Thrombosis Research* **107**(1-2): 51.

<http://www.sciencedirect.com/science/article/B6T1C-46X91V1-1/2/2d6b61127c97c8fdc2f7422e4d5f4f67>

Fifteen patients from five families with laboratory data suggesting factor X (FX) deficiency were screened for causative mutations by conformation sensitive gel electrophoresis (CSGE) followed by sequencing. All exonic and flanking intronic regions of factor X gene were amplified using PCR. After heteroduplex formation, samples were analyzed onto a polyacrylamide gel for possible mismatch. An abnormal CSGE profile indicating an heteroduplex was identified in 10/15 cases. All the 10 patients with a pattern of migration suggesting a mismatch had a laboratoristic pattern of FX deficiency whereas the five cases with a normal CSGE aspect referred to the normal components of the families who did not carry any FX defect. Sequencing demonstrated that the 10 exons, which showed a suspect CSGE pattern, harbored a mutation responsible for the factor X defect. Of the five mutation identified, two were recognized to be novel mutations (a 871C>T substitution and a 1169G>T transversion in exon 8), both located in the catalytic portion of FX. CSGE may be an effective and simple procedure for screening factor X gene mutations.

Watzke, H. H., J. Schuttrumpf, et al. (1997). "INCREASED PREVALENCE OF A POLYMORPHISM IN THE GENE CODING FOR HUMAN PROTHROMBIN IN PATIENTS WITH CORONARY HEART DISEASE." *Thrombosis Research* **87**(6): 521.

<http://www.sciencedirect.com/science/article/B6T1C-3R867FF-3/2/405f968602a8ac01ae33045d57b30fa2>

A number of genetic risk factors for the development of coronary heart disease has been identified in the past. Some of these represent polymorphisms in genes of proteins which are associated with the process of blood clotting. We investigated the distribution of a recently described G/A polymorphism in the 3'untranslated region of the human prothrombin gene (nt 20210) in 98 patients (19 female age: 53+12 SD years and 79 male, age: 49+8.5 SD years) with coronary heart disease and in 102 healthy newborns by enzymatic amplification of the genomic DNA carrying the polymorphic site and by subsequent restriction digest. The diagnosis of coronary heart disease was established by coronary angiography in all patients. The frequency of the A allele in the healthy newborns was 0.98 % (0.2 % - 3.5 %; CI 0.95) with the G/A genotype occurring in 1.96% (0.24 % - 6.9 %; CI 0.95). In the group of patients with coronary heart disease

the G/A genotype was found in 5.1 % (1.7 % - 11.4 %; CI 0.95). 94.9 % of the patients studied showed a G/G genotype. The A/A genotype was neither detected in the newborns nor in the patients with coronary heart disease. This preliminary study strongly suggest that the presence of the G/A polymorphism in the 3'untranslated region of the gene coding for human prothrombin is associated with the occurrence of coronary heart disease. (c) 1997 Elsevier Science Ltd

Xia, L., J. Gu, et al. (1996). "Preparation of an antifibrin thrombus-specific murine/human chimeric monoclonal antibody fab fragment in escherichia coli." Thrombosis Research **81**(4): 477.

<http://www.sciencedirect.com/science/article/B6T1C-3VXHGFV-M/2/c5b4494088cf39cf924c8dc23c468f8>

We have successfully developed a murine antifibrin monoclonal antibody designated SZ-63 with property of binding specifically with thrombus both in vitro and in vivo. In order to reduce its immunogenicity and molecular weight, a murine/human chimeric Fab fragment of the antibody was prepared. mRNA was selected on oligo (dT) cellulose from total RNA isolated from SZ-63 hybridoma cells. cDNAs coding for heavy and light variable regions were amplified by reverse transcriptase polymerase chain reaction. The amplified fragments were cloned and sequenced. The nucleotides of SZ-63 VH and VL are 354 and 321 respectively. The variable genes were then linked with human IgG [gamma]1 CH and [kappa] CL genes. Expression vector pHEN1-63 Fab/Hu was thereby constructed and chimeric Fab fragment was expressed in E. coli HB2151 cells in soluble form. Western blot and ELISA results showed that it remained the same capability of binding with cross-linked fibrin as the murine SZ-63 antibody, the content in culture is about 125 ug/L.

Toxicol. Sci. (15)

Abel, E. L., T. K. Bammler, et al. (2004). "Biotransformation of Methyl Parathion by Glutathione S-Transferases." Toxicol. Sci. **79**(2): 224-232.

<http://toxsci.oupjournals.org/cgi/content/abstract/79/2/224>

The organo(thio)phosphate esters are one of the most widely used classes of insecticides. Worldwide, organophosphate insecticides (OPs) result in numerous poisonings each year. In insects, glutathione S-transferases (GSTs) play an important role in OP resistance; limited data suggest that GST-mediated O-dealkylation occurs in humans as well. To characterize the capacity of mammalian GSTs to detoxify OPs, we investigated mammalian GST biotransformation of the widely used OP, methyl parathion (MeP). Cytosolic fractions isolated from rat, mouse, and ten individual adult human livers biotransformed 300 {micro}M MeP at rates of 2.36, 1.76, and 0.70 (mean rate) nmol desmethyl parathion/min/mg, respectively. Our study focused on human GSTs; in particular, we investigated hGSTs M1-1 and T1-1, since deletion polymorphisms occur commonly in these genes. However, we found no correlation between hGSTM1/T1 genotypes and MeP O-dealkylation activities of the ten human liver cytosolic samples. We also measured MeP O-dealkylation activities of several purified recombinant GSTs belonging to the alpha (human GSTs A1-1 and A2-2, mouse GSTA3-3, rat GSTA5-5), mu (human GSTs M1a-1a, M2-2, M3-3, M4-4), pi (human GSTP1-1, mouse GSTs P1-1, P2-2), and theta (human GSTT1-1) classes. At 1 mM glutathione and 300 {micro}M MeP concentrations, hGSTT1-

1 and hGSTA1-1 exhibited the highest O-dealkylation activities: 545.8 and 65.0 nmol/min/mg, respectively. When expression level and enzymatic activity are considered, we estimate that hGSTA1-1 is responsible for the majority of MeP O-dealkylation in human hepatic cytosol. In target organs such as brain and skeletal muscle, where hGSTT1-1 is expressed, hGSTT1-1-mediated biotransformation of MeP may be important.

Faiola, B., A. K. Bauer, et al. (2003). "Variations in Prkdc and Susceptibility to Benzene-Induced Toxicity in Mice." Toxicol. Sci. **75**(2): 321-332.

<http://toxsci.oupjournals.org/cgi/content/abstract/75/2/321>

Benzene, a carcinogen that induces chromosomal breaks, is strongly associated with leukemias in humans. Possible genetic determinants of benzene susceptibility include proteins involved in repair of benzene-induced DNA damage. The catalytic subunit of DNA-dependent protein kinase (DNA-PKcs), encoded by Prkdc, is one such protein. DNA-PKcs is involved in the nonhomologous end-joining (NHEJ) pathway of DNA double-strand break (DSB) repair. Here we compared the toxic effects of benzene on mice (C57BL/6 and 129/Sv) homozygous for the wild-type Prkdc allele and mice (129/SvJ) homozygous for a Prkdc functional polymorphism that leads to diminished DNA-PK activity and enhanced apoptosis in response to radiation-induced damage. Male and female mice were exposed to 0, 10, 50, or 100 ppm benzene for 6 h/d, 5 d/week for 2 weeks. Male mice were more susceptible to benzene toxicity compared with females. Hematotoxicity was evident in all male mice but was not seen in female mice. We observed similar, large increases in both micronucleated erythrocyte populations in all male mice. Female mice had smaller but significant increases in micronucleated cells. The p53-dependent response was induced in all strains and genders of mice following benzene exposure, as indicated by an increase in p21 mRNA levels in bone marrow that frequently corresponded with cell cycle arrest in G2/M. Prkdc does not appear to be a significant genetic susceptibility factor for acute benzene toxicity. Moreover, the role of NHEJ, mediated by DNA-PK, in restoring genomic integrity following benzene-induced DSB remains equivocal.

Farraj, A. K., J. R. Harkema, et al. (2004). "Allergic Rhinitis Induced by Intranasal Sensitization and Challenge with Trimellitic Anhydride but Not with Dinitrochlorobenzene or Oxazolone in A/J Mice." Toxicol. Sci. **79**(2): 315-325.

<http://toxsci.oupjournals.org/cgi/content/abstract/79/2/315>

Allergic airway diseases induced by low molecular weight (LMW) chemicals, including trimellitic anhydride (TMA), are characterized by airway mucus hypersecretion and an infiltration of eosinophils and lymphocytes. Many experimental models have linked LMW chemical-induced allergic airway disease to Th2 cytokines. Most murine models, however, use dermal exposure to sensitize mice. The present study was designed to test the hypothesis that intranasal sensitization and challenge with the known chemical respiratory allergen TMA, but not the nonrespiratory sensitizers dinitrochlorobenzene (DNCB) and oxazolone (OXA), will induce characteristic features of LMW chemical-induced allergic airway disease in the nasal and pulmonary airways. A/J mice were intranasally sensitized and challenged with TMA, DNBCB, or OXA. Only mice that were intranasally sensitized and challenged with TMA had a marked allergic rhinitis with an influx of eosinophils, lymphocytes, and plasma cells, increased intraepithelial mucusubstances, and a regenerative hyperplasia. Cytokine mRNA levels in the nasal airway of TMA treated mice also revealed an increase in the mRNA levels of the Th2 cytokines IL-4, IL-5, and IL-13, but no change in the level of the Th1 cytokine IFN- γ . No lesions were found in the nasal airways of mice exposed to DNBCB or OXA. TMA increased lung-derived IL-5 mRNA while DNBCB and OXA caused no change in lung-derived cytokine mRNA levels. Both TMA and

DNCB caused increases in total serum IgE, unlike OXA-exposed mice. However, no adverse alterations were found microscopically in the lungs of mice treated with TMA, DNCB, or OXA. This study is the first to demonstrate that intranasal administration of a known chemical respiratory allergen is an effective method of sensitization resulting in the hallmark features of allergic rhinitis after challenge with a concomitant increase in nasal airway-derived Th2 cytokine mRNA, lung-derived IL-5 mRNA, and total serum IgE. In contrast, DNCB and OXA failed to elicit the pathologic changes in the nasal airways and cytokine changes in the lung. This model may be useful for identifying other chemical respiratory allergens.

Gurel, V., D. A. Sens, et al. (2005). "Post-Transcriptional Regulation of Metallothionein Isoform 1 and 2 Expression in the Human Breast and the MCF-10A Cell Line." *Toxicol. Sci.*: kfi155.

<http://toxsci.oupjournals.org/cgi/content/abstract/kfi155v1>

Studies have shown using immunohistochemical staining that the MT-1 and MT-2 proteins (MT-1/2) are overexpressed in a substantial sub-set of ductal breast cancers, that overexpression occurs early in the disease process, and is indicative of a poor prognosis. Normal ductal breast epithelium fails to immunostain for the MT-1/2 protein where as the myoepithelial cells of the ducts stain intensely. There is no information regarding the expression of the mRNAs for the 8 active MT-1 and MT-2 genes in normal breast duct epithelium. Microdissection of normal breast samples was used to obtain total RNA from enriched populations of ductal epithelium and myoepithelium. Analysis by RT-PCR demonstrated that the identity of the MT isoform-specific genes expressed (MT-2A and MT-1X) and their relative levels of expression were similar between the myoepithelial and ductal components. These findings indicate that the ductal and myoepithelial components express similar amounts of MT-2A and MT-1X mRNAs, but have distinctly different expression of the MT-1/2 protein. Confluent cultures of MCF-10A breast epithelial cells were exposed to Cd+2 to test for evidence of post-transcriptional regulation of MT-1/2 protein accumulation in ductal epithelium. It was demonstrated that Cd+2 elicited only a marginal induction of MT-1E, MT-1X or MT-2A mRNAs; where as, there was a marked increase in MT-1/2 protein, reaching levels of 6% of total cell protein under conditions of extended exposure. This study suggests that the mechanism underlying the finding of increased MT-1/2 protein expression in ductal breast cancer may involve in part the post-transcriptional regulation of MT-1/2 protein expression.

Hamadeh, H. K., P. R. Bushel, et al. (2002). "Gene Expression Analysis Reveals Chemical-Specific Profiles." *Toxicol. Sci.* **67**(2): 219-231.

<http://toxsci.oupjournals.org/cgi/content/abstract/67/2/219>

The application of gene expression profiling technology to examine multiple genes and signaling pathways simultaneously promises a significant advance in understanding toxic mechanisms to ultimately aid in protection of public health. Public and private efforts in the new field of toxicogenomics are focused on populating databases with gene expression profiles of compounds where toxicological and pathological endpoints are well characterized. The validity and utility of a toxicogenomics is dependent on whether gene expression profiles that correspond to different chemicals can be distinguished. The principal hypothesis underlying a toxicogenomic or pharmacogenomic strategy is that chemical-specific patterns of altered gene expression will be revealed using high-density microarray analysis of tissues from exposed organisms. Analyses of these patterns should allow classification of toxicants and provide important mechanistic insights. This report provides a verification of this hypothesis. Patterns of gene expression corresponding to liver tissue derived from chemically exposed rats revealed similarity in gene expression profiles between animals treated with different agents from a common class of compounds, peroxisome

proliferators [clofibrate (ethyl-p-chlorophenoxyisobutyrate), Wyeth 14,643 ([4-chloro-6(2,3-xylidino)-2-pyrimidinylthio]acetic acid), and gemfibrozil (5-2[2,5-dimethylphenoxy]2-2-dimethylpentanoic acid)], but a very distinct gene expression profile was produced using a compound from another class, enzyme inducers (phenobarbital).

Hilscherova, K., P. D. Jones, et al. (2004). "Assessment of the Effects of Chemicals on the Expression of Ten Steroidogenic Genes in the H295R Cell Line Using Real-Time PCR." *Toxicol. Sci.* **81**(1): 78-89.

<http://toxsci.oupjournals.org/cgi/content/abstract/81/1/78>

The potential for a variety of environmental contaminants to disturb endocrine function in wildlife and humans has been of recent concern. While much effort is being focused on the assessment of effects mediated through steroid hormone receptor-based mechanisms, there are potentially several other mechanisms that could lead to endocrine disruption. Recent studies have demonstrated that a variety of xenobiotics can alter the gene expression or activity of enzymes involved in steroidogenesis. By altering the production or catalytic activity of steroidogenic or steroid-catabolizing enzymes, these chemicals have the potential to alter the steroid balance in organisms. To assess the potential of chemicals to alter steroidogenesis, an assay system was developed using a human adrenocortical carcinoma cell line, the H295R cell line, which retains the ability to synthesize most of the important steroidogenic enzymes. Methods were developed, optimized, and validated to measure the expression of 10 genes involved in steroidogenesis by the use of real-time quantitative reverse transcriptase PCR. The effects of several model chemicals known to alter steroid metabolism, both inducers and inhibitors, were assessed. Similar expression patterns were observed for chemicals acting through common mechanisms of action. Time-course studies demonstrated distinct time-dependent expression profiles for chemicals able to modulate steroid metabolism. The assay, which allows simultaneous analysis of the expression of numerous steroidogenic enzymes, would be useful as a sensitive and integrative screen for the many effects of chemicals on steroidogenesis.

Johnson, V. J., S.-H. Kim, et al. (2005). "Aluminum-Maltolate Induces Apoptosis and Necrosis in Neuro-2a Cells: Potential Role for p53 Signaling." *Toxicol. Sci.* **83**(2): 329-339.

<http://toxsci.oupjournals.org/cgi/content/abstract/83/2/329>

Aluminum maltolate (Al-malt) causes neurodegeneration following *in vivo* exposure, and apoptosis plays a prominent role. The objective of this study was to define the form of cell death induced by Al-malt and to establish an *in vitro* model system amenable to mechanistic investigations of Al-malt-induced cell death. Neuro-2a cells, a murine neuroblastoma cell line, were treated with Al-malt for 24 h, following which mode of cell death and alterations in apoptosis-related gene expression were studied. Al-malt concentration-dependently increased cell death. The mode of cell death was a combination of apoptosis and necrosis. Treatment with Al-malt resulted in caspase 3 activation and the externalization of phosphatidyl serine, both indicative of apoptosis. In addition, nuclear condensation and fragmentation were evident. Interestingly, pretreatment with cycloheximide (CHX), a potent protein synthesis inhibitor markedly reduced Al-malt-induced apoptosis, indicating that altered gene expression was critical for this form of cell death. Pretreatment with CHX had no effect on necrosis induced by Al-malt. Analysis of gene expression showed that p53 mRNA was increased following treatment with Al-malt. This increase was accompanied by a marked inhibition of Bcl2 expression and an increase in BAX expression, a pattern of gene expression suggestive of a pro-apoptotic shift. Results show for the first time that p53 is induced by Al in neuron-like cells and suggest that the p53-dependent intrinsic pathway may be responsible for Al-induced apoptosis. Future studies investigating the role of p53

in AI neurotoxicity both in vivo and in vitro are warranted.

Kato, Y., K. Haraguchi, et al. (2003). "Effects of Polychlorinated Biphenyls, Kanechlor-500, on Serum Thyroid Hormone Levels in Rats and Mice." *Toxicol. Sci.* **72**(2): 235-241.

<http://toxsci.oupjournals.org/cgi/content/abstract/72/2/235>

Effects of a commercial polychlorinated biphenyls mixture, Kanechlor-500 (KC500), on the levels of serum thyroid hormones such as total thyroxine (T4) and triiodothyronine (T3) were examined comparatively in male Wistar rats and ddy mice. Serum T4 levels were significantly decreased in both rats and mice 4 days after a single ip injection of KC500 (100 mg/kg body weight), whereas decreased levels of T3 were observed in mice but not in rats. In addition, no significant change in the level of serum thyroid stimulating hormone was observed in either rats or mice. Hepatic UDP-glucuronosyltransferases (UDP-GTs) UGT1A1 and UGT1A6, which efficiently mediate glucuronidation of T4 and promote the excretion of the hormones, were induced by KC500 in rats but not in mice. Hepatic microsomal cytochrome P450 (P450) content and the microsomal activity for 7-ethoxy-, 7-pentoxo-, and 7-benzoyloxy-resorufin dealkylations were significantly increased by KC500 in both rats and mice, although the magnitude of increase in the enzyme activities was higher in rats than in mice. The difference in the increase in the activity of microsomal enzymes, including UDP-GT and P450, between KC500-treated rats and mice was not correlated with that in the level of hepatic methylsulfonyl-PCB metabolites. In the present study, we found for the first time that the decrease in serum T4 levels by KC-500 in mice occurred without increase in hepatic UDP-GTs, UGT1A1 and UGT1A6, responsible for T4 glucuronidation. The present findings further suggested that although the decrease in serum T4 levels in KC500-treated rats would occur at least in part through the induction of the UDP-GTs, it might not be dependent on only the increase in the enzymes.

Kuruvilla, S., C. W. Qualls, Jr., et al. (2003). "Effects of Minimally Toxic Levels of Carbonyl Cyanide P-(Trifluoromethoxy) Phenylhydrazone (FCCP), Elucidated through Differential Gene Expression with Biochemical and Morphological Correlations." *Toxicol. Sci.* **73**(2): 348-361.

<http://toxsci.oupjournals.org/cgi/content/abstract/73/2/348>

Uncouplers of oxidative phosphorylation have relevance to bioenergetics and obesity. The mechanisms of action of chemical uncouplers of oxidative phosphorylation on biological systems were evaluated using differential gene expression. The transcriptional response in human rhabdomyosarcoma cell line (RD), was elucidated following treatment with carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP), a classical uncoupling agent. Changes in mitochondrial membrane potential were used as the biological dosimeter. There was an increase in membrane depolarization with increasing concentrations of FCCP. The concentration at 75% uncoupling (20 {micro}M) was chosen to study gene expression changes, using cDNA-based large-scale differential gene expression (LSDGE) platforms. At the above concentration, subtle light microscopic and clear gene expression changes were observed at 1, 2, and 10 h. Statistically significant transcriptional changes were largely associated with protein synthesis, cell cycle regulation, cytoskeletal proteins, energy metabolism, apoptosis, and inflammatory mediators. Bromodeoxyuridine (BrdU) and propidium iodide (PI) assays revealed cell cycle arrest to occur in the G1 and S phases. There was a significant initial decrease in the intracellular adenosine triphosphate (ATP) concentrations. The following seven genes were selected as potential molecular markers for chemical uncouplers: seryl-tRNA synthetase (Ser-tRS), glutamine-hydrolyzing asparagine synthetase (Glut-HAS), mitochondrial bifunctional methylenetetrahydrofolate dehydrogenase (Mit BMD), mitochondrial heat shock 10-kDa protein (Mit HSP 10), proliferating cyclic nuclear antigen (PCNA), cytoplasmic beta-actin (Act B), and

growth arrest and DNA damage-inducible protein 153 (GADD153). Transcriptional changes of all seven genes were later confirmed with reverse transcription-polymerase chain reaction (RT-PCR). These results suggest that gene expression changes may provide a sensitive indicator of uncoupling in response to chemical exposure.

Lambert, A. L., J. B. Mangum, et al. (2003). "Ultrafine Carbon Black Particles Enhance Respiratory Syncytial Virus-Induced Airway Reactivity, Pulmonary Inflammation, and Chemokine Expression." Toxicol. Sci. **72**(2): 339-346.

<http://toxsci.oupjournals.org/cgi/content/abstract/72/2/339>

Exposure to particulate matter (PM) may exacerbate preexisting respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD), bronchitis, and pneumonia. However, few experimental studies have addressed the effects of PM on lower respiratory tract (LRT) viral infection. Respiratory syncytial virus (RSV) is a major etiological agent for LRT infections in infants, the elderly, and the immunocompromised and may lead to chronic wheezing and the development of asthma in children. In this study, we examined the effects of carbon black (CB) on RSV-induced pulmonary inflammation, chemokine and cytokine expression, and airway hyperresponsiveness in a mouse model of RSV. Female BALB/c mice were instilled via the trachea (i.t.) with 1×10^6 plaque forming units (pfu) RSV or with uninfected culture media. On day 3 of infection, mice were i.t. instilled with either 40 μ g ultrafine CB particles or with saline. End points were examined on days 4, 5, 7, and 14 of RSV infection. Viral titer and clearance in the lung were unaffected by CB exposure. Neutrophil numbers were elevated on days 4 and 7, and lymphocyte numbers were higher on days 4 and 14 of infection in CB-exposed, RSV-infected mice. CB exposure also enhanced RSV-induced airway hyperresponsiveness to methacholine, bronchoalveolar lavage (BAL) total protein, and virus-associated chemokines monocyte chemoattractant protein (MCP-1), macrophage inflammatory protein (MIP-1 α), and regulated upon activation, normal T cell expressed and secreted (RANTES). MIP-1 α mRNA expression was increased in the alveolar epithelium, where ultrafine particles deposit in the lung. These data demonstrate a synergistic effect of ultrafine CB particles on RSV infection, and suggest a potential mechanism for increased respiratory infections in human populations after PM exposure.

Loertscher, J. A., T.-M. Lin, et al. (2002). "In Utero Exposure to 2,3,7,8-Tetrachlorodibenzo-p-dioxin Causes Accelerated Terminal Differentiation in Fetal Mouse Skin." Toxicol. Sci. **68**(2): 465-472.

<http://toxsci.oupjournals.org/cgi/content/abstract/68/2/465>

2,3,7,8 Tetrachlorodibenzo-p-dioxin (TCDD), a ubiquitous environmental toxin, has been shown to cause a human skin pathology called chloracne. The majority of laboratory mouse strains, with the exception of mice bearing a mutation in the hairless gene, fail to display overt signs of chloracne upon exposure to TCDD. As a result, only minimal data exist on the effects of TCDD in adult haired mice and no data exist on the effects of TCDD in developing mouse skin. Here we report that TCDD affects the temporal expression of protein markers of keratinocyte terminal differentiation during murine skin morphogenesis. Immunohistochemical analysis of E16 mice reveals accelerated expression of the intermediate filament-associated protein filaggrin in response to TCDD. At a later developmental time and after birth, expression of filaggrin and loricrin is indistinguishable between treatment and control groups. At E16 expression of keratins 5, 6, and 10 are unaltered in TCDD-exposed individuals and TUNEL analysis shows no apoptotic cells in the basal and spinous layers of either treatment or control groups. At E16, immunohistochemical analysis of AhR-null mouse skin reveals accelerated filaggrin expression in both vehicle and TCDD exposed animals. We therefore hypothesize that AhR acts as a

modulator of late stage keratinocyte terminal differentiation.

Merrill, C. L., H. Ni, et al. (2002). "Etomoxir-Induced Oxidative Stress in HepG2 Cells Detected by Differential Gene Expression Is Confirmed Biochemically." *Toxicol. Sci.* **68**(1): 93-101.

<http://toxsci.oupjournals.org/cgi/content/abstract/68/1/93>

Although they are known to be effective antidiabetic agents, little is published about the toxic effects of carnitine palmitoyltransferase-1 (CPT-1) inhibitors, such as etomoxir (ET). These compounds inhibit mitochondrial fatty acid {beta}-oxidation by irreversibly binding to CPT-1 and preventing entry of long chain fatty acids into the mitochondrial matrix. Treatment of HepG2 cells with 1 mM etomoxir for 6 h caused significant modulations in the expression of several redox-related and cell cycle mRNAs as measured by microarray analysis. Upregulated mRNAs included heme oxygenase 1 (HO1), 8-oxoguanine DNA glycosylase 1 (OGG1), glutathione reductase (GSR), cyclin-dependent kinase inhibitor 1A (CDKN1 [p21waf1]) and Mn⁺ superoxide dismutase precursor (SOD2); while cytochrome P450 1A1 (CYP1A1) and heat shock 70kD protein 1 (HSPA1A) were downregulated. Real time quantitative PCR (RT-PCR) confirmed the significant changes in 4 of 4 mRNAs assayed (CYP1A1, HO1, GSR, CDKN1), and identified 3 additional mRNA changes; 2 redox-related genes, {gamma}-glutamate-cysteine ligase modifier subunit (GCLM) and thioredoxin reductase (TXNRD1) and 1 DNA replication gene, topoisomerase II{alpha} (TOP2A). Temporal changes in selected mRNA levels were examined by RT-PCR over 11 time points from 15 min to 24 h postdosing. CYP1A1 exhibited a 38-fold decrease by 4 h, which rebounded to a 39-fold increase by 20 h. GCLM and TXNRD1 exhibited 13- and 9-fold increases, respectively at 24 h. Etomoxir-induced oxidative stress and impaired mitochondrial energy metabolism were confirmed by a significant decrease in reduced glutathione (GSH), reduced/oxidized glutathione ratio (GSH/GSSG), mitochondrial membrane potential (MMP), and ATP levels, and by concurrent increase in oxidized glutathione (GSSG) and superoxide generation. This is the first report of oxidative stress caused by etomoxir.

Richards, V. E., B. Chau, et al. (2004). "Hepatic Gene Expression and Lipid Homeostasis in C57Bl/6 Mice Exposed to Hydrazine or Acetylhydrazine." *Toxicol. Sci.* **82**(1): 318-332.

<http://toxsci.oupjournals.org/cgi/content/abstract/82/1/318>

Hydrazine (HD) and acetylhydrazine (AcHD) are metabolites of the antituberculosis drug isoniazid (INH) that have been implicated in INH-induced liver damage. The hepatotoxicity of AcHD and HD were compared in adult male C57Bl/6J mice by evaluating hepatic histopathology, plasma biochemistry, and hepatic gene expression. By all measures, HD had significantly greater effects than AcHD. There was no evidence of liver damage following exposure to AcHD (300 mg/kg, po). However, HD at this dose caused marked hepatic necrosis, macrovesicular degeneration, and steatosis. Lipid accumulation was initiated 2 h after HD exposure, with hepatic macrovesicular degeneration evident after 4 h, and severe necrosis by 36 h. Gene expression profiles were compared 24 h following 100 mg/kg po of HD or AcHD. HD changed the hepatic expression of more genes than AcHD, particularly lipid synthesis, transport, and metabolism genes that may be involved in steatosis. Hepatic expression of genes regulated by peroxisome proliferator activated receptors (PPAR) and sterol regulatory element binding protein (SREBP) transcription factors was increased only by HD. The hepatotoxicity and hepatic gene expression profile of HD, but not AcHD, indicate that exposure to HD initiates a process whereby the production and intracellular transport of hepatic lipids is favored over the removal of fatty acids and their metabolites.

Vickers, A. E. M., M. Saulnier, et al. (2004). "Organ Slice Viability Extended for Pathway Characterization: An in Vitro Model to Investigate Fibrosis." *Toxicol. Sci.* **82**(2): 534-544.

<http://toxsci.oupjournals.org/cgi/content/abstract/82/2/534>

Liver slice viability is extended to 96 h for rat, expanding the use of this in vitro model for studying mechanisms of injury and repair, including pathways of fibrosis. The contributing factors to increased organ slice survival consist of the use of a preservation solution for liver perfusion and slice preparation, obtaining rats that are within the weight range of 250-325 g, placing a cellulose filter atop the titanium mesh roller-insert to support the slice, and maintaining the slices in an optimized culture medium which is replaced daily. The liver slices remain metabolically active, synthesizing adenosine triphosphate (ATP), glutathione, and glycogen, and exhibit preserved organelle integrity and slice morphology. Slice preparation results in 2-cut surfaces which likely triggers a repair and regenerative response. The fibrogenic pathways are evident by the activation of stellate cells, the proliferation of myofibroblast-like cells, and an increased collagen deposition by 48 h. Markers indicative of activated stellate cells, {alpha}-smooth muscle actin, collagen 1a1, desmin, and HSP47 are substantiated by real time-PCR. Increased staining of {alpha}-smooth muscle actin initially around the vessels and by 72-96 h in the tissue is accompanied by increased collagen staining. Microarray gene expression revealed extracellular matrix changes with the up-regulation of cytoskeleton, filaments, collagens, and actin genes; and the down-regulation of genes linked with lipid metabolism. The improvements in extending liver slice survival, in conjunction with its three-dimensional multi-cellular complexity, increases the application of this in vitro model for investigating pathways of injury and repair, and fibrosis.

Zodrow, J. M. and R. L. Tanguay (2003). "2,3,7,8-Tetrachlorodibenzo-p-dioxin Inhibits Zebrafish Caudal Fin Regeneration." *Toxicol. Sci.* **76**(1): 151-161.

<http://toxsci.oupjournals.org/cgi/content/abstract/76/1/151>

Adult zebrafish completely regenerate their caudal fins following partial amputation. Fin regrowth can easily be monitored in vivo and regenerating tissues can be used to study this dynamic developmental process. In this study we determined that fin regeneration is significantly affected by exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Zebrafish caudal fins were partially amputated, and the fish received intraperitoneal (ip) injection of 2.8, 14, or 70 ng/g weight TCDD or vehicle control. By 7 days postamputation, fish exposed to the highest concentration of TCDD regenerated 15% of their fin compared to 65% regrowth in control fish. To determine if this effect was stage specific, zebrafish were exposed to 70 ng/g TCDD on 1, 2, 3, or 4 days postamputation. Fin regeneration was significantly inhibited at all time points following TCDD exposure. TCDD exposure also induced hyperpigmentation in de novo tissue. Zebrafish were dosed with BrdU, following fin amputation and TCDD exposure, to study changes in cell proliferation. By 4 days postamputation, cell proliferation rates were significantly lower in TCDD-exposed fish. TCDD toxicity is mediated through the aryl hydrocarbon receptor (AHR), and RT-PCR experiments confirmed AHR2, ARNT2b, and TCDD-dependent CYP1A expression in the regenerating tissue. These results demonstrate that zebrafish caudal fin regeneration is a unique model to investigate molecular mechanism(s) of TCDD toxicity.

Hwan Kim, S., K. Ok Hong, et al. (2004). "Abrogation of cisplatin-induced hepatotoxicity in mice by xanthorrhizol is related to its effect on the regulation of gene transcription." Toxicology and Applied Pharmacology **196**(3): 346.

<http://www.sciencedirect.com/science/article/B6WXH-4C0V5S4-1/2/63ea8338a7b897b93357b23949f74a86>

Cisplatin is a widely used anticancer drug, but at high dose, it can produce undesirable side effects such as hepatotoxicity. Because *Curcuma xanthorrhiza* Roxb. (Zingiberaceae) has been traditionally used to treat liver disorders, the protective effect of xanthorrhizol, which is isolated from *C. xanthorrhiza*, on cisplatin-induced hepatotoxicity was evaluated in mice. The pretreatment of xanthorrhizol (200 mg/kg/day, po) for 4 days prevented the hepatotoxicity induced by cisplatin (45 mg/kg, ip) with statistical significance. Interestingly, it abrogated cisplatin-induced DNA-binding activity of nuclear factor-kappaB (NF-[kappa]B), which consequently affected mRNA expression levels of NF-[kappa]B-dependent genes, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2), even in part. It also attenuated the cisplatin-suppressed DNA-binding activity of activator protein 1 (AP-1). Using differential display reverse transcription-polymerase chain reaction (DDRT-PCR), seven upregulated genes including S100 calcium binding protein A9 (S100A9) mRNA and antigenic determinant for rec-A protein mRNA and five downregulated genes including caseinolytic protease X (ClpX) mRNA and ceruloplasmin (CP) mRNA by cisplatin were identified. Although these mRNA expression patterns were not totally consistent with gel shift patterns, altered expression levels by cisplatin were reversed by the pretreatment of xanthorrhizol. In conclusion, the ability of xanthorrhizol to regulate the DNA-binding activities of transcription factors, NF-[kappa]B and AP-1, could be one possible mechanism to elucidate the preventive effect of xanthorrhizol on cisplatin-induced hepatotoxicity. Furthermore, genes identified in this study could be helpful to understand the mechanism of cisplatin-induced hepatotoxicity. Finally, the combination treatment of xanthorrhizol and cisplatin may provide more advantage than single treatment of cisplatin in cancer therapy.

Kenyon, N. J., R. W. Ward, et al. (2003). "Airway fibrosis in a mouse model of airway inflammation." Toxicology and Applied Pharmacology **186**(2): 90.

<http://www.sciencedirect.com/science/article/B6WXH-47RYSNF-4/2/e14863329ee59f7df018f4cf3cd54ff1>

BALB/c mice were sensitized to ovalbumin by systemic injection and then exposed for up to 8 weeks to ovalbumin aerosols in whole body chambers. A pattern of airway inflammation, mucous cell hypertrophy and hyperplasia, and airway remodeling with submucosal fibrosis was observed as lesions evolved over time. Larger conducting airways were removed from the lungs by microdissection. Airway fibrosis was quantified by direct assay for collagen content, which was significantly increased after 4 and 8 weeks of exposure to ovalbumin aerosol. Based upon PCR analysis of mRNA levels in the airways, most of the newly synthesized collagen was Type I. Relaxin, administered by continuous infusion over the second half of a 4-week exposure to ovalbumin, was able to inhibit the accumulation of collagen in the airways of exposed mice. Thus, stimulation of collagen degradation by an activator of collagen breakdown by matrix metalloproteinases appears to be an effective therapeutic strategy in prevention of airway fibrosis in this animal model. Whole body plethysmography of unrestrained mice indicated functional changes in airway reactivity in the lungs of exposed animals occurring in conjunction with the reported structural changes. This result indicates that the ovalbumin-exposed mouse may be a suitable model for examining structure-function relationships in the lungs of animals with a predictable time course of airway inflammation, remodeling, and fibrosis and for testing potential new drugs for treatment of asthma or chronic bronchitis at a mechanistic level.

Konno, Y., M. Sekimoto, et al. (2004). "Sex difference in induction of hepatic CYP2B and CYP3A subfamily enzymes by nicardipine and nifedipine in rats." Toxicology and Applied Pharmacology **196**(1): 20.

<http://www.sciencedirect.com/science/article/B6WXH-4BT1SB3-1/2/d033257ac2462d714c4155e378292b73>

Male and female of F344 rats were treated per os with nicardipine (Nic) and nifedipine (Nif), and changes in the levels of mRNA and protein of hepatic cytochrome P450 (P450) enzymes, CYP2B1, CYP2B2, CYP3A1, CYP3A2, CYP3A9, and CYP3A18 were examined. Furthermore, hepatic microsomal activities for pentoxifyresorufin O-dealkylation (PROD) and nifedipine oxidation, which are mainly mediated by CYP2B and CYP3A subfamily enzymes, respectively, were measured. Analyses of RT-PCR and Western blotting revealed that Nic and Nif induced predominantly CYP3A and CYP2B enzymes, respectively. As for the gene activation of CYP2B enzymes, especially CYP2B1, Nif showed high capacity in both sexes of rats, whereas Nic did a definite capacity in the males but little in the females. Gene activations of CYP3A1, CYP3A2, and CYP3A18 by Nic occurred in both sexes of rats, although that of CYP3A9 did only in the male rats. Although gene activations of CYP3A1 and CYP3A2 by Nif were observed in both sexes of rats, a slight activation of the CYP3A9 gene occurred only in female rats, and the CYP3A18 gene activation, in neither male nor female rats. Thus, changes in levels of the mRNA or protein of CYP2B and CYP3A enzymes, especially CYP2B1 and CYP3A2, were closely correlated with those in hepatic PROD and nifedipine oxidation activities, respectively. The present findings demonstrate for the first time the sex difference in the Nic- and Nif-mediated induction of hepatic P450 enzymes in rats and further indicate that Nic and Nif show different specificities and sex dependencies in the induction of hepatic P450 enzymes.

Lai, Y.-L., S. C. Yu, et al. (2003). "RNA interference prevents lipopolysaccharide-induced preprotachykinin gene expression." Toxicology and Applied Pharmacology **193**(1): 47.

<http://www.sciencedirect.com/science/article/B6WXH-49NXGG3-4/2/57529a79531b0125153204071dc3125c>

We showed previously that lipopolysaccharide (LPS) induces noncholinergic airway hyperreactivity to capsaicin via an upregulation of tachykinin synthesis. This study was designed to test whether double-stranded preprotachykinin (ds PPT) RNA, RNA interference (RNAi), prevents the LPS-induced alterations. First, cultured primary nodose ganglial cells of newborn Brown-Norway rats were divided into four groups: control; LPS; LPS+RNAi; and LPS+RNAi+liposome. Second, young Brown-Norway rats for the in vivo study were divided into three groups (control; LPS; and LPS+RNAi), and ds PPT RNA was microinjected bilaterally into the nodose ganglia in the LPS+RNAi group. Then, ganglial cells were collected from the culture whereas the nodose ganglia and lungs were sampled from the animals, and PPT mRNA and substance P (SP) levels were analyzed. Also, airway reactivity to capsaicin was performed in vivo. LPS induced significant increases in PPT mRNA and SP levels in vitro and in vivo and an increase in airway reactivity to capsaicin in vivo. However, ds PPT RNA, but not scrambled RNA, prevented all LPS-induced alterations. The effect of ds PPT RNA was not enhanced by liposome in vitro. Therefore, we demonstrated that the local application of RNAi prevents effectively the activation of the noncholinergic system modulating the lungs/airways.

Patterson, R. M., R. Stachlewitz, et al. (2003). "Induction of apoptosis by 2,3,7,8-tetrachlorodibenzo-p-dioxin following endotoxin exposure." Toxicology and Applied Pharmacology **190**(2): 120.

<http://www.sciencedirect.com/science/article/B6WXH-48XD53J-2/2/8a01d8f42ed8df66bb286a26860e1b70>

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potent and persistent environmental toxin that induces hepatotoxicity and increases endotoxin-induced liver injury. The objective of this study was to evaluate whether TCDD could modulate apoptosis and cytokine-controlled apoptotic signaling pathways following lipopolysaccharide (LPS) exposure in female B6C3F1 mice. The effects of TCDD treatment were most dramatic late in the time course (10-14 days posttreatment). Serum enzyme activities were elevated at day 10 (100 [mu]g TCDD/40 [mu]g LPS treatment) and day 14 (100 [mu]g TCDD/saline treatment), indicating peak liver damage occurred at those times. Histological examination of perfused livers showed an increase in apoptotic cells at day 14 in animals treated with 10 [mu]g TCDD. Caspase-1 activity was suppressed at 14 days in mice treated with 100 [mu]g TCDD/40 [mu]g LPS and 100 [mu]g TCDD/4 [mu]g LPS compared to the respective corn oil (CO)/LPS-treated controls. Caspase-3 activity was suppressed at 14 days in 100 [mu]g TCDD/saline-100 [mu]g TCDD/40 [mu]g LPS- and 100 [mu]g TCDD/4 [mu]g LPS-treated mice compared to respective CO/saline- or CO/LPS-treated control mice. At 40 [mu]g LPS, caspase activity was stimulated in TCDD (100 [mu]g)-exposed mice at 3 and 7 days and then suppressed at 10 and 14 days. Western blot analysis, electrophoretic mobility shift assay, and ELISA did not show any effect by TCDD (100 [mu]g) on I[kappa]B-[beta] and I[kappa]B-[alpha] protein expression or on DNA binding activity of the nuclear NF[kappa]B protein. These data indicate that TCDD induces apoptosis 14 days posttreatment; however, we found no evidence of suppression of the antiapoptotic transcription factor NF[kappa]B.

Rojas-Garcia, A. E., M. J. Solis-Heredia, et al. "Genetic polymorphisms and activity of PON1 in a Mexican population." Toxicology and Applied Pharmacology **In Press, Corrected Proof**

<http://www.sciencedirect.com/science/article/B6WXH-4F0118T-1/2/342e096c045e31d69f1b45f05721a129>

Human paraoxonase (PON1) plays a role in detoxification of organophosphorus (OP) compounds by hydrolyzing the bioactive oxons, and in reducing oxidative low-density lipoproteins, which may protect against atherosclerosis. Some PON1 polymorphisms have been found to be responsible for variations in catalytic activity and expression and have been associated with susceptibility to OP poisoning and vascular diseases. Both situations are of public health relevance in Mexico. Therefore, the aim of this study was to evaluate PON1 phenotype and the frequencies of polymorphisms PON1 -162, -108, 55, and 192 in a Mexican population. The studied population consisted of unrelated individuals (n = 214) of either gender, 18-52 years old. Serum PON1 activity was assayed using phenylacetate and paraoxon as substrates. PON1 variants, -162, 55, and 192, were determined by real-time PCR using the TaqMan System, and PON1 -108 genotype by PCR-RFLP. We found a wide interindividual variability of PON1 activity with a unimodal distribution; the range of enzymatic activity toward phenylacetate was 84.72 to 422.0 U/mL, and 88.37 to 1645.6 U/L toward paraoxon. All four PON1 polymorphisms showed strong linkage disequilibrium (D% >90). PON1 polymorphisms -108, 55, and 192 were independently associated with arylesterase activity; whereas the activity toward paraoxon was related only with PON1 192 polymorphism, suggesting that this polymorphism is determinant to infer PON1 activity. A better understanding of the phenotype and genotypes of PON1 in Mexican populations will facilitate further epidemiological studies involving PON1 variability in OP poisoning and in the development of atherosclerosis.

Zheng, Y. i.-M., K. R. Henne, et al. (2003). "Genotyping and site-directed mutagenesis of a cytochrome P450 meander Pro-X-Arg motif critical to CYP4B1 catalysis." Toxicology and Applied Pharmacology **186**(2): 119.

<http://www.sciencedirect.com/science/article/B6WXH-47RYSNF-3/2/0246741551aadbdef0ffe5508494ff4c>

CYP4B1 isoforms from rodents and other common laboratory animals are involved in the bioactivation of a range of protoxins, including 2-aminofluorene, 4-ipomeanol, and valproic acid. However, an earlier study provided evidence for a human allele encoding a nonfunctional CYP4B1 enzyme due to a Pro427Ser transversion in the meander region of the protein. In the present study, the CYP4B1 gene from several racial groups, Caucasians, African-Americans, and Hispanics, and from six nonhuman primate species was genotyped using a PCR-Hinf1 restriction enzyme fragment length polymorphism assay or by direct sequencing. All human populations examined were found to possess only the Ser allele at codon 427 (1279TCT) and all of the nonhuman primate species possessed only the Pro (CCT) allele. Therefore, an inactivating 1279C->T mutation in the human CYP4B1 gene likely arose following divergence of the Homo and Pan clades. Amino acid sequence alignments revealed further that this key Pro residue is located two amino acid residues N-terminal to the distal Arg of a Glu-Arg- triad thought to participate in heme binding and/or redox partner interactions. Mutation of the corresponding Arg424 residue in rabbit CYP4B1 to Leu, but not His, resulted in a loss of lauric acid hydroxylase activity and ability to generate a reduced-CO binding spectrum. These data provide additional evidence for the importance of this meander region Pro-X-Arg motif in CYP4B1 heme binding and catalytic function.

Toxicology in Vitro (6)

Hildebrand, H., U. Schmidt, et al. (1999). "An in vitro model for peroxisome proliferation utilizing primary hepatocytes in sandwich culture." Toxicology in Vitro **13**(2): 265.

<http://www.sciencedirect.com/science/article/B6TCP-3WRB2B0-6/2/a26a09192f8c3aa3813cdac0fc601a51>

Peroxisome proliferators comprise a group of structurally diverse chemicals which share as a common biologic effect the induction of peroxisomal fatty acid degrading enzymes. Concomitantly, the number and size of peroxisomes within hepatocytes increases. Following chronic administration some peroxisome proliferators act as non-genotoxic hepatocarcinogens in susceptible species such as rodents. To establish an in vitro model for the toxicological investigation of peroxisome proliferation, primary hepatocytes of rats, dogs and humans were cultivated in an organotypic cell culture model (sandwich model). By employing a panel of diverse compounds in this model a graded response was observed in the induction of carnitine acetyl transferase (CAT), the activity of which was determined as an endpoint. The following results were obtained in the order of decreasing inducing potential for rat hepatocytes: FOE 3798>nafenopin>fenofibrate (ciprofibrate>bezafibrate >> DEHP[ap]ETYA>DEHA. Induction of CAT activity was generally higher than reported in earlier cell culture systems, probably reflecting the effect of the extracellular matrix provided by the collagen gel sandwich. In parallel, transcription of the rat CYP4A1 gene was induced by a similar order of magnitude as measured by TaqMan RT-PCR. In accordance with literature data, human and dog hepatocytes did not display such a strong and graded response but rather were not susceptible to this effect. In addition, 3H-thymidine incorporation data demonstrated that nafenopin was able to induce DNA synthesis in rat hepatocytes whereas it did not in human hepatocytes.

Pawlowski, S., M. Islinger, et al. (2000). "Temperature-dependent vitellogenin-mRNA expression in primary cultures of rainbow trout (*Oncorhynchus mykiss*) hepatocytes at 14 and 18[deg]C." Toxicology in Vitro **14**(6): 531.

<http://www.sciencedirect.com/science/article/B6TCP-41CP350-7/2/eb6babd6ef637553d04350ff95f28b85>

In order to study the influence of temperature on vitellogenin gene and estrogen receptor gene expression in primary hepatocytes from rainbow trout (*Oncorhynchus mykiss*), cells were exposed to 17[beta]-estradiol, bisphenol-A and nonylphenol for 48 and 96 hr. Induction of vitellogenin-mRNA expression was detected in a non-radioactive dot blot/RNase protection assay and by RT-PCR. In the dot blot/RNase protection assay, the estrogenic potentials of bisphenol-A and nonylphenol were about 104- to 105-fold and 105-fold lower than that of 17[beta]-estradiol, respectively. The relative estrogenic potential did not show any difference between 14 and 18[deg]C. In contrast, at 18[deg]C, RT-PCR analysis revealed increased amounts of vitellogenin- and estrogen receptor-mRNA after 12 and 24 hr of exposure to 17[beta]-estradiol, if compared to 14[deg]C. Owing to increased vitellogenin gene expression at 18[deg]C, the sensitivity of primary hepatocytes to 17[beta]-estradiol and bisphenol-A could be increased.

Pichowski, J. S., M. Cumberbatch, et al. (2000). "Investigation of induced changes in interleukin 1[beta] mRNA expression by cultured human dendritic cells as an in vitro approach to skin sensitization testing." Toxicology in Vitro **14**(4): 351.

<http://www.sciencedirect.com/science/article/B6TCP-40SFFYB-8/2/cac096480d04ea1da0a1560ee674dd2d>

It has been reported previously that in vitro treatment of human blood derived dendritic cells (DC) with contact allergens provokes the elevated expression of mRNA for interleukin (IL) 1[beta], under conditions where similar treatment of cells with the non-sensitizing skin irritant sodium lauryl sulfate (SLS) did not alter IL-1[beta] mRNA levels (Reutter et al., 1997). The purpose of the present investigation was to evaluate further this phenomenon and to explore the potential utility of this approach for the purpose of skin sensitization testing. Human peripheral blood progenitor cells prepared from healthy adult volunteers were cultured in the presence of IL-4 and granulocyte/macrophage colony stimulating factor. After 5 days of culture, the majority of cells had a Langerhans cell-like phenotype, with characteristic dendritic morphology and cell surface expression of CD83, major histocompatibility complex class II and CD1a determinants. These blood-derived DC were cultured in the presence of the contact allergen 2,4-dinitrofluorobenzene (DNFB), SLS or vehicle alone and mRNA expression for IL-1[beta], IL-6 and IL-18 was analysed by semiquantitative reverse transcriptase polymerase chain reaction. Constitutive expression of all three cytokines was observed for DC isolated from all donors examined. Exposure to DNFB resulted in upregulation of IL-1[beta] mRNA (two- to threefold) in cells derived from three out of eight donors whereas IL-6 and IL-18 were largely unaffected by allergen exposure. In contrast, SLS treatment did not induce IL-1[beta] mRNA expression in any of the donors investigated. Analysis of cytokine mRNA expression using the protocol described by Reutter et al. (1997), did not increase the sensitivity of measurement of induced cytokine expression. Although selected upregulation of IL-1[beta] by blood derived DC has been confirmed, a wider range of contact allergens and irritants need to be assessed before this approach could be considered for hazard identification.

Reutter, K., D. Jager, et al. (1997). "In vitro model for contact sensitization: II. Induction of IL-1[beta] mRNA in human blood-derived dendritic cells by contact sensitizers." Toxicology in Vitro **11**(5): 619.

<http://www.sciencedirect.com/science/article/B6TCP-3RSP2T3-15/2/5423ea258d97109a15cf3901c1a05d42>

Epidermal mRNA for interleukin 1[beta] (IL-1[beta]) has been shown to be increased following exposure of mouse skin to sensitizing compounds. In addition, this early upregulation of IL-1[beta] was specific for contact sensitizers, while expression of IL-1[beta] was unaffected by irritants. Langerhans cells are the major source of IL-1[beta] within the epidermis in the induction phase of skin sensitization. Since the isolation of Langerhans cells from skin biopsies results only in low frequencies, we decided to use dendritic cells (DCs) generated from peripheral blood as Langerhans cell equivalents to investigate the ability of five contact sensitizers and one irritant to induce IL-1[beta] gene expression in vitro. For our studies we cultivated DCs in serum-free medium supplemented with granulocyte/macrophage-colony stimulation factor (GM-CSF) and interleukin 4 (IL-4). The DCs showed a typical dendritic morphology, a characteristic expression of surface markers and high stimulatory capacity for autologous T cells. 5-day-old DCs were incubated with subtoxic concentrations of the contact sensitizers pentadecyl-catechol, 2,4,6-trinitrobenzene sulfonic acid, 2,4-dinitrofluorobenzene, NiSO₄, K₂Cr₂O₇ and the irritant sodium dodecyl sulfate. IL-1[beta] mRNA expression was detected by using the reverse transcriptase-polymerase chain reaction (RT-PCR) technique and non-radioactive hybridization procedures. For all contact sensitizers, expression of IL-1[beta] mRNA increased, whereas treatment with the irritant SDS had no significant effect on IL-1[beta] expression. Thus we developed an in vitro system, which may be useful to evaluate allergic potentials of chemicals and products.

Shimizu, M., K. Ohta, et al. (2002). "Sulfation of bisphenol A abolished its estrogenicity based on proliferation and gene expression in human breast cancer MCF-7 cells." *Toxicology in Vitro* **16**(5): 549.

<http://www.sciencedirect.com/science/article/B6TCP-46M1JR0-9/2/585dfd18036804d829dc600bc864ddfb>

Bisphenol A, an endocrine-disrupting chemical, is widely used in many consumer products. We previously showed the sulfoconjugation of bisphenol A catalyzed by a human thermostable phenol sulfotransferase, ST1A3. The estrogenic potency of bisphenol A sulfate was compared with that of bisphenol A by an E-screen assay using human breast cancer MCF-7 cells. An increase in the expression level of an estrogen-responsive pS2 gene was also examined using MCF-7 cells after exposure to bisphenol A and its sulfate for their estrogenicity. Bisphenol A sulfate did not exhibit estrogenic effects at 0.1 [mu] (E-screen assay) and 1 m (pS2 gene expression) compared with bisphenol A, which exhibited the effects at 3 n (E-screen assay) and 1 [mu] (pS2 gene expression), respectively. We have therefore evaluated major roles of cytosolic phenol sulfotransferase in the human liver. Bisphenol A sulfation in human liver cytosols was inhibited by more than 90% by p-nitrophenol and quercetin, a typical substrate and specific inhibitor of phenol sulfotransferase, respectively. These results indicated that the estrogenicity of bisphenol A was abolished through its sulfation catalyzed by a human hepatic thermostable phenol sulfotransferase.

zur Nieden, N. I., L. J. Ruf, et al. (2001). "Molecular markers in embryonic stem cells." *Toxicology in Vitro* **15**(4-5): 455.

<http://www.sciencedirect.com/science/article/B6TCP-440BK3X-16/2/1c84c2410e44cd9b2163e1f12559bddd>

Embryonic stem cells are pluripotent cells derived from mouse blastocysts, which have the

capacity to differentiate in vitro into a wide variety of cell types. Based on this potential the embryonic stem cell test (EST) has been developed, which represents an assay system for the classification of compounds for their teratogenic potential, based on the morphological evaluation of contracting myocard cells compared to the cytotoxic effects on undifferentiated stem cells and adult 3T3 fibroblasts. To expand the EST, the quantitative expression of the [alpha]- and [beta]-myosin heavy chain (MHC) genes under the influence of test compounds was studied employing real-time TaqMan PCR analysis. The molecular evaluation of the MHC genes allows a higher sensitivity for the classification of substances and the transfer of the EST to the molecular level allows to start experimental procedures at day 9 of culture. Thus, the modulated EST holds promise as a new easily quantifiable in vitro screening assay in teratology.

Toxicology Letters (8)

Brauch, H., G. Weirich, et al. (2004). "VHL mutations in renal cell cancer: does occupational exposure to trichloroethylene make a difference?" Toxicology Letters **151**(1): 301.

<http://www.sciencedirect.com/science/article/B6TCR-4C47J23-6/2/d4fefa5499232039717baa44b805331c>

Occupational exposures have long been suspected to play a role in the incidence of renal cell carcinoma (RCC). Especially, the carcinogenicity of the industrial solvent trichloroethylene (TCE) has been controversially debated, both with respect to the epidemiological and the molecular studies. In order to further elucidate this issue, it appeared important to compare suitable RCC patient groups, i.e., TCE-exposed versus non-TCE-exposed patients. We evaluated RCC from a previous German study that had described differences in RCC risks between TCE-exposed (n=17) and non-exposed patients (n=21). We compared age at diagnosis and histopathologic parameters of tumors as well as somatic mutation characteristics in the kidney cancer causing VHL tumor suppressor gene. RCC did not differ with respect to histopathological characteristics in both patient groups. We noticed a younger age at diagnosis in TCE-exposed patients compared to non-exposed patients (P=0.01). Moreover, the non-TCE-exposed patients did not share the somatic VHL mutation characteristics of TCE-exposed patients such as the previously identified hot spot mutation 454 C > T P81S or multiple mutations. These data support the notion of a putative genotoxic effect of TCE leading to VHL gene damage and subsequent occurrence of RCC in highly exposed subjects.

Garrett, S. H., V. Phillips, et al. (2002). "Transient induction of metallothionein isoform 3 (MT-3), c-fos, c-jun and c-myc in human proximal tubule cells exposed to cadmium." Toxicology Letters **126**(1): 69.

<http://www.sciencedirect.com/science/article/B6TCR-44JHY8R-7/2/fd4cbec082de4a2bdf6ac0d1d7f0139>

Cadmium (Cd²⁺) has been shown to transiently increase the expression of mRNA for the third isoform of the metallothionein (MT-3) gene family in cultured human proximal tubule (HPT) cells. The goal of the present study was to further define the expression of MT-3 in mortal (HPT) and immortal (HK-2) cultures of HPT cells when exposed to lethal and sub-lethal concentrations of Cd²⁺ under both acute and chronic time periods of exposure. Expression of MT-3 mRNA and

protein was determined in cultured HPT cells and HK-2 cells using reverse-transcription-polymerase chain reaction (RT-PCR) and immuno-blotting, and expression of c-fos, c-jun and c-myc mRNA by RT-PCR. The results confirmed that exposure of the HPT cells to Cd+2 induced a transient increase in MT-3 mRNA and extended the induction to include a subsequent transient increase in the level of the MT-3 protein. The induction of MT-3 was rapid and returned to control values within 48 h of exposure despite the continued presence of lethal and sublethal concentrations of Cd+2. It was also demonstrated that the pattern of expression of MT-3 mRNA was similar to that of the early response genes, c-fos, c-jun and c-myc. It was shown that the HK-2 cells did not express MT-3 when exposed to Cd+2, but had similar expression of the c-fos, c-jun and c-myc genes. The results demonstrate that MT-3 expression is metal responsive in HPT cells.

Kojima, M., T. Masui, et al. (2004). "Lead nitrate-induced development of hypercholesterolemia in rats: sterol-independent gene regulation of hepatic enzymes responsible for cholesterol homeostasis." Toxicology Letters **154**(1-2): 35.

<http://www.sciencedirect.com/science/article/B6TCR-4D4VHW9-4/2/f49f52e6aec345d3ad0250778acb2eb>

Changes in the gene expressions of hepatic enzymes responsible for cholesterol homeostasis were examined during the process of lead nitrate (LN)-induced development of hypercholesterolemia in male rats. Total cholesterol levels in the liver and serum were significantly increased at 3-72 h and 12-72 h, respectively, after LN-treatment (100 [mu]mol/kg, i.v.). Despite the development of hypercholesterolemia, the genes for hepatic 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) and other enzymes (FPPS, farnesyl diphosphate synthase; SQS, squalene synthase; CYP51, lanosterol 14[alpha]-demethylase) responsible for cholesterol biosynthesis were activated at 3-24 h and 12-18 h, respectively. On the other hand, the gene expression of cholesterol 7[alpha]-hydroxylase (CYP7A1), a catabolic enzyme of cholesterol, was remarkably suppressed at 3-72 h. The gene expression levels of cytokines interleukin-1[beta] (IL-1[beta]) and TNF-[alpha], which activate the HMGR gene and suppress the CYP7A1 gene, were significantly increased at 1-3 h and 3-24 h, respectively. Furthermore, gene activation of SREBP-2, a gene activator of several cholesterologenic enzymes, occurred before the gene activations of FPPS, SQS and CYP51. This is the first report demonstrating sterol-independent gene regulation of hepatic enzymes responsible for cholesterol homeostasis in LN-treated male rats. The mechanisms for the altered-gene expressions of hepatic enzymes in LN-treated rats are discussed.

Lafuente, M. J., X. Casterad, et al. (2002). "Pi*S and Pi*Z alpha 1 antitrypsin polymorphism and the risk for asbestosis in occupational exposure to asbestos." Toxicology Letters **136**(1): 9.

<http://www.sciencedirect.com/science/article/B6TCR-46W1CF4-2/2/3b1471822f16320970b2fa425a5d6686>

Alpha 1 antitrypsin is a highly polymorphic anti-elastase enzyme, especially active in the protection of alveoli and liver. Here we studied the distribution of two deficient alleles Pi*Z and Pi*S, in 194 asbestos workers, of whom 100 were asbestosis cases, and 94 were controls without disease (exposed controls, EC). A second group of controls without asbestos exposure (non-exposed controls, NEC; n=122) was also included. Multivariate analysis adjusted by age and smoking habit showed ninefold risk for asbestosis in Pi*Z heterozygous individuals and 5.9-fold risk for Pi*S homozygous although differences were only significant in the first case (cases vs. EC: OR 8.9; p=0.04). Considering both genotypes (Pi*Z heterozygous, Pi*S homozygous) we obtained an OR of 8 (p=0.01). Our results suggest that the alpha 1 antitrypsin polymorphisms,

especially Pi*Z, could help to predict asbestosis risk and confirm the high prevalence of the Pi*S allele in Spain.

Navarra, P., P. Puccetti, et al. (1995). "Anticancer drug toxicity via cytokine production: the hydroxyurea paradigm." Toxicology Letters **82-83**: 167.

<http://www.sciencedirect.com/science/article/B6TCR-3VXBPDC-VI2/c3c847fefb17707e3b0f904ac98b5ef5>

Our previous observations on the toxic effects of hydroxyurea (HU) in adrenalectomized (ADX) rats prompted us to suggest that these effects might be mediated by an increased synthesis of proinflammatory cytokines. This study was conducted to determine whether HU stimulates cytokine gene expression in vivo. The polymerase chain reaction (PCR) technique was used to assess levels of mRNA for interleukin-1[alpha] (IL-1[alpha]), tumor necrosis factor (TNF) and interleukin-4 (IL-4) in spleen cells from intact and ADX rats treated with HU or vehicle. In ADX rats, expression of proinflammatory-cytokine mRNA was markedly increased by HU, but no expression of these genes was seen in intact animals after treatment. In the latter rats, cytokine-gene expression seemed to be down-regulated by HU-induced elevations in plasma corticosterone levels, since IL-1[alpha] and TNF transcripts could be detected only after corticosterone levels had returned to normal (24 h after treatment). Interestingly, IL-4 mRNA could not be detected in either treated or untreated ADX rats, indicating that expression of this gene is closely related to circulating levels of corticosterone. These findings strongly suggest that the increased toxicity displayed by HU in ADX animals is mediated by stimulation of cytokine synthesis in vivo.

Seidel, B., L. Jiang, et al. (2000). "Differentially displayed genes in neuroblastoma cells treated with a mitochondrial toxin: evidence for possible involvement of ICAM-1 in 3-nitropropionic acid-mediated neurodegeneration." Toxicology Letters **115(3)**: 213.

<http://www.sciencedirect.com/science/article/B6TCR-408BJ93-5/2/dff3d60f00a9885cd5eab36f451c52a8>

The mitochondrial toxin 3-nitropropionic acid (3-NPA) causes neurodegeneration in the basal ganglia and neurological symptoms resembling Huntington's disease (HD) when applied to primates or rodents, and therefore might be used as an animal model for this disorder. For that reason, the molecular mechanisms involved in 3-NPA-induced neurodegeneration are of considerable interest. In our model, murine neuroblastoma cells (Neuro-2a) were treated with different doses of 3-NPA, and changes in gene expression were analyzed by means of mRNA differential display (DDRT-PCR). Using 18 primer combinations, we have identified a set of 33 candidate cDNAs deriving from 29 excised DDRT bands whose expression appeared to be changed in response to the 3-NPA insult (mostly elevated). DNA sequencing revealed that novel, as well as previously described genes, are included in this panel. Amongst the known cDNAs, the differential mRNA expression of the ribosomal proteins S6 and L40, of the protein kinase A (PKA) catalytic beta subunit and of the intercellular adhesion molecule ICAM-1 could be verified using Northern hybridization and RT-PCR, respectively. Furthermore, ICAM-1 expression could also be shown to increase at the protein level, which points to a possible function for this molecule in neuronal cells in the course of neurodegeneration. The results may prove useful in elucidating the multiple processes causing neurodegeneration subsequent to lesions by mitochondrial toxins and excitotoxins as well.

Somji, S., M. Ann Sens, et al. (2002). "Expression of hsp 90 in the human kidney and in proximal tubule cells exposed to heat, sodium arsenite and cadmium chloride." Toxicology Letters **133**(2-3): 241.

<http://www.sciencedirect.com/science/article/B6TCR-460DFXH-1/2/f2249d82b2c2600a64d52706a2d75616>

The expression of heat shock protein (hsp) 90[alpha] and [beta] mRNA and protein were determined in the human kidney and in human proximal tubule (HPT) cells exposed to lethal and sub-lethal concentrations of Cd²⁺ under both acute and extended conditions of exposure. Using immunohistochemical analysis, it was demonstrated that hsp 90 was widely distributed in the human adult and fetal kidney. Moderate to strong staining was observed in the straight portions of the distal and proximal tubules, the distal convoluted tubule, the collecting ducts and the parietal epithelium of Bowmans capsule in the glomerulus. Moderate staining was observed in the proximal convoluted tubule of the cortex and the thick loops of Henle within the medulla. In addition, the fetal kidney demonstrated strong staining of the blastema, the 'S-shaped' bodies, and the developing glomeruli. Analysis of hsp 90[alpha] and [beta] mRNA expression in total RNA isolated from in situ microdissected proximal tubules or HPT cells demonstrated similar expression levels of both the [alpha] and [beta] isoforms in this tubule segment. It was demonstrated that HPT cells exhibited the classic heat shock response when subjected to a physical (heat) or chemical stress (NaAsO₂). Heat stress, elevated temperature at 42.5 [deg]C for 1 h, caused a modest increase in both hsp 90[alpha] and [beta] mRNA and protein. Similar results were obtained when the cells were subjected to a classic chemical stress of exposure to 100 [mu]M NaAsO₂ for 4 h. In contrast, acute exposure of HPT cells to 53.4 [mu]M CdCl₂ for 4 h resulted in no consistent increase in hsp 90[alpha] and [beta] mRNA or protein. Chronic exposure to Cd²⁺ likewise failed to increase either hsp 90 mRNA or protein expression, even at concentrations of Cd²⁺ that were lethal to the cells during the time course. This study shows that the HPT has a high basal expression of hsp 90, which is not induced by Cd²⁺ exposure.

Somji, S., J. H. Todd, et al. (2000). "Expression of heat shock protein 60 in human proximal tubule cells exposed to heat, sodium arsenite and CdCl₂." Toxicology Letters **115**(2): 127.

<http://www.sciencedirect.com/science/article/B6TCR-408BJC1-5/2/e344ad1d859faea13b8624a03df873dd>

The expression of hsp 60 mRNA and protein were determined in human proximal tubule cells (HPT) exposed to lethal and sub-lethal concentrations of Cd²⁺ under both acute and extended conditions of exposure. It was demonstrated that HPT cells exhibited the classic heat shock response when subjected to a physical (heat) or chemical stress (sodium arsenite). Heat stress, elevated temperature at 42.5[deg]C for 1 h, caused an increase in both hsp 60 mRNA and protein following removal of the stress. Similar results were obtained when the cells were subjected to a classic chemical stress of exposure to 100 [mu]M sodium arsenite for 4 h. Acute exposure of HPT cells to 53.4 [mu]M CdCl₂ for 4 h also resulted in an increase in hsp 60 mRNA and protein following removal of the metal. An extended exposure to Cd²⁺ was modeled by treating the cells continuously with Cd²⁺ at both lethal and sub-lethal levels over a 16-day time course. It was demonstrated that chronic exposure to Cd²⁺ failed to increase either hsp 60 mRNA or protein expression in HPT cells, even at concentrations of Cd²⁺ that were lethal to the cells during the time course. In fact, hsp 60 protein levels were decreased compared to controls at lethal levels of Cd²⁺ exposure. These findings suggest that hsp 60 expression may have two distinct roles when the human proximal tubule cell is exposed to Cd²⁺. A protective role through hsp 60 induction when the proximal tubule cell is acutely exposed to Cd²⁺ and a deleterious role when hsp 60 protein is down-regulated during extended exposure to Cd²⁺.

Clissa, P. B., G. D. Laing, et al. (2001). "The effect of jararhagin, a metalloproteinase from Bothrops jararaca venom, on pro-inflammatory cytokines released by murine peritoneal adherent cells." Toxicon **39**(10): 1567.

<http://www.sciencedirect.com/science/article/B6TCS-43K2G13-G/2/04a86f0b5a781c7f1a11bb747bdd3512>

The release of pro-inflammatory cytokines (IL-1[beta], IL-6 and TNF-[alpha]) from murine peritoneal adherent cells (MPAC) was studied after exposure to jararhagin, a metalloproteinase/disintegrin isolated from Bothrops jararaca venom. MPACs were treated with LPS (lipopolysaccharide), jararhagin, or EDTA-inactivated jararhagin for up to 24 h. Following incubation, the culture supernatant was assayed by ELISA for the presence of cytokines, while the cells were analysed for viability and cytokine mRNA expression. The cells exposed to native jararhagin released TNF-[alpha] and IL-1[beta] after 4 and 24 h respectively. When MPACs were exposed to Jararhagin treated with EDTA, TNF-[alpha] and IL-1[beta] production was sustained throughout the culture period and IL-6 production was observed. TNF-[alpha], IL-6 and IL-1[beta] mRNA were detected 4 h after stimulation with either native or EDTA-treated jararhagin. Addition of jararhagin to LPS stimulated cells resulted in a dramatic decrease in the release of IL-6 and TNF-[alpha]. RT-PCR showed that this inhibition does not occur at the transcriptional level and further experiments showed that jararhagin degraded soluble cytokines by proteolytic activity. This study suggests that jararhagin induces TNF-[alpha], IL-1[beta] and IL-6 expression, which may be rapidly degraded by its proteolytic activity.

DaSilva, L., D. Cote, et al. (2003). "Pulmonary gene expression profiling of inhaled ricin." Toxicon **41**(7): 813.

<http://www.sciencedirect.com/science/article/B6TCS-48KF64N-1/2/4d3374021a5e286c7c37ba59eb8ef6e1>

Aerosol exposure to ricin causes irreversible pathological changes of the respiratory tract resulting in epithelial necrosis, pulmonary edema and ultimately death. The pulmonary genomic profile of BALB/c mice inhalationally exposed to a lethal dose of ricin was examined using cDNA arrays. The expression profile of 1178 mRNA species was determined for ricin-exposed lung tissue, in which 34 genes had statistically significant changes in gene expression. Transcripts identified by the assay included those that facilitate tissue healing (early growth response gene (egr)-1), regulate inflammation (interleukin (IL)-6, tristetraproline (ttp)), cell growth (c-myc, cytokine-inducible SH2-containing protein (cish)- 3), apoptosis (T-cell death associated protein (tdag)51, pim-1) and DNA repair (ephrin type A receptor 2 (ephA2)). Manipulation of these gene products may provide a means of limiting the severe lung damage occurring at the cellular level. Transcriptional activation of egr-1, cish-3, c-myc and thrombospondin (tsp)-1 was already apparent when pathological and physiological changes were observed in the lungs at 12 h postexposure. These genes may well serve as markers for ricin-induced pulmonary toxicity. Ongoing studies are evaluating this aspect of the array data and the potential of several genes for clinical intervention.

Ivanovski, G., F. Gubensek, et al. (2002). "mRNA secondary structure can greatly affect production of recombinant phospholipase A2 toxins in bacteria." *Toxicon* **40**(5): 543.

<http://www.sciencedirect.com/science/article/B6TCS-44HTMX5-1/2/07784f5c6b087787f50076c99156cd40>

The neurotoxic activity of ammodytoxin A (AtxA), a phospholipase A2 from *Vipera ammodytes* venom, has been investigated by protein engineering. With the aim of obtaining AtxA as a non-fused protein in the bacterial cytoplasm and avoiding problems with incomplete cleavage in vivo of the initial Met preceding the first residue (Ser1), a double mutant (S1A/E4Q) was prepared and expressed in *Escherichia coli*. Immunoblotting of the bacterial lysate showed that the mutant was synthesized at a low level not exceeding 0.5% of total cell protein. Analysis of the potential secondary structure of the mutant mRNA in the translation initiation region suggested that the Ala1 (GCC) and Leu2 (CUG) codons used are likely to be involved in a hairpin structure with the Thr13 (ACG) and Gly14 (GGG) codons, hindering effective translation at the ribosome. To weaken this structure (by ΔG of about 20 kJ/mol) the same double mutant was prepared using another mutagenic oligonucleotide with silent mutations in the Ala1 (GCU) and Leu2 (UUG) codons. The mutant was successfully produced at a level of approximately 15% of total protein, with the initial Met completely removed in the bacterial cell. Such an approach could be important in solving similar problems in bacterial production of other toxic proteins.

Lima, C., P. Bianca Clissa, et al. (2003). "Characterisation of local inflammatory response induced by *Thalassophryne nattereri* fish venom in a mouse model of tissue injury." *Toxicon* **42**(5): 499.

<http://www.sciencedirect.com/science/article/B6TCS-49HMS1P-1/2/1204fc67d1441720fc61488796a69425>

The *Thalassophryne nattereri* fish venom induces a severe burning pain, oedema, and necrosis observed both clinically and experimentally. The present study was carried out in order to describe the pattern of local acute inflammatory response after *T. nattereri* venom injection. Our findings show that the edematogenic response induced by *T. nattereri* venom in footpad of mice was dose- and time dependent, and remained significantly elevated over 48 h after injection. Analysis of footpad homogenates were tested for the presence of TNF- α , IL-1 β and IL-6, and demonstrated augmented levels of these cytokines. Our results showed that the injection of venom developed an inadequate cellular inflammatory response evidenced by poor infiltration of mononuclear cells, preceded by decreased number of these cells in peripheral blood. In contrast, we observed an early intense recruitment of neutrophil to peritoneal cavity, accompanied by a significant decrease in the number of mononuclear cells. A drastic increase in the total amount of cells, mainly in neutrophils, followed by mononuclear cell recruitment was observed 24 h. In addition, we also demonstrated that *T. nattereri* venom affects the viability of mononuclear cells (J774A1) in culture. We conclude that the scarcity of inflammatory cellular influx into local lesions (intraplantar) induced by *T. nattereri* venom could be a consequence of an impaired blood flow in venules at injured tissue and cytotoxic effect of the venom on inflammatory cells can contribute to this impairment.

Forcic, D., R. Zgorelec, et al. (2001). "Incidence of hepatitis C virus RNA in anti-HCV negative plasma pools in Croatia." Transfusion and Apheresis Science **24**(3): 269.

<http://www.sciencedirect.com/science/article/B6W7X-43K2PMM-F/2/493f7b1827cca548500415d64502cec8>

Transfusion Science (1)

Legler, T. J., J. Riggert, et al. (1999). "Quantitative PCR for counting residual white blood cells in blood products." Transfusion Science **20**(2): 107.

<http://www.sciencedirect.com/science/article/B6TCT-3WK3RR8-3/2/2bfaa4fe0dfe4bed917a5aac1e99cee2>

Leukocyte depleted blood components are frequently used to reduce alloimmunization and the risk of transfusion transmitted infection. Counting residual white blood cells in filtered blood products requires sensitive and reliable techniques. After separation of white blood cells from 500 [mu]l of 20 non-filtered and 54 filtered blood products we used polymerase chain reaction (PCR) and fluorimetric detection for the quantification of genomic DNA. The results were compared with results from Nageotte chamber counting. The accurate limit of detection of PCR was determined at 1 WBC/[mu]l (intra-assay coefficient of variation: 16.3%). PCR correlated well with Nageotte chamber counts ($r=0.77$, $p=74$). Concordant results were obtained in 51 filtered and 20 non-filtered blood products. Discrepant results were obtained in 3 filtered whole blood units: In these blood products >12 WBC/[mu]l were counted in Nageotte chamber and PCR gave a negative result. After component preparation fresh-frozen plasma and red cell concentrates of these units contained <1 WBC/[mu]l using both methods. In conclusion we describe a quantitative PCR method which had about the same sensitivity and specificity as Nageotte chamber testing. However, PCR is more laborious than the standard method. As well, as reliable PCR testing requires expensive instruments and staff experienced in molecular biology, the standard method is more cost effective.

Transplant Immunology (13)

de Mattos, A. M., M. M. Meyer, et al. (1997). "Interleukin-12 p40 m-RNA expression in human kidney allograft biopsies." Transplant Immunology **5**(3): 199.

<http://www.sciencedirect.com/science/article/B6W6V-436FSD6-4S/2/10ad7b759cbe4bf8f79ff220b2aa2574>

Interleukin-12 (IL-12) is a heterodimeric cytokine implicated in the early differentiation of naive T-lymphocytes into the Th1 subset. IL-12 is important for induction of the cellular immune response against viruses, intracellular parasites and neoplasms. Its role in alloresponsiveness has not been fully elucidated. Preliminary data in the literature point toward the prevalence of Th1 lymphocytes

in processes of allograft rejection. In attempt to further investigate the expression of this cytokine during episodes of cellular rejection of renal allografts, we searched for IL-12 message in human kidney allograft biopsies using the reverse transcriptase-polymerase chain reaction technique. Twenty-three allograft core biopsies from 19 patients were obtained precutaneously for clinical indications in 18 cases, and as part of an investigational protocol in five cases. A portion of the tissue was used for RNA extraction using the guanidium-thiocyanide phenol-chloroform method. Histology was performed on the remaining core material. Ten mg of total RNA were used for reversed transcription. PCR of the c-DNAs was done for 40 cycles using primers for the p40 subunit of IL-12 and GAPDH which was used as a control. PCR products were photographed after electrophoresis, transferred to a nylon membrane and hybridized with a radiolabelled cloned human IL-12 p40 1 kb c-DNA fragment. Autoradiographies were developed after 20-min exposure. All samples were run in triplicate. IL-12 p40 m-RNA was expressed in all 17 biopsies showing acute cellular rejection as well as in all three biopsies showing focal interstitial fibrosis. No message was found in the presence of normal allograft histology. This is the first in vivo report of IL-12 p40 subunit m-RNA expression during renal allograft rejection in humans. The role of this Th1 cytokine in the alloresponse deserves further investigation.

Diamond, L. E., K. R. McCurry, et al. (1995). "Human CD59 expressed in transgenic mouse hearts inhibits the activation of complement." *Transplant Immunology* 3(4): 305.

<http://www.sciencedirect.com/science/article/B6W6V-436FRRN-1B/2/30f98d3a8b21b5eab25ea9475255db2f>

Porcine-to-human xenotransplantation offers a potential solution to the critical shortage of human organs. The major immunological barrier to xenotransplantation between these species is a rapid rejection process mediated by preformed natural antibodies and complement. Xenogeneic organ grafts are especially susceptible to complement mediated injury because complement regulatory proteins, which ordinarily protect cells from inadvertent injury during the activation of complement, function poorly in regulating activation of heterologous complement. Removal of xenoreactive antibodies or systemic inhibition of complement activity has been shown to prolong graft survival. As an alternative to the systemic inhibition of complement activity, we have established a model system using transgenic animals to test whether the expression of human membrane bound complement regulatory proteins on mouse endothelial cells can inhibit the activation of human complement. CD59, which acts at the terminal stage of complement activation by inhibiting the formation of the membrane attack complex, was used as a paradigm for this model. A CD59 construct containing the putative CD59 gene promoter linked to the CD59 coding region was used to demonstrate expression of the human CD59 protein in various tissues of transgenic mice, including endothelial cells in the heart. In addition, we show that the transgenic CD59 protein is biologically active as determined by the ability to inhibit the formation of membrane attack complex in transgenic mouse hearts perfused ex vivo with human plasma. These results demonstrate that expression of membrane bound complement regulatory proteins can achieve complement inhibition in a xenogeneic organ and suggest that this approach may be useful for successful xenotransplantation between discordant species.

Doumaid, K., P. P. M. C. van Miert, et al. (2000). "Modulation of the T cell receptor beta chain repertoire after heart transplantation." *Transplant Immunology* 8(2): 83.

<http://www.sciencedirect.com/science/article/B6W6V-414N51F-1/2/11487ee88e54a7f5c3450ee2790ae05b>

Background: In a previous study it was shown that pre-transplant blood transfusion was associated with a better clinical outcome after heart transplantation (HTx). In this study the effect

of heart transplantation (HTx) on the T cell receptor V beta chain (TCRV[beta]) repertoire was investigated. Therefore, we analyzed the TCRV[beta] repertoire of patients after HTx to see whether a correlation with clinical outcome could be observed. Methods: Patients were analyzed at four different time points: pre-HTx, less than 1 month post-Htx, between 1 month and 2.5 month post-Htx and more than 2.5 months post-HTx. CD4+ and CD8+ T cells were purified from patient peripheral blood mononuclear cells (PBMC). TCR beta chain usage was analyzed semiquantitatively by Southern blot analysis. Results: HTx affected the TCRV[beta] repertoire in both the CD4+ and CD8+ T cell compartments in all patients. Changes in the TCRV[beta] repertoire were most pronounced within the CD8+ T cell subset. Interestingly, one patient showed modulation in TCRV[beta] chain usage predominantly in the CD4+ T cell compartment. Conclusions: Modulation of TCRV[beta] chain usage was detected in all patients analyzed. No clear-cut relation was observed between TCRV[beta] modulation after transplantation and clinical outcome. In some cases modulations appeared to concur with observed immunological events (clinically and/or in-vitro).

Ermolli, M., M. Schumacher, et al. (2003). "Differential expression of MMP-2/MMP-9 and potential benefit of an MMP inhibitor in experimental acute kidney allograft rejection." Transplant Immunology **11**(2): 137.

<http://www.sciencedirect.com/science/article/B6W6V-46RF030-3/2/f248b8f2f4f5cc79ecd57618679c9011>

Acute cellular allograft rejection is characterized by leukocyte invasion and tissue destruction, associated with qualitative and quantitative alterations in the extracellular matrix (ECM) compartment. Metabolism of ECM proteins is mainly regulated by matrix metalloproteinases (MMP), that are zinc depended endoproteinases. MMP, especially basement membrane degrading MMP-2 and MMP-9, also facilitate tissue invasion of leukocytes. In addition, MMP-2 exerts a direct pro-inflammatory effect upon glomerular mesangial cells. Therefore, the investigation of the role of MMP in transplant rejection may lead to novel approaches in the therapy of rejection processes. To our knowledge, this is the first study of acute allograft rejection, formally addressing expression and activity of MMP, including the effect of a MMP inhibiting agent. For our studies, we used the orthotopic kidney allograft model in the stringent Dark Agouti-to-Lewis rat strain combination. Animals were divided into four groups: group A, healthy untreated Lewis rats (n=3); group B, sham operated Lewis rats (n=3); group C, transplanted Lewis rats treated with vehicle solution only (n=12); group D, transplanted Lewis rats treated with MMP inhibitor BB-94 (n=12). Respective animals were treated once daily intraperitoneally with BB-94 (30 mg/kg) or vehicle solution only. Treatment lasted from the third preoperative day until the end of the experiment, the time of severe rejection at day +7. Acute kidney allograft rejection led to alterations in the expression and activity of MMP. Overall MMP activity slightly increased despite severe destruction of kidney histology. The MMP inhibitor BB-94 successfully inhibited MMP activity to a high extent. MMP expression did not show uniform findings, since acute rejection led to differential expression of MMP-2 and MMP-9. During the rejection process, MMP-9 showed a small but significant increase, whereas MMP-2 production decreased substantially. Interestingly, BB-94 was able to keep proteinuria at a low level in transplanted animals. In conclusion, MMP--especially MMP-9--appear to represent new mediators involved in acute kidney transplant rejection.

Holweg, C. T. J., A. H. M. M. Balk, et al. (2004). "Intragraft heme oxygenase-1 and coronary artery disease after heart transplantation." Transplant Immunology **13**(4): 265.

<http://www.sciencedirect.com/science/article/B6W6V-4DBJ85M-1/2/fcde1cac16f9310f369939865be981d6>

Peri-operative tissue injury triggers the development of Transplant Coronary Artery Disease (TCAD). Animal studies have shown that induction of heme oxygenase (HO)-1 protects the donor organ from the development of TCAD. To investigate the role of HO-1 in TCAD after clinical heart transplantation, we measured intragraft mRNA expression of HO-1, HIF-1[alpha], TGF-[beta], FLIP, and the Bcl-2/Bax balance. Immunohistochemical staining of HO-1 was performed to determine its origin. Myocardial biopsies taken at the end of the transplantation procedure (time 0), at 1 week and at 10 months after transplantation were studied from recipients with or without angiographic signs of accelerated TCAD, diagnosed after 1 year. At time 0, no differences in mRNA expression for any of the measured parameters were found between TCAD positive and negative patients. At 1 week, mRNA expression of HO-1 and TGF-[beta] was higher in grafts that developed accelerated TCAD ($p=0.001$ and $p=0.0002$). These higher mRNA levels were accompanied by a pro-apoptotic shift in Bcl-2/Bax ($p=0.02$), suggesting proneness for apoptosis via the mitochondrial pathway. Immunohistochemical staining showed that HO-1 was mainly produced by infiltrating macrophages. At 10 months, again HO-1 and TGF-[beta] levels were high in TCAD positive patients ($p=0.02$ and $p=0.05$), but the expression of apoptotic markers was comparable at this time point. Our results suggest that a higher HO-1 by macrophages in our patient population might be an adaptive response to tissue injury and inflammation, reflecting damage due to the transplantation procedure that finally results in TCAD.

Holweg, C. T. J., A. M. A. Peeters, et al. (2003). "Recipient gene polymorphisms in the Th-1 cytokines IL-2 and IFN-[gamma] in relation to acute rejection and graft vascular disease after clinical heart transplantation." *Transplant Immunology* **11**(1): 121.

<http://www.sciencedirect.com/science/article/B6W6V-46NX397-4/2/949f1e99cfc9fd7fd8c270550649beb4>

IL-2 and IFN-[gamma] are associated with acute rejection (AR) and graft vascular disease (GVD) after clinical heart transplantation. Polymorphisms in the genes of IL-2 (T-330G in the promoter) and IFN-[gamma] (CA repeat in the first intron) influence the production levels of these cytokines. Therefore, these polymorphisms might have an effect on the outcome after transplantation. To investigate possible effects of genetic variations in IL-2 and IFN-[gamma] genes on AR and GVD, we analyzed the IL-2 T-330G and the IFN-[gamma] CA repeat polymorphism in DNA of 301 heart transplant recipients. No associations were found for allele or genotype distributions between patients with or without AR (IL-2 allele frequency: $P=0.44$, genotype distribution: $P=0.46$; IFN-[gamma] allele frequency $P=0.10$, genotype distribution 12 repeats allele: $P=0.21$). Also, no associations were found analyzing the number (0 vs. 1 vs. [ges]1) of AR (IL-2 allele frequency: $P=0.59$; genotype distribution: $P=0.37$; IFN-[gamma] allele frequency: $P=0.27$, genotype distribution 12 repeats allele: $P=0.41$) or analyzing the polymorphisms in patients with AR within the first month or thereafter (IL-2 allele frequency: $P=0.45$, genotype distribution: $P=0.38$; IFN-[gamma] allele frequency: $P=0.21$, genotype distribution 12 repeats allele: $P=0.41$). Analyzing both polymorphisms in relation to GVD, resulted in comparable allele and genotype distributions (IL-2 allele frequency: $P=0.75$; genotype distribution: $P=0.77$; IFN-[gamma] allele frequency: $P=0.70$, genotype distribution 12 repeats allele: $P=0.63$). In conclusion, we did not detect an association between the IL-2 T-330G promoter polymorphism and CA repeat polymorphism in the first intron of the IFN-[gamma] gene and AR or GVD after heart transplantation.

Kovarik, J., M. Koulmanda, et al. (1997). "The role of cytokines during rejection of foetal pig and foetal mouse pancreas grafts in nonobese diabetic mice." *Transplant Immunology* **5**(4): 307.

<http://www.sciencedirect.com/science/article/B6W6V-436FSJ9-5H/2/054cb171b5951d0376c896011a762401>

The rejection of discordant foetal pig islet xenografts in nonimmunosuppressed nonobese diabetic (NOD) mice is dominated by polymorphonuclear cell infiltration whereas allografts are almost exclusively infiltrated by mononuclear cells. To determine if this variation is due to different proinflammatory factors generated at the graft site, we analysed graft-site mRNA expression of various cytokines, and the eosinophil attractant chemokine, eotaxin, in a renal subcapsular islet transplant model using organ cultured foetal pig (xenograft) and foetal BALB/c (allograft) pancreas in prediabetic NOD mice. Using semiquantitative RT-PCR on samples recovered at multiple time points during the first 15 post-transplantation days from mice transplanted with either allogeneic or xenogeneic tissue, we found increased expression of IL-2, IL-4, TNF-[beta] and IL-10 mRNAs at the peak of the cellular infiltrate (on day 5) in both xenografts and allografts but, in contrast to the allografts, no enhanced transcription of IFN-[gamma] mRNA in the rejecting xenografts. When an allograft and a xenograft were placed at the opposite pole of the same kidney the histological appearance of the rejecting allograft site resembled the xenograft site with significant numbers of eosinophils in both, and enhanced expression of eotaxin and iNOS. Additionally, the xenograft response, unlike the allograft response, was marked by an early increased expression of TNF-[alpha] and IL-6 (day 3) and an almost complete absence of IFN-[gamma] expression. The results suggest a distinct cell-mediated mechanism for rejection of local pancreas xenografts compared to the rejection of foetal pancreas allografts.

Roman, P. (2002). "Platelet antigens. The role of human platelet alloantigens (HPA) in blood transfusion and transplantation." Transplant Immunology **10**(2-3): 165.

<http://www.sciencedirect.com/science/article/B6W6V-45TY3YH-H/2/dbdd2db806a1469824b30238183bf8ac>

In this review, we describe the platelet surface molecules with special focus on the polymorphic glycoproteins giving rise to the human platelet alloantigen (HPA) system. We list the platelet glycoprotein complexes GPIa/IIa, GPIIb/IIIa, GPIb/V/IX and some other molecules, the corresponding genes that encode them and we describe their polymorphisms as well as their physiological function. Based on data obtained by serological and molecular methods, we explain how ancestral HPA alloepitopes have developed into the modern variants. We also describe the tissue distribution of these proteins, which seems to be wider than thought initially, and discuss the significance of the HPA encoding genes distribution in various populations. Methods for their determination are then described briefly. Since HPA alloepitopes can induce antibodies with resulting clinical conditions such as: post-transfusion refractoriness to platelets (PTR); post-transfusion thrombocytopenic purpura (PTTP); and fetomaternal alloimmune thrombocytopenia (FMAIT), the mechanism of this alloimmunization and its prevention is described. Although the humoral arm is more important from the clinical viewpoint, the activation of the cytotoxic arm by HPA alloepitopes is also possible. These polymorphisms also seem to have a role in certain other clinical circumstances, therefore their impact on cardiovascular diseases and haemostatic disorders as well as their role in the transplantation of solid organs and bone marrow is addressed. We conclude that during the last decades, the research of the platelet membrane molecules contributed considerably to the diagnostics, prevention and therapy of the blood coagulation and haemostatic disorders, to the management of the neonatal thrombocytopenias, transfusion-related thrombocytopenias, refractoriness to platelet transfusions and autoimmune disorders. It also changed our view on the role of HPA alloepitopes in bone marrow and solid organ transplantation. In the future, this accumulated knowledge will be useful for the development of the cell-based therapies and immune modulation of both acquired and hereditary diseases.

Shoker, A. S., Z.-R. Lun, et al. (2002). "Cytokine gene expression by alloactivated cells in SCID mice." Transplant Immunology **10**(4): 311.

<http://www.sciencedirect.com/science/article/B6W6V-4645DB6-1/2/703aeb7a61289f7d63ca726e6102b278>

Objectives: The severe combined immune deficient (SCID) mouse provides a neutral environment to study human immune responses. We therefore tested human gene expression of Interleukin (IL) 2, 4 and 10, interferon gamma (IFN[gamma]); transforming growth factor beta 1 (TGF[beta]1); and CD40 ligand (CD40L) in splenic extracts of SCID mice after engraftment of PBLs from two persons (direct MLR) or one person plus allopeptides (indirect MLR) in the presence or absence of cyclosporin A (CsA) or FK506. **Methods:** Cytokine gene expression was detected by RT and quantitative (for IFN[gamma], TGF[beta]1 and CD40L) PCR. All cells, allopeptides, CsA (25 mg/kg/day for 7 days) or FK 506 (0.5 mg/kg/day for 7 days) were administered intraperitoneally (IP). **Results:** In both direct and indirect MLR the numbers of SCID mice expressing the human cytokine genes varied between 33% for IL4 and 100% for IL10, IFN[gamma], TGF[beta]1, and CD40L. There was significant interpersonal variation in levels of gene expression. Concomitant CsA or FK506 administration for 7 days did not abrogate early or late (1 week after discontinuation of CsA or FK506) cytokine gene expression in either the direct or indirect MLR, but paradoxically enhanced levels of IFN[gamma], TGF[beta]1 and CD40L gene expression in some experiments. **Conclusions:** The results explain late rejection after rapid calcineurin inhibitor withdrawal or reduction, and illustrate the potential use of SCID mice as a surrogate model to study graft outcome by determination levels of gene expression and sensitivity to immunosuppressive agents in the in vivo alloresponse.

Shoker, A. S., Z.-R. Lun, et al. (2001). "Analysis of the CD40/CD40L role in the sustenance of alloreactive antibody production." *Transplant Immunology* 8(4): 219.

<http://www.sciencedirect.com/science/article/B6W6V-42JHD55-1/2/8cd26458bf2a034d07e1dde729b29529>

CD40 ligand (CD40L) is important for T/B lymphocyte interaction. To understand the cellular basis of humoral allosensitization we, therefore: (1) measured CD40L protein and gene expression in sensitized and non-sensitized uremic unactivated peripheral CD4+ T lymphocytes; (2) studied the impact of blocking the CD40/CD40L pathway on alloreactive antibody (allo-Ab) production by engrafted sensitized PBLs into severe combined immunodeficient (SCID) mice after in vitro preactivation with IL2/LPs/HLA class II allopeptides and adjuvants as a potent stimulus to produce allo-Ab (Shoker et al. Transplantation 1999;68;1188); and (3) studied the modifying effect of CD40/CD40L blockade on T helper type I and II cytokine gene expression in the respective mice spleen. The CD40L protein was measured by flow cytometry and the gene expression was measured by quantitative RT-PCR. Alloreactive antibodies (alo-Abs) produced by sensitized PBLs engrafted into SCID mice with and without blockade of the CD40 receptor were measured by the PRA-STAT ELISA method. The modifying effects of CD40 blocking on allo-Ab production and cytokine gene expression by the engrafted cells measured by RT-PCR were then compared. There was no detectable CD40L protein expression in either the uremic or the control groups. The CD40L gene expression of 0.04 ± 0.02 attomoles (aM) in the sensitized group was significantly higher than in the non-sensitized patients (0.009 ± 0.007 aM, P+ T cells (0.016 ± 0.004 aM, Pn=10); decreased the mean $\pm$ S.D. optic density of allo-Ab to 0.1 ± 0.13 and the mean $\pm$ S.D. PRA to 12 ± 16). In the presence of the control Ab, allo-Ab production in SCID sera was present in 100% of the 10 SCID mice tested; the mean $\pm$ S.D. PRA was 75 ± 20 , and the mean $\pm$ S.D. OD activity was 0.412 ± 0.17 . All cytokine genes were, otherwise, expressed in the presence or absence of CD40 blockade. The results suggest a potential role of an enhanced CD40/CD40L interaction in the sustenance of alloreactive antibody production without significant deviation to T helper-like I or II responses. Blocking the CD40/CD40L pathway may have a potential therapeutic benefit to treat sensitized uremic patients.

Takatsuki, M., S. Uemoto, et al. (2001). "Analysis of alloreactivity and intra-graft cytokine profiles in living donor liver transplant recipients with graft acceptance." Transplant Immunology 8(4): 279.

<http://www.sciencedirect.com/science/article/B6W6V-42JHD55-8/2/bfdea831c17749443cddb058edbef1b>

Although some previous studies have indicated the possibility of immunosuppression withdrawal in clinical liver transplantation, the mechanism of graft acceptance is not clear. The aim of this study is to elucidate the alloreactivity against the donor and intra-graft cytokine profiles in living donor liver transplant (LDLT) recipients with graft acceptance. In October 1999, we had 23 patients who survived without immunosuppression after LDLT with a median drug-free period of 25 months (range: 3-69 months). They consisted of six patients who were electively weaned by an elective weaning protocol and 17 either forcibly or accidentally weaned patients due to various causes but mainly due to infection. We evaluated the alloreactivity against the donor in these patients by a mixed lymphocyte reaction and intra-graft cytokine profiles by real-time reverse transcriptase-polymerase chain reaction. The development of donor-specific hyporeactivity was observed in the patients with graft acceptance. The cytokine pattern in the supernatant of the culture medium revealed a down regulation of T helper (Th) 1 cytokine INF [gamma] against the donor while no significant difference was seen in Th2 cytokine IL-10. Regarding the intra-graft cytokine profiles, we could find no amplification of Th1 cytokines (IL-2, INF [gamma]) and IL-4 while some of the patients revealed a gene expression of IL-10 with no significant difference from that of the normal, untransplanted liver specimen. In addition, no difference was observed in any other cytokines (IL-1[beta], IL-8, IL-15, TNF[alpha]) compared with those of the normal controls. We propose that the down regulation of Th1 cytokine is one possible mechanism of graft acceptance in LDLT recipients.

Toyoda, M., K. Galfayan, et al. (1997). "Cytomegalovirus infection induces anti-endothelial cell antibodies in cardiac and renal allograft recipients." Transplant Immunology 5(2): 104.

<http://www.sciencedirect.com/science/article/B6W6V-436FS91-46/2/36c87014cc584b1abb38c428b08286f3>

Cytomegalovirus (CMV) infection represents a significant morbidity factor for transplant recipients. CMV infection has an association with the development of allograft rejection (AR) through graft endothelial cell (EC) damage, but the mechanisms are not yet clear. There are few reports addressing the role of humoral immunity in vascular EC injury mediated CMV infection whereas many reports are available regarding the mechanism(s) of CMV-associated allograft EC injury mediated by cellular immunity. Here we examine the incidence of CMV infection in 40 cardiac and 25 renal allograft recipients using polymerase chain reaction (PCR) techniques. We also monitored sera for the development of anti-EC antibodies (AECA) using an ELISA with human umbilical vein ECs as targets, and IL-2 levels using an ELISA. AECA levels (immunoglobulin-G and immunoglobulin-M) were significantly elevated in allograft recipients who demonstrated CMV-PCR positivity when compared with the CMV-PCR negative group (IgG: 23.1 $\pm$ 16.4 vs 4.7 $\pm$ 4.5, p [tau]0.0001; IgM: 47.0 $\pm$ 53.6 vs. 7.0 $\pm$ 11.2, p[tau]0.0001). Serum AECA (IgG and IgM) levels increased one to four weeks after CMV DNA was detected and elevated AECA levels persisted for at least one to two months, and sometimes for several months. Elevated AECA levels correlated well with serum IL-2 levels. These results suggest that CMV infection is associated with an increased humoral immune response to EC antigens, which may be a risk factor for vascular rejection, chronic rejection and decreased allograft survival.

Zuo, X.-J., Y. Matsumura, et al. (1995). "Cytokine gene expression in rejecting and tolerant rat lung allograft models: analysis by RT-PCR." Transplant Immunology 3(2): 151.

Cytokine gene expression is a critical component of the lung allograft rejection (AR) response and tolerance development in rat models. In order to determine the specificity of cytokine gene expression for AR and tolerance, we examined cytokine (interleukin-2) (IL-2), (gamma-interferon) ([gamma]-IFN), IL-4, IL-10 and tumor necrosis factor-[alpha] (TNF-[alpha]) and control (cyclophilin) mRNA levels in two models of rat lung allograft rejection by RT-PCR (reverse transcriptase polymerase chain reaction), Southern blotting. The first model (WKY->F344) develops a mild to moderate lymphocytic infiltrate on days 14-21 post-transplant (stage II-III AR), which spontaneously resolves by day 35 post-transplant with subsequent development of allograft tolerance (grafts surviving without evidence of AR for > 140 days). Conversely, F344->WKY develops a similar lymphocytic infiltrate by day 14 post-transplant, but by day 21 post-transplant the graft shows severe AR (stage III-IV) and has haemorrhagic infarction with alveolar haemorrhage. Methods: RNA was extracted from allografts removed on days 3, 7, 13, 21, 35 and 42 post-transplant. Five animals for each group (WKY->F344) and F344->WKY) were examined at each time point, except that no animals in the F344->WKY were examined on day 42. cDNA was synthesized from total extracted RNA and primers specific for rat TNF-[alpha], rat IL-2, rat [gamma]-IFN, rat IL-4, rat IL-10 and rat cyclophilin were used for gene-specific amplification. (TNF-[alpha], [gamma]-IFN, IL-10, 20 cycles; IL-2, IL-4, 30 cycles; cyclophilin, 20 cycles). The cycles numbers chosen for comparison were found to be optimal during preliminary experiments and occurred during the exponential phase of amplification. PCR products were electrophoresed on a polyacrylamide gel and silver-stained. Gels were subsequently electrotransferred to nylon membranes which were probed with murine cDNAs specific for IL-2, [gamma]-IFN IL-4, IL-10 and TNF-[gamma]. Results: Cyclophilin gene expression was similar for both models at all time points tested; this also served as an internal standard for RT-PCR. In the WKY->F344 tolerance model, TNF-[alpha] mRNA levels were not detectable on days 3 and 7 post-transplant, were at very low levels on day 14 and were undetectable on day 21 post-transplant. In marked contrast, the F344->WKY rejection model showed TNF-[alpha] mRNA present on day 3 which increased markedly on day 7 and peaked on day 14 post-transplant. TNF-[alpha] mRNA levels decreased on days 21 and 35 post-transplant, a time when the lung was undergoing AR. The pattern of IL-2 and [gamma]-IFN mRNA expression was similar to that for TNF-[alpha]. However, IL-2 mRNA was clearly detectable in the WKY->F344 tolerance model on day 7 and [gamma]-IFN was not present until day 14 post-transplant. The F344->WKY rejection model showed very high levels of IL-2 and [gamma]-IFN on day 3 which peaked on day 14. The ratio of IL-2/IL 10 in the F344->WKY rejection model was more than five times that seen in the WKY->F344 tolerance model on day 3 (p WKY rejection model than in the WKY->F344 tolerance model (p F344 tolerance model than in the rejection model (p Conclusions: 1) The WKY->F344 tolerance model develops mild to moderate lymphocytic infiltrates on day 14 which is associated with low level IL-2, [gamma]-IFN and TNF-[alpha] gene expression. IL-10 and IL-4 are present at day 3; however, by day 14, IL-10 is the predominantly expressed Th2 cytokine and IL-4 is not expressed. The infiltrates ultimately resolve and the animals develop a functional tolerance to their grafts. 2) The F344->WKY rejection model shows similar lymphocytic infiltrates on day 14, but diverges to a severe rejection which is associated with very early and high levels of IL-2, IL-4, [gamma]-IFN and TNF-[alpha] mRNA expression. IL-10 is not expressed on day 3, and only poorly expressed on day 14. 3) These data suggest that delayed and low level expression of cytokines can be seen in animals developing tolerance to their grafts while early and abundant expression of cytokines (IL-2, IL-4, [gamma]-IFN and TNF-[alpha]) is associated with AR and ultimate graft loss. It also appears that, in this system, IL-10 expression is critical for development of tolerance while IL-4 expression occurs early in both the rejection and tolerant models, but it expressed poorly in the tolerant models on day 14.

Transplantation Proceedings (9)

Adorno, D., A. Canossi, et al. (1999). "The role of [beta]-pleated sheet DRB1 differences in acute rejection after cadaveric renal transplant." Transplantation Proceedings **31**(1-2): 730.

<http://www.sciencedirect.com/science/article/B6VJ0-3W9KWTk-CD/2/762c7bd20180a9f9a434ccf3f0cc583e>

Behboo, R., C. Ricordi, et al. (1997). "Aminoguanidine inhibits the generation of nitric oxide in vitro and prolongs islet xenograft survival in rats." Transplantation Proceedings **29**(4): 2152.

<http://www.sciencedirect.com/science/article/B6VJ0-3X2B8S5-7G/2/a1ff887787c18d45657a04086f495a3>

Fischer, L., M. Sterneck, et al. (1999). "Hepatitis G virus infection in liver transplant recipients." Transplantation Proceedings **31**(1-2): 496.

<http://www.sciencedirect.com/science/article/B6VJ0-3W9KWTk-7P/2/6b4f41d4cef57e746d563d8f64e5d4a4>

Fukuda, Y., S. Hoshino, et al. (2001). "HLA-DMB polymorphism in living-related kidney transplantation." Transplantation Proceedings **33**(7-8): 3304.

<http://www.sciencedirect.com/science/article/B6VJ0-44N9JF0-3V/2/be7fc0c09fc1dd548310681548543e67>

Lazzereschi, D., M. Forni, et al. (2000). "Efficiency of transgenesis using sperm-mediated gene transfer: generation of hDAF transgenic pigs." Transplantation Proceedings **32**(5): 892.

<http://www.sciencedirect.com/science/article/B6VJ0-40WDV4D-13/2/62d4f2fe60a8491e9f0bcd049c5637a7>

Nakajima, H. and T. Oka (1997). "Analysis of biochemical and biological functions of Fas-Ligand (FasL) and fas on activated T cells in allo-immune response." Transplantation Proceedings **29**(1-2): 1096.

<http://www.sciencedirect.com/science/article/B6VJ0-3VN9BV8-1D/2/c4584f01210976fd9f5ef8d90f63f280>

Rangel, E. B., L. A. Moura, et al. (2004). "Expression of cyclooxygenases during renal allograft rejection." Transplantation Proceedings **36**(4): 838.

<http://www.sciencedirect.com/science/article/B6VJ0-4CKJVPX-G/2/ffb82bc6c324ec525600051754e99a9b>

The aim of this work was to determine the expression of cyclooxygenases (COX-1 and COX-2) during acute human renal allograft rejection. RT-PCR and immunohistochemistry were performed. The COX-2 mRNA was more abundant than COX-1 mRNA in the group with acute rejection ($P = .04$). COX-2 protein was more abundant than COX-1 protein in the group with acute rejection, including podocytes ($P = .003$), and interstitial cells ($P < .001$). In conclusion, COX-2 which is up-regulated during acute human renal allograft rejection, may play a role in renal inflammation.

Takada, M., H. Toyama, et al. (2004). "Augmentation of interleukin-10 in pancreatic islets after brain death." Transplantation Proceedings **36**(5): 1534.

<http://www.sciencedirect.com/science/article/B6VJ0-4CV04HC-2Y/2/03294eeaac0dd59ba74b9cc1c32a30cd>

Clinical islet transplantation is now established as a treatment for patients with type I diabetes. Although organs from brain-dead (BD) donors are the main source for clinical transplantation, marginal status after BD produces deterioration of the organs followed by molecular activation. The effect of brain-death (BD) induction on the immunological status of donor islets was investigated using a rodent model of BD. BD animals showed decreased levels of peripheral white blood cells (WBC) compared to controls, indicating the extravasation of these cells (7270 $\pm$ 500 vs 9570 $\pm$ 370, respectively). In a densitometric study of RT-PCR products, the Th2 cytokine (IL-10) was significantly up-regulated in BD (2.91 $\pm$ 0.26 vs 1.76 $\pm$ 0.40), but a Th1 cytokine (IL-2) showed minimal change. Increased expression of IL-10 may inhibit macrophage function. As the marginal status after BD deteriorates, the islets of these donors may display early graft loss or poor long-term function. Integrative studies of immunomodulation might be necessary to eliminate islet infiltrates.

Zhang, H. Q., H. Lu, et al. (2002). "Identification of novel genes associated with immunosuppression in renal transplant patients." Transplantation Proceedings **34**(7): 2733.

<http://www.sciencedirect.com/science/article/B6VJ0-475HWXG-33/2/247cb29c3bf5d4a0488d0ea2ed7c0be4>

Trends in Genetics (2)

Czank, A. (1996). "One-tube direct PCR from whole *Drosophila melanogaster* adults." Trends in Genetics **12**(11): 457.

<http://www.sciencedirect.com/science/article/B6TCY-4C89G52-D/2/4caa610d0886ded94e7e6386a1ba5188>

Illuxley, C., E. D. Green, et al. (1990). "Rapid assessment of *S. cerevisiae* mating type by PCR." Trends in Genetics **6**: 236.

<http://www.sciencedirect.com/science/article/B6TCY-47DVCJN-6W/2/7a3fcd38382e85a1427fa1c35366ec1>

Tubercle and Lung Disease (1)

Miorner, H., U. Sjobring, et al. (1995). "Diagnosis of tuberculous meningitis: A comparative analysis of 3 immunoassays, an immune complex assay and the polymerase chain reaction." Tubercle and Lung Disease **76**(5): 381.

<http://www.sciencedirect.com/science/article/B6WXJ-4D6XP4P-3/2/51098ba49d1f656d04e4cb38a6ea1e0f>

Objective: To compare 3 immunoassays, an immune complex assay, and an application of the polymerase chain reaction (PCR) for the diagnosis of tuberculous meningitis (TBM). Material: Cerebrospinal fluid (CSF) from 33 patients with TBM and from 34 control patients with infectious and non-infectious CNS diseases was analysed. Results: The antibody immunoassays were either nonspecific or insensitive. However, detection of mycobacterial IgG immune complexes correlated strongly with infection, as they were detected in the CSF from 64% of the patients with TBM compared to only 3 (9%) of the control samples. PCR analysis, using *Mycobacterium tuberculosis*-specific oligonucleotide primers, also strongly correlated with infection, as DNA was amplified from 54% of the samples from patients with TBM, but from only 2 (6%) of the control samples. Both 'false positive' samples were also positive in the immune complex assay and came from 2 patients with otogenic brain abscesses. It is conceivable that these patients suffered from otogenic tuberculosis with secondary non-mycobacterial meningitis. When combining the immune complex assay with DNA-detection by PCR, 100% of the culture positive and 74% of culture negative samples were found to be positive, while maintaining a high specificity. Conclusion: Parallel analysis to detect mycobacterial immune complexes and *M. tuberculosis*-specific DNA by PCR from the CSF of patients may offer a sensitive and specific tool for the diagnosis of TBM.

Tuberculosis (4)

Badewa, A. P., L. J. Quinton, et al. "Chemokine receptor 5 and its ligands in the immune response to murine tuberculosis." Tuberculosis In Press, Corrected Proof

<http://www.sciencedirect.com/science/article/B6W XK-4FRKVJR-2/2/9f54e8034d300928d820472abfa51e7c>

SummarySetting: The ability of chemokines such as macrophage inflammatory protein (MIP)-1[alpha], MIP-1[beta], and regulated-upon-activation, normal T cell expressed and secreted (RANTES), to attract and activate T cells and monocytes, the building blocks of the granuloma, suggests that these chemokines may have a role in modulating immune responses to

Mycobacterium tuberculosis infection. Objective: We hypothesized that the chemokine receptor 5 (CCR5) ligands, MIP-1[alpha], MIP-1[beta] and RANTES, are virulence correlates in M. tuberculosis infection and are indispensable to granuloma formation. Design: The ability of virulent (H37Rv) and avirulent (H37Ra) strains of M. tuberculosis to induce chemokine production in vivo and in vitro was determined at protein and mRNA levels. We also compared bacterial burden, and granuloma numbers and size in H37Rv-infected CCR5-/- or wild-type C57BL/6 mice. Results: In vivo, lung mRNA and protein measurements of MIP-1[alpha], MIP-1[beta] and RANTES indicate significantly higher (p values (days 14-28) in the H37Rv-infected than the H37Ra-infected mice. This is consistent with a higher infection burden of the virulent strain. However, in vitro alveolar macrophage stimulation by H37Rv or H37Ra yielded no significant differences in production of the three chemokines at all time points. Histological analysis of granulomas did not show any significant differences in granuloma numbers, size and M. tuberculosis growth in CCR5-/- compared to wild-type mice. Conclusions: The production of the CCR5 ligands, MIP-1[alpha], MIP-1[beta], and RANTES, does not clearly correlate with virulence of M. tuberculosis. These ligands and their receptors may not be indispensable to the development of granulomas in murine tuberculosis.

Inwald, J., K. Jahans, et al. (2003). "Inactivation of the Mycobacterium bovis homologue of the polymorphic RD1 gene Rv3879c (Mb3909c) does not affect virulence." Tuberculosis **83**(6): 387.

<http://www.sciencedirect.com/science/article/B6WXK-4B0KWVV-3/2/adeef1a017b1c9ddc81b9d54350279d4>

The RD1 locus is deleted from all strains of Mycobacterium bovis BCG but present in virulent isolates of M. bovis and Mycobacterium tuberculosis. The RD1 gene Rv3879c encodes a proline- and alanine-rich protein that shows sequence polymorphism across members of the M. tuberculosis complex. The role of this protein in virulence was investigated by deleting the Rv3879c homologue from M. bovis (Mb3909c) and testing the virulence of the mutant in the guinea pig model. The M. bovis [Delta]Mb3909c mutant was not attenuated in the guinea pig model, showing that this gene does not encode a virulence factor and plays no role in the attenuation caused by loss of RD1.

Jureen, P., J. Werngren, et al. (2004). "Evaluation of the line probe assay (LiPA) for rapid detection of rifampicin resistance in Mycobacterium tuberculosis." Tuberculosis **84**(5): 311.

<http://www.sciencedirect.com/science/article/B6WXK-4BRB74S-1/2/3eab7d0e8d31f1e55705ddc237f53856>

Resistant Mycobacterium tuberculosis has become a serious threat to public health. In order to inhibit spreading and give effective treatment, it is of great importance, as early as possible, to detect drug-resistant bacteria. To evaluate the usefulness of the Line Probe Assay (INNO-LiPATM Rif.TB) for rapid detection of rifampicin resistance, we used 52 clinical isolates of M. tuberculosis from the national strain collection at the Swedish Institute for Infectious Disease Control and the drug susceptible reference strain H37Rv. By using BACTEC 460 methodology, 27 of these strains were determined as resistant to rifampicin and 26 as sensitive to rifampicin. Mutations known to give resistance to rifampicin were detected by LiPA in all 27 rifampicin-resistant strains. Among the 26 susceptible strains, 24 had the wild-type pattern in LiPA, while in two, mutations were seen. The LiPA correctly identified the M. tuberculosis complex in all samples. The high accuracy and simplicity of LiPA makes it a very promising method for the early identification of rifampicin resistance in M. tuberculosis.

Wedlock, D. N., M. A. Skinner, et al. (2003). "Vaccination with DNA vaccines encoding MPB70 or MPB83 or a MPB70 DNA prime-protein boost does not protect cattle against bovine tuberculosis." Tuberculosis **83**(6): 339.

<http://www.sciencedirect.com/science/article/B6W XK-49FGP7B-2/2/85fb80117c918d3c07af2e7b6614493e>

Setting: Bovine tuberculosis is a problem in a number of countries and protection of cattle by vaccination could be an important control strategy. Objectives: To determine the ability of DNA vaccines, which express the mycobacterial antigens MPB83 and MPB70 and a DNA prime-protein boost strategy to stimulate immune responses in cattle and protect against bovine tuberculosis. Design: Groups of cattle (n=10) were vaccinated with MPB83 DNA, MPB70 DNA, or MPB70 DNA followed by MPB70 protein or injected with BCG or control plasmid DNA. Animals were challenged intratracheally with virulent *Mycobacterium bovis* at 13 weeks and protection assessed 17 weeks later at postmortem. Results: In contrast to the strong cellular immune responses induced by BCG, the DNA vaccines induced minimal interferon-gamma (IFN- γ) and interleukin-2 (IL-2) responses. Cattle primed with MPB70 DNA and boosted with MPB70 protein induced a strong antibody response and a weak IFN- γ response. BCG gave significant reduction in four pathological parameters of disease while the DNA vaccines and MPB70 DNA/protein did not protect animals against challenge with *M. bovis*. Moreover, cattle vaccinated with MPB70 DNA/protein had a significantly higher proportion of animals with severe lung lesions (>100 lesions) than the MPB70 DNA alone or the control group. Increased bovine PPD-specific IL-4 mRNA expression in cattle, post-challenge, correlated with the presence of tuberculous lung lesions. Conclusion: Vaccination of calves with MPB70 or MPB83 DNA vaccines or with a more immunogenic MPB70 DNA prime-protein boost strategy did not induce protection against bovine tuberculosis.

Ultrasound in Medicine & Biology (1)

Zhang, Z.-J., J. Huckle, et al. (2003). "The effects of pulsed low-intensity ultrasound on chondrocyte viability, proliferation, gene expression and matrix production." Ultrasound in Medicine & Biology **29**(11): 1645.

<http://www.sciencedirect.com/science/article/B6TD2-4B3N204-F/2/1257b33945bdacd1048b3a717c23ede1>

This study was designed to examine the effects of pulsed low-intensity ultrasound (PLIUS) on chondrocyte viability, proliferation, matrix production and gene expression. Chondrocytes were isolated from the distal part of the sternum of 16-day-old chick embryos and cultured in alginate beads. PLIUS at 2 mW/cm² (group PLIUS2) and 30 mW/cm² (group PLIUS30) was applied to chondrocytes for a single 20-min treatment. A control group was treated without PLIUS. The viability of chondrocytes was not affected by exposure to PLIUS. PLIUS influenced chondrocyte proliferation in an intensity-dependent manner. By day 7 after application of PLIUS, the gene expression and synthesis of aggrecan was the same as in the controls. At this same time point, the expression and synthesis of type II collagen was not different between the controls and PLIUS30, but was increased in PLIUS2. PLIUS was shown to inhibit the expression of type X collagen. This inhibition of chondrocyte hypertrophy may prove to be significant in the

management of cartilage degeneration. (E-mail: spencer@helix.nih.gov)

Urologic Oncology (4)

Chen, W.-C., H.-C. Wu, et al. (2002). "p21 gene codon 31 polymorphism is associated with bladder cancer." Urologic Oncology 7(2): 63.

<http://www.sciencedirect.com/science/article/B6TD3-45BCDY7-4/2/2d7d14673c3aea4196579a7ec67b8ca4>

The function of p21 is related to cell apoptosis, progression and malignancies. It is thought that p21 is related to cancer formation but is not related to tumor grade. We aimed to investigate the polymorphism of p21 codon 31 as a candidate for the genetic marker of bladder cancer and its progression. The distribution was analyzed in 53 bladder cancer patients, 119 healthy controls in Taiwanese patients. Polymerase chain reaction based restriction analysis was used for the study of the association of p21 codon31 polymorphism with bladder cancer. There was a significant difference in p21 codon 31 polymorphism between the control and the cancer patients (pp<0.05). Serine heterozygote was more prominent in the invasive group with 25 to 1% respectively when compared with the non-invasive group. The polymorphism of p21 codon 31 is associated with bladder cancer. An individual possessing one allele of arginine form in p21 codon 31 has a higher risk of developing bladder cancer than the serine form. Although the mechanism is unclear, our results show p21 gene is associated with tumor grade.

McGarvey, T. W. and S. B. Malkowicz (2000). "Steady state expression of cell-cycle regulatory genes in prostate carcinoma cell lines." Urologic Oncology 5(2): 71.

<http://www.sciencedirect.com/science/article/B6TD3-3XWJR6F-4/2/63ae78d8fadd5388b6f344d744dca85c>

The transforming growth factor- β (TGF- β) signal transduction system has been reported to play a role in prostate tumorigenesis and the regulation of cell cycle-related gene expression including the cyclins, cyclin dependent kinases (Cdks), and Cdk inhibitors. The objective of this investigation was to examine the expression of TGF- β receptors I and II and five cell cycle-related genes--Cdk-4, p15, p21(WAF1/CIP1), p27, and cyclin E--in three prostate carcinoma cell lines and normal prostate by quantitative reverse transcriptase (RT)/polymerase chain reaction (PCR). The expression of the TGF- β receptor II was reduced by 5.5- and 2.2-fold in the LNCaP and DU145 cells, respectively, compared with normal prostate tissue. A similar decrease was observed for the TGF- β receptor I transcript in the LNCaP cells. In addition, 20-fold less of the TGF- β inducible p15inkb transcript was produced by the LNCaP and DU145 cell lines compared with the PC-3 cell line. The p21(WAF1/CIP1) transcript, although present, was only 6% of normal in the DU145 and PC-3 cell lines. Furthermore, the steady state levels of p21(WAF1/CIP1) mRNA significantly increased within 15 minutes after the addition of exogenous TGF- β to PC-3 cells. Likewise, addition of TGF- β antibodies to the PC-3 cells significantly reduced p21(WAF1/CIP1) transcript levels to less than 2% of normal. This suggests that p21(WAF1/CIP1) expression in PC-3 cells is related not only to p53 induction but may function through alternative pathways including TGF- β . We conclude that the expression of specific cell cycle-related genes may be entirely or partially regulated by alterations in TGF- β

pathway and may play a role in prostate carcinoma development.

McGarvey, T. W., R. D. Meng, et al. (2001). "Growth inhibitory effect of p21 and p53 containing adenoviruses on transitional cell carcinoma cell lines in vitro and in vivo." Urologic Oncology 6(4): 155.

<http://www.sciencedirect.com/science/article/B6TD3-438KG8V-6/2/d51b3c09dae45f3b7e0a015aae9093fe>

Altered p53 expression has been demonstrated in the majority of advanced transitional cell carcinoma (TCC) of the bladder tumors. The objective of this investigation was to examine the effect of the introduction of a p53 or p21(WAF1/CIP1) adenovirus on the proliferation and apoptosis of various human TCC cell lines in vitro and in vivo. Proliferation was measured by ³H-thymidine incorporation. Apoptosis was measured by DNA fragmentation and bax expression. We also examined the effect of ex vivo introduction of the p21(WAF1/CIP1) or the p53 gene on growth of the T24 TCC cells and UMUC-3 TCC cells introduced subcutaneously into athymic nude mice. We found that although the effect of the p21-adenovirus on the proliferation of various TCC lines varied with each individual cell line, there was a substantial growth inhibition observed (greater than 80% growth inhibition) in seven of the eight TCC cell lines at the highest viral dosage. In contrast, after 24 h, the highest dosage of the p53-adenovirus produced only a heterogeneous decrease in proliferation compared to the highest dose of the p21(WAF1/CIP1)-adenovirus (40-90%). In ex vivo experiments, no tumors were found in nude mice injected subcutaneously with either TCC cell line exposed in vitro to the AdSCMV-p21(WAF1/CIP1) or AdSCMV-p53 viruses before three weeks. There was a threefold decrease in tumor square area at week 5 in the Ad5CMV-p21(WAF1/CIP1) or Ad5CMV-p53 TCC cells injected mice (p(WAF1/CIP1) pathway. Thus, the restoration of p21(WAF1/CIP1) function in this tumor system may be a beneficial therapeutic strategy.

Perabo, F. G. E., R. H. Mattes, et al. (2001). "Soluble Fas and Fas-ligand in bladder cancer in vitro and in vivo." Urologic Oncology 6(4): 163.

<http://www.sciencedirect.com/science/article/B6TD3-438KG8V-7/2/828ed8a7c4cade9e460d86b1ea359766>

Circulating soluble Fas (sFas) and expression of Fas-ligand on cancer cells are mechanisms of immune escape. The aim of the present study was to investigate expression and production of Fas and Fas-ligand on bladder cancer cell lines of different grade as a basic mechanism of their secretion in vivo. sFas and sFas-ligand serum levels of patients with different stage of bladder cancer were examined to determine the possible clinical use of these molecules as tumor markers. Bladder cancer cell lines RT4 (G1), RT112 (G1), T24 (G3) and SUP (G4) were analyzed by flowcytometry for Fas and Fas-ligand expression. To determine if the Fas-ligand gene is transcribed in these bladder cancer cell lines, RT-PCR was performed on mRNA extracted from these cell lines. Production of sFas and sFas-ligand was examined in cell culture supernatants of the cancer cells as well as in the serum of 62 patients with bladder cancer by a specific ELISA test. We demonstrate that Fas is expressed in similar levels on all human bladder carcinoma cell lines. In T24 (G3) and SUP (G4) cell lines we were able to detect the Fas-ligand protein, whereas no Fas-ligand protein could be found in RT4 and RT112 (G1) cells. Fas-ligand mRNA was expressed in all bladder cancer cell lines. Furthermore, all bladder cancer cell lines produce sFas but no sFas-ligand in spite of mRNA expression. The range of sFas levels in the serum of all patients with bladder cancer was large and did not show a correlation to the histopathological stage of bladder cancer. Although there is in vitro evidence that sFas and Fas-ligand play a role in bladder cancer, no correlation between the sFas and s Fas-ligand serum levels and the

histopathological stage of bladder cancer could be found. Therefore, serum sFas and sFas-ligand have to date limited clinical relevance.

Urologic Oncology: Seminars and Original Investigations (5)

Dahiya, R. (1996). "DNA repair genes in human fetal and adult prostatic tissues and cancer cell lines using differential RT-PCR." Urologic Oncology: Seminars and Original Investigations 2(2): 52.

<http://www.sciencedirect.com/science/article/B6TD3-3Y45XM7-2/2/afb5e9e74e38bc17a38ed76ec601dc36>

The present study was designed to investigate the mRNA expression of four DNA repair genes (XPCC, hMSH2, XRCC 1, and ERCC 1) in human fetal and adult prostatic tissues and cancer cell lines using differential reverse transcriptase-polymerase chain reaction (RT-PCR). For this purpose, total RNA from four human prostate cancer cell lines (LNCaP, PC-3, DU-145, and ND-1) and human fetal (n = 10) and adult (n = 10) prostates was extracted and mRNA expression was analyzed for four DNA repair genes by RT-PCR using specific oligonucleotides. For quantitation, we used [beta]-actin as an internal standard in each tube as baseline gene expression. The results of these experiments suggest that human prostate cancer cell lines (LNCaP, PC-3, DU-145, and ND-1) have 2- to 10-fold lower mRNA expression for all four DNA repair genes compared with human fetal and adult prostate tissues. Expression of DNA repair gene XPCC was about 10- to 15-fold lower in prostate cancer cells compared with fetal and adult prostatic tissues. This study, for the first time, demonstrates that mRNA levels of DNA repair genes in human prostate cancer cells are significantly lower compared with the adult prostate.

Lin, C.-C., H.-C. Wu, et al. (2003). "CYP17 gene promoter allelic variant is not associated with prostate cancer." Urologic Oncology: Seminars and Original Investigations 21(4): 262.

<http://www.sciencedirect.com/science/article/B6TD3-49D1VPF-5/2/653ff1e959227082e653bd84ec0a5f24>

Prostate cancer is the most common urological malignancy in Taiwan. The formation of prostate cancer has been reported to be associated with androgen. Two key steps in the sex steroid synthesis are mediated by the enzyme cytochrome P450c17[alpha] which is encoded in the CYP17 gene. Our aim was to investigate whether a polymorphism of CYP17 gene could be used as a genetic marker for associating prostate cancer. In this study, we compared the frequency of the C/T polymorphism of CYP17 gene 5'-UTR promoter region between 93 patients with prostate cancer and 121 healthy male volunteers (age, >60 years). The result revealed no significant association between the CYP17 genotype and prostate cancer (P = .781). Therefore, CYP17 C/T polymorphism is not a valid genetic marker for prostate cancer. Although a possible interaction between CYP17 gene C/T polymorphism and SP-1 transcription factor has been reported in the literature, we did not find any evidence for this the difference among clinical staging, pathological grading, or responsiveness to hormonal therapy in prostate cancer.

Nomura, T., H. Mimata, et al. (2004). "Cisplatin inhibits the expression of X-linked inhibitor of apoptosis

protein in human LNCaP cells." Urologic Oncology: Seminars and Original Investigations **22**(6): 453.

<http://www.sciencedirect.com/science/article/B6TD3-4F26RJ6-6/2/aa07e348a5ae24bf4f00baf41bd62a8c>

The purpose of the present study is to investigate the role of X-linked inhibitor of apoptosis protein (XIAP) in the regulation of apoptosis induced by cisplatin in human prostate cancer cell line (LNCaP). We examined the effects of cisplatin on cell growth and apoptosis in LNCaP by 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt (WST-1), flow cytometric analyses, and caspase-3 activity assay. In addition, to clarify the roles of the XIAP, we established clonal cell lines that overexpressed XIAP. The effects of cisplatin on the XIAP expression in the induction of apoptosis in LNCaP were examined by RT-PCR and immunoblot analyses. Although the growth rates were reduced in a dose- and time-dependent manner by cisplatin in LNCaP sublines, the anti-proliferative effects of cisplatin were significantly decreased in XIAP stably overexpressing cell lines. In addition, we found that cisplatin-induced apoptosis following activation of caspase-3, and that the overexpression of XIAP inhibited apoptosis by attenuating caspase-3 activity. Interestingly, treatment of LNCaP cells with 10 and 100 [μ]M cisplatin for 48 h significantly decreased the expression of XIAP at both the protein and mRNA levels in a dose-dependent manner. Furthermore, 10-[μ]M cisplatin treatment of LNCaP decreased XIAP mRNA and protein in a time-dependent manner. These results suggest that cisplatin induces apoptosis by the inhibition of XIAP expression, and that XIAP plays an important role in the regulation of cisplatin-induced apoptosis in LNCaP cells. The ability of cisplatin to down-regulate XIAP may be an important mechanism in chemosensitivity.

Price, D. K., W. L. Woodard, et al. (1995). "Simultaneous detection of circulating prostate specific antigen (PSA)-expressing and prostate specific membrane antigen (PSM)-expressing cells by a multiplex reverse transcriptase polymerase chain reaction (RT-PCR) assay." Urologic Oncology: Seminars and Original Investigations **1**(6): 226.

<http://www.sciencedirect.com/science/article/B6TD3-3XSK186-J/2/b3bebdd1111509c488d01d50a9742e3b>

It has yet to be determined whether the detection of prostate specific antigen (PSA)-expressing or prostate specific membrane antigen (PSM)-expressing cells in the circulation of prostate cancer patients is a more accurate predictor of clinical outcome. A method of evaluating both markers simultaneously would aid in the determination of the clinical relevance of reverse transcriptase polymerase chain reaction (RT-PCR) as a staging tool for prostate cancer. We describe the development of a multiplex RT-PCR assay that simultaneously detects the presence of both PSA-expressing cells and PSM-expressing cells, as well as a ubiquitously expressed internal control all within a single reaction. Both PSA cDNA and PSM cDNA were concurrently amplified by multiplex PCR using LNCaP mRNA as the starting template. When used as part of a nested PCR system, the multiplex RT-PCR assay identified one prostate cancer cell when placed in a background of one million cultured B lymphocytes. The multiplex assay was then applied to mRNA isolated from metastatic prostate cancer patients and from healthy male and female volunteers. While all were positive for the internal control G3PDH, three of seven prostate cancer patients were positive for both PSA and PSM expression and two more were positive for either PSA or PSM. None of the male or female volunteers were positive for either PSA or PSM. Multiplex RT-PCR allows for the amplification of both PSA and PSM cDNA within a single RT-PCR reaction, and this approach should allow a consistent comparison of the clinical utility of both PSA and PSM markers as staging tools and predictors of response to therapy.

Steiner, M. S., D. J. Satterwhite, et al. (1995). "Molecular insights into altered cell cycle regulation and genitourinary malignancy." Urologic Oncology: Seminars and Original Investigations **1**(1): 3.

<http://www.sciencedirect.com/science/article/B6TD3-3XY2J8K-H/2/08257c8be9de6c487878b53c9518fa1e>

The basis for malignancy is the deregulation of the cell cycle resulting in uncontrolled cellular proliferation. This review examines our current understanding of peptide growth factors, stimulatory and inhibitory signaling cascades, protooncogenes and tumor suppressor genes, cyclin dependent kinases and cyclins as they relate to the cell cycle. The epidermal growth factor and transforming growth factor [beta] transduction pathways are presented to illustrate the complex interactions required for normal signaling and control of the cell cycle. The second part of the review explores the relevance of these pathways and their alterations in the genitourinary malignancies of the prostate, bladder, kidney and testis.

Urology (11)

El-Sakka, A. I., M. U. Hassan, et al. (1998). "Possible molecular mechanisms of cryoablation-induced impotence in a rat model." Urology **52**(6): 1144.

<http://www.sciencedirect.com/science/article/B6VJW-3WDDGNS-1M/2/3ac7f158bfd045c70076caecde7492dd>

Objectives. Cryoablation of the prostate has been reported to induce impotence as a consequence of cavernosal nerve injury. This study is designed to investigate the early and late effects of cavernosal nerve cryoablation on growth factor expression and erectile function in a rat model. **Methods.** Forty male rats were divided into two groups (n = 20 each). The first group underwent unilateral cavernosal nerve freezing (experimental group). Before their euthanization at 1 and 3 months (10 rats each), erectile function was assessed by electrostimulation of the cavernous nerves. The second group served as the control and was killed at the same time points. Western blot and reverse transcriptase-polymerase chain reaction (RT-PCR) techniques were used to identify protein and gene expression of nerve growth factor (NGF), transforming growth factor-alpha (TGF-alpha), epidermal growth factor (EGF), and insulin-like growth factor-1 (IGF-1) in the rat penis and pelvic ganglia. **Results.** Electrostimulation of the frozen nerve after 3 months revealed a significantly higher maximal intracavernosal pressure and a shorter latency period than in the 1-month group. At 3 months, immunoblot showed upregulation of NGF, TGF-alpha, and the precursor form of IGF-1 protein expression in the penile tissue; RT-PCR showed downregulation of NGF gene expression in the pelvic ganglia of the frozen side. **Conclusions.** The results show that erectile function decreased at 1 month and then partially recovered 3 months after cavernosal nerve freezing. This alteration in erectile function was associated with differential gene and protein expression of the growth factors (NGF, TGF-alpha, EGF, and IGF-1). Further studies are required to elucidate the potential role of these growth factors in the prevention and treatment of cryoablation-induced impotence.

Fujimoto, N., A. Mizokami, et al. (2001). "Different expression of androgen receptor coactivators in human prostate." Urology **58**(2): 289.

<http://www.sciencedirect.com/science/article/B6VJW-4599BFT-1R/2/d6d23f7d131e3b705c3a6683ed9014f5>

Objectives. To investigate the expression of androgen receptor (AR) coactivators in the human prostate for a better understanding of androgen action in prostate cancer. **Methods.** Using reverse transcriptase-polymerase chain reaction, we examined the expression levels of AR coactivators (ARA55, SRC1, ARA54, TIF2, RAC3) in four prostate cancer cell lines (DU145, PC3, LNCaP, and LN-TR2), nine benign prostatic tissue samples, and 21 prostate cancer tissue specimens. **Results.** In the cell lines, SRC1 was expressed ubiquitously at almost equal amounts. Contrary to this, ARA55, ARA54, TIF2, and RAC3 displayed cell line-specific expression. In the LN-TR2 cells, established from LNCaP cells by long-term treatment with tumor necrosis factor-alpha, the expression levels of ARA55 and TIF2 were much higher than those in the LNCaP cells. In every prostatic tissue specimen, the expression levels of TIF2 and RAC3 were very low. The expression levels of ARA55 and SRC1 were higher in the cancer specimens with a higher grade or poor response to endocrine therapy than in those with a lower grade or good response to endocrine therapy. **Conclusions.** Prostate cancer cells express AR coactivators. Long-term stimulation by tumor necrosis factor-alpha could increase ARA55 and TIF2 expression in LNCaP cells. The different expression of coactivators may contribute to the different response of prostate cancer to androgenic stimulation or endocrine therapy.

Gonzalzo, M. L., M. Nakayama, et al. (2004). "Detection of GSTP1 methylation in prostatic secretions using combinatorial MSP analysis." *Urology* **63**(2): 414.

<http://www.sciencedirect.com/science/article/B6VJW-4BP0PD8-21/2/34f6cb2301034d625abb7f63b8897502>

ObjectivesTo evaluate the utility of methylation-specific polymerase chain reaction analysis of the pi-class glutathione-S-transferase (GSTP1) gene promoter in prostatic secretions for cancer detection and prognostication. **Methods**Prostatic secretions were obtained from a total of 100 radical prostatectomy specimens immediately after surgical extirpation. GSTP1 promoter methylation was assessed by methylation-specific polymerase chain reaction analysis using two different primer sets. Correlations between GSTP1 promoter methylation and clinical and pathologic variables were examined. **Results**The sensitivity for detection of GSTP1 methylation in prostatic secretions from men with clinically localized prostate cancer using two different primer sets was 76% and 54%. Methylation of the GSTP1 promoter was detected by both primer sets in 44% and by at least one primer set in 86% of the prostatic secretion specimens. The degree of methylation detected in the prostatic secretions was associated with the extent of cancer (predominant involvement of one or both sides of the gland; $P = 0.02$) and increasing age ($P = 0.009$). **Conclusions**Genomic DNA with GSTP1 promoter methylation can be detected in prostatic secretion specimens from the great majority of men with localized prostate cancer. Assays of GSTP1 promoter methylation in prostatic massage fluid or ejaculate may therefore serve as useful adjuncts to existing methods for prostate cancer screening and prognostication.

Keay, S., C. O. Zhang, et al. (1999). "Polymerase chain reaction amplification of bacterial 16RNA genes in prostate biopsies from men without chronic prostatitis." *Urology* **53**(3): 487.

<http://www.sciencedirect.com/science/article/B6VJW-3VXRW2V-8/2/3d1d7018f61031c0f19ea03ff430c4da>

Objectives. A previously reported study using nested polymerase chain reaction (PCR) analysis indicated the presence of DNA from a variety of prokaryotic microorganisms in 77% of

transperineal prostate biopsies from patients with chronic nonbacterial prostatitis. Because that study did not include a control group, we investigated whether microbial DNA could also be found in transperineal prostate biopsies obtained from men who did not have a history of prostatitis. **Methods.** Transperineal biopsies of both lobes of the prostate were obtained under ultrasound guidance from 9 patients with localized adenocarcinoma of the prostate. DNA was extracted from the prostatic tissue and two-round amplification performed using nested primers from a highly conserved region of the bacterial 16s rRNA gene. Amplified DNA was purified and sequenced, and sequences obtained were compared to bacterial rRNA genes recorded in GenBank. **Results.** Eleven of 18 biopsy specimens from 8 of 9 patients were positive for bacterial DNA by PCR. Sequence data indicated a predominant organism in 8 of 11 specimens, with greater than 95% homology to DNA from several different genera of bacteria, including *Escherichia* and *Bacteroides*. All 9 control samples from the instruments before biopsy were negative. **Conclusions.** The presence of bacterial 16s rRNA genes in prostatic tissue is not specific for chronic prostatitis and occurred in most of our patients with localized prostate cancer. Whether the presence of such bacteria is related to the development of prostatic diseases such as prostatitis or prostatic cancer will require carefully controlled trials, including appropriate control groups examined identically.

Lin, C.-C., H.-C. Wu, et al. (2003). "Vascular endothelial growth factor gene-460 C/T polymorphism is a biomarker for prostate cancer." *Urology* **62**(2): 374.

<http://www.sciencedirect.com/science/article/B6VJW-494YTJB-1T/2/9d8f6639a9a445f78233425a59474cba>

ObjectivesTo use the Bst U I polymorphism as a genetic marker in the search for the association between patients with prostate cancer and normal control subjects. The formation or progression of prostate cancer is presumed to be associated with a polymorphism of the vascular endothelial growth factor (VEGF) gene. The most frequently seen polymorphism is Bst U I (C to T) located at the -460th nucleotide upstream of the VEGF gene. **Methods**A normal control group of 119 healthy people and 96 patients with prostate cancer were examined. The polymorphism was seen after polymerase chain reaction-based restriction analysis. **Results**The analysis revealed significant differences between normal individuals and patients with cancer ($P = 0.110$, Fisher's exact test). **Conclusions**The Bst U I polymorphism of the VEGF gene is a suitable genetic marker of prostate cancer but cannot be used in the prediction of the outcome of patients who have received hormonal therapy.

Miller, J., M. Hesse, et al. (2004). "Congenital unilateral ureteropelvic junction obstruction of the rat: a useful animal model for human ureteropelvic junction obstruction?" *Urology* **63**(1): 190.

<http://www.sciencedirect.com/science/article/B6VJW-4BHSXTW-22/2/2038a918747430cf9837ce10240bca26>

ObjectivesTo investigate the expression of endothelin-1 (ET-1) and adrenomedullin (ADM) in the renal pelvis, stenotic ureteropelvic junction, and ureter of 20 male Wistar rats with congenital unilateral ureteropelvic junction obstruction; the normal contralateral kidneys served as controls. The molecular pathophysiology of congenital ureteropelvic junction obstruction is still unclear. The implication of altered peptidergic innervation is under discussion. Our study group has recently been able to demonstrate a significant increase in ET-1 and a significant decrease in ADM in prestenotic and stenotic tissue, but not in the remainder of the ureter, compared with controls. **Methods**Twenty animals were killed, and samples of the renal pelvis, ureteropelvic junction, upper ureter, middle part of the ureter, and lower ureter were immediately snap-frozen and stored in liquid nitrogen. Total RNA was extracted, and subsequently 1 μ g of RNA was

reversely transcribed. mRNA expression of ET-1 and ADM was determined semiquantitatively using on-line polymerase chain reaction. The expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was determined to relate the specific mRNA expression to the expression of a housekeeping gene. Results We found a significant increase in the expression of ET-1 in the obstructed junctions related to GAPDH (P Conclusions Alterations in the local production of peptidergic neurotransmitters, especially ET-1, may contribute to the molecular pathogenesis of ureteropelvic junction obstruction. Results previously obtained in the stenotic tissue from children were confirmed in the stenotic tissue from the rat model. We hypothesize that the alterations are disease-, but not age-specific.

Price, D. K., D. R. Clontz, et al. (1998). "Detection and clearance of prostate cells subsequent to ultrasound-guided needle biopsy as determined by multiplex nested reverse transcription polymerase chain reaction assay." *Urology* **52**(2): 261.

<http://www.sciencedirect.com/science/article/B6VJW-3W847WP-J/2/8c4f52e112274c9f71fc67e1cf9288f3>

Objectives. To determine if circulating prostate cells are detectable subsequent to transrectal ultrasound (TRUS)-guided biopsy, and if so, whether cells remain in circulation for up to 4 weeks. **Methods.** Blood samples were drawn from 90 patients with elevated serum prostate-specific antigen (PSA) levels and/or abnormal digital rectal examination. Two samples were drawn from all patients immediately prior to TRUS and 30 minutes postbiopsy. Blood samples were also obtained 1 week postbiopsy from 83 patients, and 1 month postbiopsy from 61 patients. Multiplex nested reverse transcription polymerase chain reaction assay (RT-PCR) for PSA and prostate-specific membrane antigen (PSM) was performed on total ribonucleic acid (RNA) from each sample. Results were reported as positive if at least one of the targets was detected. **Results.** Of 45 patients with biopsy-proven adenocarcinoma, 22 were RT-PCR positive prebiopsy and all remained positive 30 minutes postbiopsy. Of 23 patients with adenocarcinoma who were RT-PCR negative prebiopsy, 5 (22%) converted to positive 30 minutes postbiopsy (P P **Conclusions.** Detection of circulating prostate cells subsequent to biopsy occurred in 11 of 55 (20%) previously RT-PCR negative patients, a proportion twice that reported in the literature. We attribute this higher proportion to the simultaneous detection of PSA and PSM mRNA in our multiplex assay. Conversion rates were similar in patients regardless of biopsy result. Testing of serial postbiopsy blood demonstrates clearing of these cells by 4 weeks in most patients.

Tsui, K.-H., A.-J. Cheng, et al. (2004). "Association of nucleophosmin/B23 mRNA expression with clinical outcome in patients with bladder carcinoma." *Urology* **64**(4): 839.

<http://www.sciencedirect.com/science/article/B6VJW-4DKC7JC-1N/2/201c50f9730a2582cc909e412f4e5bdd>

Objectives To determine whether nucleophosmin/B23 mRNA expression in bladder carcinoma predicts recurrence, progression, and survival. **Methods** Real-time reverse transcriptase-polymerase chain reaction was performed on 50 fresh cancer specimens. The change in the cycle of threshold (Ct) was the difference in the Ct values derived from the nucleophosmin/B23 gene assayed and the 18S ribosomal RNA control [Ct (18S) - Ct (nucleophosmin/B23)]. **Results** Fifty patients diagnosed with bladder cancer were followed up postoperatively for a median of 24 months. Overexpression of nucleophosmin/B23 mRNA was observed in 37.1% of patients with Stage pT1 and 73.3% of those with pT2-T4 disease. Nucleophosmin/B23 overexpression was not associated with tumor grade (P = 0.163) but was associated with bladder cancer recurrence (68.2%) and progression (88.9%) when adjusted for the effects of clinical stage. Multivariate analysis revealed that the overall tumor stage and

nucleophosmin/B23 mRNA overexpression were important prognostic indicators for bladder carcinoma (P Conclusions Overexpression of nucleophosmin/B23 mRNA was independently associated with bladder cancer recurrence and progression. In patients with muscular invasion disease, overexpression of nucleophosmin/B23 mRNA was associated with the greatest risk of recurrence and progression, suggesting a potential rationale for early definitive therapy in these patients.

Wu, G., M. Iwamura, et al. (1998). "Characterization of the cell-specific expression of parathyroid hormone-related protein in normal and neoplastic prostate tissue." *Urology* **51**(5, Supplement 1): 110.

<http://www.sciencedirect.com/science/article/B6VJW-3V3MWCB-N/2/7bb1cb93ea0586c8bdf9424aaa8a0144>

Objectives. Parathyroid hormone-related protein (PTHrP) is a primary factor in the pathogenesis of malignancy-associated hypercalcemia. By alternative splicing, the human PTHrP gene can generate three different species of mRNA that encode three initial translational isoforms of 139, 173, and 141 amino acids. We recently reported that PTHrP was present in normal prostatic neuroendocrine cells and was overexpressed in prostate cancer tissue as demonstrated by immunostaining. This study was undertaken to further clarify the complex expression of PTHrP gene in normal prostate tissue and prostate cancer. **Methods.** PTHrP mRNA in samples prepared from normal prostate tissue, prostate cancer, and three prostate cancer cell lines, PC3, LNCaP, and DU145 was assessed using Northern hybridization. Expressed PTHrP isoforms were deduced from differential reverse transcription-polymerase chain reaction (RT-PCR) assays with exon-specific primers. Further localization of different species of PTHrP mRNA was performed using nonradioactive in situ hybridization with exon-specific probes on consecutive sections of normal and neoplastic prostate tissue. **Results.** Northern hybridization showed that the PTHrP expression level was higher in prostate cancer than in normal prostate tissue. All three PTHrP isoforms could be detected in normal prostate tissues and prostate cancer with differential RT-PCR. Further analysis using in situ hybridization with exon-specific probes revealed that all three PTHrP isoforms were present in prostatic neuroendocrine cells and only PTHrP-1-139 isoform could be clearly detected in prostate cancer tissue. Two androgen-insensitive cell lines, PC3 and DU145, derived from a bone metastasis and a brain metastasis, respectively, expressed all three mRNA species encoding for the three isoforms, but DU145 cells expressed less than PC3 cells. Androgen-sensitive LNCaP cells exhibited a low level of expression of mRNA species encoding for PTHrP-1-139 and PTHrP-1-173, and no expression of PTHrP1-141 isoform. **Conclusions.** All three initial translational isoforms of PTHrP are produced by prostatic neuroendocrine cells. The mature products of PTHrP might exert their effects on other prostatic epithelial cells in a paracrine fashion and also participate in the homeostatic regulation of the ejaculate. In prostate cancer, differential expression of these three isoforms is evident and PTHrP-1-139 isoform is more abundant than the other two forms. These findings are valuable for designing future research studies to further elucidate the biological functions of PTHrP in normal prostatic glands and prostate cancer.

Yoshida, K.-i., M. Yano, et al. (1999). "CAG repeat length in the androgen receptor gene is enhanced in patients with idiopathic azoospermia." *Urology* **54**(6): 1078.

<http://www.sciencedirect.com/science/article/B6VJW-3Y51HYK-12/2/06dd4b234b5363126c8803f7775d71d9>

Objectives. To determine whether the number of CAG repeats in the androgen receptor gene is enhanced in patients with idiopathic azoospermia. **Methods.** Using the polymerase chain reaction,

the number of CAG repeats was assayed in 41 patients with idiopathic azoospermia and in 48 normozoospermic fertile men. Results. In the control group, the CAG repeat length ranged from 17 to 30 (mean 23.9 +/- 2.9); in the azoospermic group, the CAG repeat length ranged from 20 to 34 (mean 26.5 +/- 3.5). The difference between the two groups was statistically significant ($P = 0.0013$). None of the men in the control group had a CAG repeat length greater than 31; four of the azoospermic men had 34 CAG repeats. Conclusions. Results suggest that an increase in the number of CAG repeats in the androgen receptor gene to 31 or greater may be associated with the etiology of at least some cases of idiopathic azoospermia.

Yuan, S.-S. F., Y.-F. Chung, et al. (2004). "Aberrant expression and possible involvement of the leptin receptor in bladder cancer." Urology **63**(2): 408.

<http://www.sciencedirect.com/science/article/B6VJW-4BP0PD8-20/2/87a12e7f99967fcb59c54655ae5c63ac>

ObjectivesTo explore the expression patterns and possible involvement of leptin and its receptor in the pathogenesis of urinary bladder cancer, with a focus on transitional cell carcinoma. **Methods**Using reverse transcription-polymerase chain reaction, immunoblotting, and immunohistochemistry techniques, we correlated the expression patterns of leptin and its receptor with the occurrence of transitional cell carcinoma. We also applied transient transfection followed by BrdU labeling and immunofluorescent staining to address the effect of the leptin receptor on bladder cancer cell growth. **Results**Although leptin was not detected in the bladder tissue specimens, a decreased expression of the leptin receptor was observed in most cancer tissue specimens we analyzed. Furthermore, the forced expression of the leptin receptor in T24 bladder cancer cells prevented them from entering the S phase. **Conclusions**Our data demonstrated for the first time that the leptin receptor is aberrantly expressed in bladder cancer tissue and is possibly involved in the carcinogenesis of bladder cancer.

Vaccine (33)

Amaral-Doel, C. M. F., N. E. Owen, et al. (1993). "Detection of foot-and-mouth disease viral sequences in clinical specimens and ethyleneimine-inactivated preparations by the polymerase chain reaction." Vaccine **11**(4): 415.

<http://www.sciencedirect.com/science/article/B6TD4-476W4JB-1R/2/acceccbed4d0a871d521cd36f27c2df5>

The polymerase chain reaction method (PCR) has been applied to the diagnosis of foot-and-mouth disease viral RNA in tissues and, particularly, oesophageal-pharyngeal fluid (probang) samples from cattle. Using primer sets which corresponded to conserved regions of the VP1 sequence of the viral genome, it was possible to amplify sequences regardless of the serotype/strain of the virus. In comparison with infectivity assays, the PCR was generally more sensitive although there were a number of examples where only infectivity was detectable. In experiments with uninfected probang samples deliberately seeded with a dilution series of virus, the PCR proved to be approximately 104 times more sensitive than infectivity assays. This greater sensitivity was attributed, in part, to the ability of the PCR to amplify specifically from non-infectious RNA preparations. This enabled the identification, by sequencing, of viral RNA from

chemically inactivated virus concentrates typical of those used for commercial vaccine production. Amplification of specific PCR products was also achieved with virus eluted from commercial vaccine, including preparations which had been stored for more than 10 years at 4[deg]C. The PCR technique is of considerable value, therefore, both as a complement to infectivity assays and as a powerful tool in vaccine-associated studies.

Brown, D. R., K. H. Fife, et al. (2004). "Early assessment of the efficacy of a human papillomavirus type 16 L1 virus-like particle vaccine." Vaccine **22**(21-22): 2936.

<http://www.sciencedirect.com/science/article/B6TD4-4BJDCVS-1/2/d775c7f62c37bf361274118bc63204ab>

A post hoc analysis was performed using combined data from two Phase I tolerability/immunogenicity studies of monovalent human papillomavirus type 11 (HPV11) or HPV16 L1 virus-like particle (VLP) vaccines. The goal was to determine if the HPV16 L1 VLP vaccine protected against HPV16 infection. Vaccine or placebo was given at 0, 2 and 6 months. HPV16 infection was defined by positive polymerase chain reaction (PCR) results following vaccination. The incidence of HPV infection was observed to be 0 cases per 100 person-years at risk in the vaccine group, and 5 cases per 100 person-years at risk in the control group. These results support the institution of larger efficacy trials for HPV L1 VLP vaccines.

Clarke, S. C., M. A. Diggle, et al. (2003). "Analysis of PorA variable region 3 in meningococci: implications for vaccine policy?" Vaccine **21**(19-20): 2468.

<http://www.sciencedirect.com/science/article/B6TD4-47T1S71-2/2/3ce53320cfa04cee9d00fc1379225648>

Outer membrane protein (OMP) vaccines are being developed against *Neisseria meningitidis* serogroup B which may provide protection against common circulating serotypes and serosubtypes in some countries. However, limited data is available in Europe from genosubtyping meningococci. We therefore undertook a retrospective analysis of the three main variable regions, VR1, VR2 as well as VR3, of the *porA* gene from *N. meningitidis* isolated from different countries, mainly from Scotland and Sweden. Analysis of this gene showed that, amongst 226 strains studied, there were a total of 78 different strains. No new VR1 or VR2 alleles were found but five new VR3 alleles are described. Our data indicates the importance of analysing the VR3 region of *PorA* in addition to VR1 and VR2 and also highlights, in general terms, the need for genosubtyping meningococci. Such analyses have major implications for the design of new meningococcal vaccines.

de Coana, Y. P., J. Rodriguez, et al. (2003). "A highly infective *Plasmodium vivax* strain adapted to *Aotus* monkeys: Quantitative haematological and molecular determinations useful for *P. vivax* malaria vaccine development." Vaccine **21**(25-26): 3930.

<http://www.sciencedirect.com/science/article/B6TD4-48J4K43-1/2/1d3fefbbaa79f9506bd48e8036898d32>

The New World primate *Aotus nancymae* is susceptible to infection by the human malaria parasite *Plasmodium vivax* and has therefore been recommended by the World Health Organization as a model for malaria vaccine candidate evaluation. We report the isolation,

adaptation, titration and genetic characterization of a *P. vivax* wild strain in splenectomized *A. nancymae* monkeys. Parasitemia remained high after 22 passages, reaching 7.88% by Giemsa and Acridine Orange staining and Real-Time PCR determination, making this *P. vivax* strain a highly infective and reliable asset to be used in *P. vivax* biological studies and vaccine development.

Djavani, M., C. Yin, et al. (2000). "Murine immune responses to mucosally delivered *Salmonella* expressing Lassa fever virus nucleoprotein." *Vaccine* **18**(15): 1543.

<http://www.sciencedirect.com/science/article/B6TD4-3Y6Y3MY-J/2/feabdd71820a2989f2fc4762ed65c7c8>

Arenaviruses are emerging pathogens known to infect via the mucosa, however no formal attempts to make mucosal vaccines have been undertaken. Here we describe a recombinant *aroA* attenuated *Salmonella typhimurium* that expresses the nucleoprotein (NP) gene of Lassa fever virus (LAS). The complete NP gene was cloned downstream of the bacterial *groEL* promoter and integrated into the *aroA* locus of *S. typhimurium*. Lassa NP protein was detected in whole cell extracts from the recombinant *Salmonella* by immunoblot analysis with serum from Lassa-infected people. Mice were inoculated by intragastric intubation with 5×10^9 *S. typhimurium* and boosted with the same recombinant *Salmonella* 21 days after the primary inoculation. Both local mucosal IgA and serum immunoglobulins against Lassa NP were observed. Splenic cytotoxic T-lymphocyte responses to LAS NP were detected after the boost and they cross-reacted with target cells infected with the related arenavirus, lymphocytic choriomeningitis virus. Recombinant *Salmonella* elicits humoral and cell mediated immune responses against Lassa fever virus in mice and should be considered as a potential vaccine strategy in man.

Erkkila, L., K. Laitinen, et al. (2002). "Experimental *Chlamydia pneumoniae* infection in NIH/S mice: effect of reinoculation with chlamydial or cell preparation on culture, PCR and histological findings of lung tissue." *Vaccine* **20**(17-18): 2318.

<http://www.sciencedirect.com/science/article/B6TD4-458P8WG-6/2/b1b975ed22ba05e30646101099a14ce4>

The cellular components present in chlamydial preparations may contribute to the course of the experimental infection. NIH/S mice were inoculated and reinoculated intranasally with *Chlamydia pneumoniae* or a cellular preparation. The mock inoculation induced only mild histological changes in the lungs, which possibly induced partial protection against subsequent *C. pneumoniae* infection and, when given as reinoculation, possibly reactivated the culture-negative infection as culture-positive. In addition, serum antibodies against mouse heat shock protein 60 (Hsp60) were found in a few mice. In conclusion, the main immunopathogenic factors in a *C. pneumoniae* mouse model are chlamydial components. However, a cellular preparation may participate in an inflammatory reaction. Autoimmunity against Hsp60 may also play a role in the pathogenesis of *C. pneumoniae* infection.

Finerty, S., C. R. Stokes, et al. (2000). "Mucosal immunization with experimental feline immunodeficiency virus (FIV) vaccines induces both antibody and T cell responses but does not protect against rectal FIV challenge." *Vaccine* **18**(28): 3254.

<http://www.sciencedirect.com/science/article/B6TD4-40JFY80-7/2/684b0978558031de9fe725d609fdbccc>

Feline immunodeficiency virus (FIV) is a natural lentiviral pathogen of cats which can be experimentally transmitted via rectal and vaginal routes -- the major routes of human immunodeficiency virus type 1 transmission in man. An important objective for lentiviral research is the development of vaccine strategies which generate good mucosal immune responses capable of giving protection from a mucosal virus challenge. The experimental vaccines employed in this study were based on (a) a peptide from the third variable region of the FIV envelope glycoprotein and (b) fixed whole FIV, Glasgow-8 strain. Adjuvants used were Quil A and cholera toxin for mucosal administration and incomplete Freund's adjuvant and immune stimulating complexes for subcutaneous injection. Mucosal immunization was given by rectal and intranasal routes. Both antibody and proliferative responses were elicited by mucosal immunization and cholera toxin was found to be a good mucosal adjuvant. The addition of a lipo thioester to the FIV peptide improved IgG and IgA responses upon parenteral administration. However, no protection from a rectal FIV challenge was achieved.

Fomsgaard, A., H. V. Nielsen, et al. (1999). "Induction of cytotoxic T-cell responses by gene gun DNA vaccination with minigenes encoding influenza A virus HA and NP CTL-epitopes." *Vaccine* **18**(7-8): 681.

<http://www.sciencedirect.com/science/article/B6TD4-3XRY69C-J/2/4235198f36596d20615cff99bb8692be>

Cytotoxic T-lymphocyte (CTL) response is an important component of anti-viral immunity. CTLs are specific to short peptides presented by MHC-I molecules and immunisation with the exact peptide sequence introduced in the cytosol is therefore a minimal approach, which potentially affords a high degree of controllability. We have examined the induction of murine CTL's by this approach using DNA plasmid minigene vaccines encoding known mouse Kk minimal CTL epitopes (8 amino acids) from the influenza A virus hemagglutinin and nucleoprotein. We here report that such an approach is feasible and that wild type influenza virus flanking amino acid sequences can influence the CTL response but are not essential for optimal CTL induction. We also examined the effect of different new amino acid sequences flanking the CTL epitopes. In one version, two CTL epitopes were linked together as 'string of beads'. This did not improve CTL induction. In another version, one CTL epitope was inserted into a known T-helper protein (HBsAg). This did significantly augment the response probably due to immunological help from HBsAg Th epitopes. Finally, the CTL inducing minigene DNA vaccines were compared with Flu-induced CTL responses and tested for their protective effect against a lethal influenza A virus infection in mice and no effect was found. We conclude that a specific and highly directed CTL induction is possible by unlinked minigene DNA immunisation, but that CTL induction solely is not always sufficient to provide protection.

Harrison, R. A., Y. Wu, et al. (1999). "DNA immunisation with *Onchocerca volvulus* chitinase induces partial protection against challenge infection with L3 larvae in mice." *Vaccine* **18**(7-8): 647.

<http://www.sciencedirect.com/science/article/B6TD4-3XRY69C-D/2/aba0509d106ec13c2e67b7f86c08ef69>

The parasitic nematode, *Onchocerca volvulus* is a major cause of blindness and dermal pathology in tropical regions. A vaccine directed to infective larvae would provide a valuable control tool alongside the current methods of chemotherapy and vector control. Previously we

have described the identification of a chitinase cDNA that is expressed in a stage specific manner by *O. volvulus* infective third stage (L3) larvae. To evaluate its host protective potential, the complete open reading frame was cloned into the eukaryotic expression plasmid pJW4303 and used to vaccinate mice by DNA immunisation with the Accell GeneGun. The survival of challenge infective larvae was monitored using implanted micropore chambers. In the first trial, mice immunised 3 times over 4 months with 1 [mu]g *O. volvulus* chitinase DNA responded with modest antibody responses dominated by IgG2a and exhibited a 36% ($p=0.189$, NS) reduction in parasite survival compared with challenge controls. In the second trial, an increased dose of DNA (5 [mu]g) and more frequent immunisations (5 times over 6 months) stimulated an IgG1 dominant response and a 53% reduction in parasite survival ($p=0.042$). Antibodies from the vaccinated mice reacted with the cuticle of post-infective L3 larvae, implying that this may be the site of immune attack following secretion of chitinase.

Hino, K., Y. Katoh, et al. (2001). "The effect of introduction of universal childhood hepatitis B immunization in South Africa on the prevalence of serologically negative hepatitis B virus infection and the selection of immune escape variants." *Vaccine* **19**(28-29): 3912.

<http://www.sciencedirect.com/science/article/B6TD4-439VBP9-H/2/2bc0b282ed3741bbb064a7a2176e24bd>

The effect of universal hepatitis B vaccination on the prevalence of serologically negative hepatitis B virus infection (HBV) and the emergence of HBsAg variants is unknown. We prospectively studied two different cohorts of 12-24 month old children in South Africa. They consisted of the unvaccinated children ($n=459$) born before the introduction of universal vaccination and the vaccinated children ($n=1213$) between 1 and 2 years after the introduction of the vaccination program. The frequency of detecting HBV DNA by PCR was reduced from 6.5% in unvaccinated children to 0.3% in vaccinated children ($P<0.00001$). There were no unique amino acid substitutions within the major hydrophilic region of the S sequence in both pre- and post-vaccination samples. Universal childhood vaccination reduced the frequency of serologically negative HBV infection and did not necessarily lead to selection of escape variants.

Hodgson, L. A., G. V. Ludwig, et al. (1999). "Expression, processing, and immunogenicity of the structural proteins of Venezuelan equine encephalitis virus from recombinant baculovirus vectors." *Vaccine* **17**(9-10): 1151.

<http://www.sciencedirect.com/science/article/B6TD4-3VSNYVN-P/2/e755a7bf81cc6df6fff65c67b41aaa02>

Recombinant baculoviruses expressing the structural proteins of Venezuelan equine encephalitis virus (VEE) have been constructed and the intracellular processing, antigenicity, and immunogenicity of the expression products have been assessed. Baculoviruses expressing the entire structural protein region (C-E3-E2-6K-E1), or the complete glycoprotein region (E3-E2-6K-E1), generated products in Sf9 cells that were accurately and completely processed, and resulted in mature proteins that were antigenically and electrophoretically indistinguishable from authentic viral proteins. These products were highly immunogenic in BALB/c mice, induced efficient VEE neutralizing responses, and protected these animals against challenge with virulent VEE. Expression of individual glycoprotein regions (E3-E2 and 6K-E1) generated products that were accurately but incompletely processed, and induced non-neutralizing antibodies that reacted more efficiently with denatured than native VEE proteins. Nonetheless, immunization with the 6K-E1 expression product provided complete protection against VEE challenge.

Hoffmann, E., S. Krauss, et al. (2002). "Eight-plasmid system for rapid generation of influenza virus vaccines." *Vaccine* **20**(25-26): 3165.

<http://www.sciencedirect.com/science/article/B6TD4-464P50V-5/2/e0c9b5fc50359c1fc16f56ca19fc34>

The antigenic variation of influenza A virus hemagglutinin (HA) and neuraminidase (NA) glycoproteins requires frequent changes in vaccine formulation. The classical method of creating influenza virus seed strains for vaccine production is to generate 6+2 reassortants that contain six genes from a high-yield virus, such as A/PR/8/34 (H1N1) and the HA and NA genes of the circulating strains. The techniques currently used are time-consuming because of the selection process required to isolate the reassortant virus. We generated the high-yield virus A/PR/8/34 (H1N1) entirely from eight plasmids. Its growth phenotype in embryonated chicken eggs was equivalent to that of the wild-type virus. By using this DNA-based cotransfection technique, we generated 6+2 reassortants that had the antigenic determinants of the influenza virus strains A/New Caledonia/20/99 (H1N1), A/Panama/2007/99 (H3N2), A/teal/HK/W312 (H6N1), and A/quail/HK/G1/97 (H9N2). Our findings demonstrate that the eight-plasmid system allows the rapid and reproducible generation of reassortant influenza A viruses for use in the manufacture of vaccines.

Hulskotte, E. G. J., G. F. Rimmelzwaan, et al. (1995). "Antigenicity and immunogenicity of recombinant envelope glycoproteins of SIVmac32H with different in vivo passage histories." *Vaccine* **13**(13): 1187.

<http://www.sciencedirect.com/science/article/B6TD4-3Y44SNT-39/2/c31b1ad2107f8e8b3106a55a6aa01626>

Shortly after infection of two rhesus monkeys (*Macaca mulatta*) either with a SIVmac32H challenge stock or with the same virus that had been passaged in another rhesus monkey for 11 months, SIV-envelope genes were cloned from their peripheral blood mononuclear cells and subsequently expressed by recombinant vaccinia viruses. The molecular weights and antigenicities of the thus produced envelope glycoproteins were largely identical to those of the native SIV. The envelope glycoprotein derived from the in vivo passaged virus proved to be poorly recognized by virus neutralizing monoclonal antibodies directed against one of the seven antigenic sites for which monoclonal antibodies were available. Immunization studies in rats showed that this protein was also less efficient in inducing antibodies against this antigenic site, and that it induced significantly lower levels of virus neutralizing antibodies than the other SIV-envelope glycoprotein. The immunogenicity of the SIV-envelope glycoprotein incorporated into immune stimulating complexes (iscoms) was compared to that of the same protein presented with Quil A or MDP-tsl.

Katow, S., H. Minahara, et al. (1997). "Identification of strain-specific nucleotide sequences in E1 and NS4 genes of rubella virus vaccine strains in Japan." *Vaccine* **15**(14): 1579.

<http://www.sciencedirect.com/science/article/B6TD4-42R6XXB-J/2/ecff4923ea6e37f2716b0857620ee969>

Strain-specific nucleotide sequences of E1 and NS4 genes in five strains of a live rubella virus vaccine manufactured in Japan were identified for comparison, using 2389 nucleotides (1443

nucleotides of the E1 gene, 41 of the 3' terminal region following the E1 gene and 905 of the NS4 gene). Sequences of the E1 gene in three strains (Matsuura, TCRB19 and To-336) were identified. Takahashi and Matsuba strains shared common sequences, but were discriminated by the sequence of the NS4 gene. These five strains showed a phylogenetic relationship with the places of their isolation. In a comparative study of three strains with their unattenuated progenitors, the nucleotides in these regions were almost conserved during the attenuation process.

Langhammer, S., U. Fiebig, et al. "Neutralising antibodies against the transmembrane protein of feline leukaemia virus (FeLV)." **Vaccine In Press, Corrected Proof**
<http://www.sciencedirect.com/science/article/B6TD4-4FD76H1-1/2/fca556f58f9f09179172716e6dca8445>

Neutralising antibodies specific for feline leukaemia virus (FeLV) were induced by immunisation with recombinant FeLV transmembrane envelope protein p15E. Epitope mapping revealed two epitopes located in similar regions to those previously identified for the porcine endogenous retrovirus (PERV). One of the epitopes has partial homology and both are located in regions corresponding to epitopes recognised by neutralising antibodies in patients infected with HIV-1.

Le Grand, R., G. Vogt, et al. (1992). "Specific and non-specific immunity and protection of macaques against SIV infection." **Vaccine** **10**(12): 873.

<http://www.sciencedirect.com/science/article/B6TD4-476VD73-2K/2/5d1baf8119ea5912a1a01448893b9bde>

The simian immunodeficiency virus is a retrovirus closely related to the human immunodeficiency viruses; it induces an AIDS-like disease in macaques, and provides therefore an obvious animal model for anti-lentiviral drug and vaccine strategy assessments. In our experiment, we immunized rhesus macaques with a purified and formalin-inactivated whole SIVmac251 antigen preparation. Most of these monkeys were still protected for more than 4 months following a heterologous SIVsm intravenous challenge. Both virus stocks, for vaccine preparation and challenge, were provided by culture supernatants of infected T cells of human origin. Four of the protected macaques were then reimmunized with the same antigen preparation and rechallenged intravenously with a homologous rhesus cell grown SIVmac251. Unexpectedly, all animals developed clinical and biological evidence of infection by day 15 after the second challenge.

Matsuo, K., T. Yoshikawa, et al. (2000). "Induction of innate immunity by nasal influenza vaccine administered in combination with an adjuvant (cholera toxin)." **Vaccine** **18**(24): 2713.

<http://www.sciencedirect.com/science/article/B6TD4-40315PM-D/2/9d5017f161cce6754b5fa1b2af8c17e2>

Inactivated influenza vaccine was administered intranasally to BALB/c mice together with an adjuvant (cholera toxin B subunit [CTB] supplemented with a trace amount of the whole toxin, CTB*) and its ability to induce innate immunity and confer protection against influenza was examined. Nasal wash virus titres 3 days after inoculation of homologous viruses were measured as an index of the ability of the vaccine to confer protection in mice immunized with either CTB*-combined vaccine or CTB* alone 1-21 days previously. The results were as follows. (1) Partial but significant reduction of the nasal-wash virus titres (prevention) was detected beginning 3 days

after the vaccination, that is, 2 days earlier than the appearance of both virus-specific antibody-forming cells (AFCs) in the nasal-associated lymphoid tissue (NALT) and virus-specific IgA antibody responses in the nasal washes of mice immunized with the CTB*-combined vaccine. (2) The protection, detected on day 3 and peaking on day 5 but lost by day 21, was also conferred in mice immunized with CTB* alone. (3) The non-specific prevention was detected at doses of more than 0.3 [μ g] of CTB*/mouse. (4) The nonspecific protection beginning 3 days after the immunization involved the enhanced expression of cytokine mRNAs (IL-15 and IL-18), considered responsible for natural killer (NK) cell activation, by the non-T cell populations in the NALT. (5) Normal NALT cells, when cultured in vitro with CTB*, secreted IL-1[β] within a few hours in culture. These results demonstrate that the CTB*-combined vaccine, when given intranasally into mice, can confer nonspecific protection against influenza beginning 3 days after the vaccination and that CTB* also possessed this ability to confer protection non-specifically and temporarily by inducing the secretion of IL-1[β], one of the most important cytokines that initiates both innate and adaptive immunity, and also NK cell activity.

Mederle, I., R. Le Grand, et al. (2003). "Mucosal administration of three recombinant Mycobacterium bovis BCG-SIVmac251 strains to cynomolgus macaques induces rectal IgAs and boosts systemic cellular immune responses that are primed by intradermal vaccination." *Vaccine* **21**(27-30): 4153.

<http://www.sciencedirect.com/science/article/B6TD4-49RCXR6-D/2/31dcc85cdf15c178e49deb4255443d15>

The widely administered Mycobacterium bovis BCG is an attractive live vector for the development of AIDS vaccines. We explored immune responses induced in cynomolgus macaques to rBCG-SIV3, a mixture of three recombinant BCG strains expressing the SIVmac251 nef, gag and env genes. After a single intradermal (ID) inoculation, circulating blood cells from rBCG-SIV3-vaccinated monkeys exhibited CTL responses targeted against the three antigens and interferon-gamma (IFN[γ]) secretion was observed. A rectal or oral boosting dose of rBCG-SIV3 elicited anti-SIV IgAs in the rectum of vaccinated monkeys and increased IFN[γ] secretion by circulating blood cells. Despite a good response against the vector, rBCG-SIV3 administration did not induce IgG antibody responses or lymphoproliferation against the SIV antigens in blood. This could be due to the lack of in vivo persistence of the recombinant BCG strains that were used. Rectal challenge with fully pathogenic SIVmac251-infected all animals. However, after viral challenge, anti-SIV cellular and antibody responses were higher in rBCG-SIV3 monkeys than in controls indicating that the vaccine induced anti-SIV CD4+ T-cell memory.

Miyashita, M., T. Joh, et al. (2002). "Immune responses in mice to intranasal and intracutaneous administration of a DNA vaccine encoding Helicobacter pylori-catalase." *Vaccine* **20**(17-18): 2336.

<http://www.sciencedirect.com/science/article/B6TD4-45C0134-1/2/817d54f7c1e2ac4d7060147399f67188>

We previously reported that the intracutaneous injection of DNA vaccines encoding Helicobacter pylori heat shock proteins elicited specific immune responses, and led to reduced infection in mice. In this study, we constructed DNA vaccine encoding H. pylori-catalase (pcDNA3.1-kat) and investigated the immune responses to intranasal and intracutaneous administration of pcDNA3.1-kat. C57/BL6 mice were immunized intracutaneously with 10 [μ g] of pcDNA3.1-kat or intranasally with 50 [μ g] of pcDNA3.1-kat. Catalase-specific IgG antibody was detected in the sera of intranasal and intracutaneous immunized mice. Both intranasal and intracutaneous immunized mice were significantly protected from colonization by H. pylori and had significantly

reduced degrees of gastritis. These results demonstrate that DNA vaccine encoding *H. pylori*-catalase can induce an immune response against *H. pylori*, and that intranasal immunization works as well as intracutaneous immunization.

Mota, J., M. Acosta, et al. "Induction of protective antibodies against dengue virus by tetravalent DNA immunization of mice with domain III of the envelope protein." ***Vaccine In Press, Corrected Proof*** <http://www.sciencedirect.com/science/article/B6TD4-4FG8859-4/2/098675861b881c92914ffae51deb0f86>

Dengue fever is a growing public health concern around the world and despite vaccine development efforts, there are currently no effective dengue vaccines. In the present study we report the induction of protective antibodies against dengue virus by DNA immunization with domain III (DIII) region of the envelope protein (E) in a mouse model. The DIII region of all four dengue virus serotypes were cloned separately into pcDNA 3 plasmid. Protein expression was tested in COS-7 cells. Each plasmid, or a tetravalent combination, were used to immunize BALB/c mice by intramuscular route. Presence of specific antibodies was evaluated by ELISA, and neutralizing antibodies were tested using a cytopathogenic effect (CPE) inhibition assay in BHK-21 cells, as well as in newborn mice challenged intracranially with dengue 2 virus. Mice immunized with individual DIII constructs or the tetravalent formulation developed antibodies against each corresponding dengue serotype. Antibody titers by ELISA were similar for all serotypes and no significant differences were observed when boosters were administered, although antibody responses were dose-dependent. CPE inhibition assays using Den-2 virus showed neutralization titers of 1:10 in mice immunized with individual DIII plasmid or those immunized with the tetravalent formulations. 43% of newborn mice challenged with Den-2 in combination with sera from mice immunized with Den-2 DIII plasmid were protected, whereas sera from mice immunized with the tetravalent formulation conferred 87% protection. Our results suggest that DIII can be used as a tetravalent DNA formulation to induce neutralizing and protective antibodies against dengue virus.

Nam, J.-H., L. S. Wyatt, et al. (1999). "Protection against lethal Japanese encephalitis virus infection of mice by immunization with the highly attenuated MVA strain of vaccinia virus expressing JEV prM and E genes." ***Vaccine* 17(3): 261.**

<http://www.sciencedirect.com/science/article/B6TD4-3VGDCJX-8/2/d558762b12c7b7503a4d2bae1835fe46>

Genes encoding the glycosylated precursor of the membrane (prM) and envelope (E) proteins of a Korean strain of Japanese encephalitis virus (JEV) were inserted into the genome of the host-range restricted, highly attenuated, and safety-tested MVA strain of vaccinia virus. MVA recombinants containing the JEV genes, under strong synthetic or modified H5 vaccinia virus promoters, were isolated. Synthesis of JEV prM and E proteins was detected by immunofluorescence microscopy, flow cytometry, and polyacrylamide gel electrophoresis. Mice inoculated and boosted by various routes with either of the MVA recombinants produced JEV neutralizing antibodies, that had titres comparable with those induced by an inactivated JEV vaccine, as well as haemagglutination-inhibiting antibodies. Mice immunized with 2×10^6 infectious units of MVA/JEV recombinants by intramuscular or intraperitoneal routes were completely protected against a 10^5 LD₅₀ JEV challenge at 9 weeks of age.

Osen, W., T. Peiler, et al. (2001). "A DNA vaccine based on a shuffled E7 oncogene of the human

papillomavirus type 16 (HPV 16) induces E7-specific cytotoxic T cells but lacks transforming activity." Vaccine **19**(30): 4276.

<http://www.sciencedirect.com/science/article/B6TD4-43G2YV1-V/2/7876d810b8bf2ce5849613e0317c6039>

Vaccination with oncogene-derived DNA for anti-cancer treatment carries a risk of de-novo tumor induction triggered by the persisting recombinant DNA. We hypothesized that an oncoprotein whose primary sequence has been rearranged ('shuffled') to maintain all possible T cell epitopes still induces cytotoxic T cells against the authentic protein but is devoid of transforming properties. As a model antigen, we used the E7 oncoprotein of the human papillomavirus (HPV) type 16, the major cause of cervical cancer. We have generated an artificial E7 molecule in which four domains were rearranged and, in order to maintain all possible T cell epitopes, certain sequences were duplicated. Upon transfection of this shuffled E7 gene (E7SH) into RMA cells, presentation of an E7 Db-restricted T cell epitope was shown by an E7-specific CTL line in vitro. Immunization of C57BL/6 mice with E7SH DNA induced E7-specific CTL and also conveyed protection against E7-positive syngeneic tumor cells. No transforming activity of E7SH DNA in NIH3T3 cells was detected, as determined by focus formation, induction of S-phase under conditions of serum deprivation and degradation of endogenous pRB. Our results suggest that DNA shuffling may become a promising concept for DNA-based anti-cancer vaccines.

Pisarev, V. M., P. Parajuli, et al. (2002). "Flt3 ligand and conjugation to IL-1[beta] peptide as adjuvants for a type 1, T-cell response to an HIV p17 gag vaccine." Vaccine **20**(17-18): 2358.

<http://www.sciencedirect.com/science/article/B6TD4-454T75N-2/2/7f7ceb0fcc5bef4ee7498f81fd84552d>

The adjuvant activity of Flt3 ligand (Flt3L) and conjugation to an interleukin (IL)-1[beta] bioactive fragment were compared, either alone or in combination, for their ability to induce T- and B-cell responses to the HGP-30 peptide sequence (amino acids 86-115 of human immunodeficiency virus (HIV) gag p17). The efficiency of HGP-30/IL-1[beta] conjugation, Flt3L administration or both as adjuvants was examined and all were found to augment similar levels of delayed type hypersensitivity (DTH) responses. In contrast, significant antigen (Ag)-specific types 1 and 2 T-cell ELISPOT responses were induced only by the combination of adjuvants. Further, in vitro sensitization with HGP-30 selectively increased Ag-specific, type 1 T-cell and cytotoxic T lymphocyte (CTL) responses to HGP-30-derived nonapeptide epitopes, while type 2 responses declined as measured in the ELISPOT assay. No serum antibodies to HGP-30 were induced unless HGP-30 was conjugated to keyhole-limpet hemocyanin. This suggests that a combination adjuvant strategy using Flt3L and conjugation to a biologically active IL-1[beta] fragment may be used to preferentially increase type 1 T-cell and CTL responses to HIV-1 gag antigenic epitopes.

Ploton, I. N., B. Wizel, et al. (1995). "Mapping of two overlapping linear epitopes in Pfg27 recognized by Plasmodium falciparum transmission-blocking monoclonal antibodies." Vaccine **13**(13): 1161.

<http://www.sciencedirect.com/science/article/B6TD4-3Y44SNT-35/2/a42277c6519c6c799cf2a5ed7260e46f>

We have reported previously the production of Plasmodium falciparum transmission-blocking monoclonal antibodies (mAb) recognizing a reduction-insensitive cross-reacting epitope in the gametocyte antigen Pfg27 and the gamete surface antigens Pfs230 and Pfs48/45. In this study, the amino acid sequence of this epitope in Pfg27 was determined. First, the epitope was localized

near the N terminus of the protein by probing recombinant overlapping fragments spanning Pfg27 with transmission-blocking mAb in immunoblot experiments. The amino acid sequence of the epitope was then determined by using overlapping synthetic peptides spanning the smallest immunoreactive recombinant fragment in an ELISA. The sequence KPLDKFGNIYDYHYEH (amino acids 10-25 in the Pfg27 sequence) was shown to contain two overlapping epitopes recognized by transmission-blocking mAb. Comparison of the sequence of the gene encoding Pfg27 in seven different *P. falciparum* strains demonstrated that these sequential epitopes are totally conserved. Immunization of mice with synthetic peptides derived from Pfg27, conjugated with keyhole limpet hemocyanin (KLH) and formulated in Freund's adjuvant or alum, resulted in the production of antibodies capable of recognizing the peptides as well as the native Pfg27.

Register, K. B. (2004). "Comparative sequence analysis of *Bordetella bronchiseptica* pertactin gene (prn) repeat region variants in swine vaccines and field isolates." *Vaccine* **23**(1): 48.

<http://www.sciencedirect.com/science/article/B6TD4-4D1YV3C-4/2/bd95d4618867d2212bc7dabb04547761>

The product of the *Bordetella bronchiseptica* pertactin gene, prn, has been implicated as an adhesin and a protective immunogen in swine. Recent studies demonstrate prn sequence heterogeneity in swine isolates and vaccine strains within and surrounding the region 1 amino acid repeat GGXXPn and the region 2 amino acid repeat PQPn. However, only a few isolates have been evaluated. Allelic variation between vaccine strains and field isolates may affect vaccine efficacy, since region 2 is known to encode an immunodominant protective epitope. In the present study, the DNA and predicted amino acid sequences of the pertactin repeat regions from a collection of 81 recent swine field isolates and 5 vaccine strains from the United States were determined. Two region 1 variants and four region 2 variants, one of which has not been previously reported, were identified, comprising four pertactin types. Four vaccines are derived from strains with a region 1 variant identical to that found in the majority of field isolates. However, only two vaccines possess the most commonly identified sequence in region 2, while two others contain a variant found in only one other swine isolate. Ribotype analysis demonstrated that although vaccines containing the novel region 2 variant fall within the same major cluster as other common swine ribotypes, they are less closely related. No relationship was observed between pertactin type and ribotype.

Sang, H., V. M. Pisarev, et al. (2003). "Regional, but not systemic recruitment/expansion of dendritic cells by a pluronic-formulated Flt3-ligand plasmid with vaccine adjuvant activity." *Vaccine* **21**(21-22): 3019.

<http://www.sciencedirect.com/science/article/B6TD4-483BMS0-1/2/5ad6e9277a82f8856e01641d31a1b120>

Regional recruitment of dendritic cells (DCs) by the local administration of granulocyte macrophage-colony stimulating factor (GM-CSF) or Flt3-ligand (Flt3L) has vaccine adjuvant activity. However, Flt3L, with its DC growth factor activity, has not been extensively studied as a vaccine adjuvant, particularly as a plasmid vector. We report that the intramuscular (IM) injection of a Flt3L plasmid (pNGVL-hFlex), when formulated in a pluronic carrier (SP1017, Supratek Pharma, Inc., Laval, Que., Canada), recruits DC to the injection site and regional lymph nodes (LNs) and augments immune responses to a p17 HIV plasmid vaccine to a greater extent than the injection of a naked DNA vaccine alone. Following IM administration of pNGVL-hFlex, Flt3L mRNA, Flt3L protein and infiltrating DC accumulate at the injection site. The number of DC in the draining LNs are also significantly increased with the greatest increase observed following injection of 2.5 [mu]g of pNGVL-hFlex formulated in 0.01% SP1017. Flow cytometric studies

demonstrate that the LN-infiltrating DC is mainly of the CD11c+CD11b- phenotype (IL-12 producing). Further, the co-injection of pNGVL3-hFlex and p17 HIV plasmids, formulated in SP1017, significantly increases the immune responses to the plasmid vaccine (pVAX-gag). The co-injection of pVAX-gag and pNGVL3-hFlex, formulated in SP1017, significantly increase delayed-type hypersensitivity responses and the numbers of antigen (Ag)-specific interferon-[gamma] secreting T cells in the spleen (Enzyme Linked Immune Spot (ELISpot) assay), compared to mice immunized with pVAX-gag formulated in SP1017 alone. We conclude that the IM injection of pNGVL-hFlex with SP1017 can increase the number of DC in draining LN and at the site of injection, thereby providing adjuvant activity for a plasmid vaccine resulting in a significantly increased, Ag-specific T cell response.

Solioz, N., U. Blum-Tirouvanziam, et al. (1999). "The protective capacities of histone H1 against experimental murine cutaneous leishmaniasis." *Vaccine* **18**(9-10): 850.

<http://www.sciencedirect.com/science/article/B6TD4-3Y0J1DX-F/2/4aa4d35072b0113ef06d9488843ff76c>

In a murine model of experimental cutaneous leishmaniasis, we investigated the protection elicited by injection of histone H1 isolated from parasites by perchloric extraction, of a H1 recombinant protein produced in *E. coli*, and of H1 long and short synthetic peptides, against infection by *L. major*. Partial protection was achieved in most of the animals as shown by reduction in lesion size, upon immunization with histone H1 or its peptides, provided that the region 1-60 was present in the molecule. These observations argue in favor of a thorough examination of the possibility of including histone H1 described here in a cocktail vaccine against human leishmaniasis.

Uno-Furuta, S., K. Matsuo, et al. (2003). "Immunization with recombinant Calmette-Guerin bacillus (BCG)-hepatitis C virus (HCV) elicits HCV-specific cytotoxic T lymphocytes in mice." *Vaccine* **21**(23): 3149.

<http://www.sciencedirect.com/science/article/B6TD4-48CFGJ-2/2/4dacec4a86a5fda72da79aa5e66c30a9>

Since virus-specific cytotoxic T lymphocytes (CTLs) play a critical role in preventing the spread of hepatitis C virus (HCV), an effective HCV vaccine should be capable of eliciting HCV-specific CTLs. In the present study, we assessed the capability of a novel recombinant vaccine using an attenuated tuberculosis bacillus, Calmette-Guerin bacillus (BCG), as a vaccine vehicle to elicit HCV-specific CTLs. BCG was engineered to express the CTL epitope of HCV-non-structure protein 5a (NS5a) as a chimeric protein with alpha antigen of mycobacteria. Immunization with this recombinant BCG elicited major histocompatibility complex class I-restricted CD8+ HCV-NS5a-specific CTLs in mice. Immunized mice showed a substantial reduction in the vaccinia virus titer compared with control mice when the immunized mice were challenged with a recombinant vaccinia virus expressing HCV-NS5a genes. These findings provide evidences for the possibility of BCG as a vaccine vector and its continued exploration as a vehicle for eliciting HCV-specific immunity.

Vanegas, R. A., N. E. Street, et al. (1997). "In a vaccine model, selected substitution of a highly stimulatory T cell epitope of hen's egg lysozyme into a *Salmonella* flagellin does not result in a homologous, specific, cellular immune response and may alter the way in which the total antigen

is processed." Vaccine **15**(3): 321.

<http://www.sciencedirect.com/science/article/B6TD4-3RJX9N5-F/2/ab29550391320d85682e55cc5f1bb584>

A 13 amino acid peptide corresponding to a potent BALB/c mouse T cell epitope of hen's egg lysozyme (HEL) was substituted singly at five sites in the d flagellin of *Salmonella muenchen*. The resulting chimeric proteins were unable to expand T cells capable of being stimulated by the HEL epitope and induced T cell populations which either failed to respond or responded at a low level to a normally highly stimulatory flagellin T cell epitope that was present in all chimeras. The results suggested that substitution of a T cell epitope in flagellin may alter the processing of the resulting immunogen.

Vaughan, K., G. H. Rhodes, et al. (2005). "DNA immunization against respiratory syncytial virus (RSV) in infant rhesus monkeys." Vaccine **23**(22): 2928.

<http://www.sciencedirect.com/science/article/B6TD4-4F37HJM-1/2/f40668a399c5cb372be17349c72e3516>

A DNA vaccine was tested in infant Rhesus macaques to evaluate its safety, immunogenicity and protective efficacy. Monkeys were vaccinated and challenged with a clinical isolate of human RSV. Vaccinated animals developed humoral and cellular responses following inoculation with plasmid DNA encoding the fusion (F) and nucleoprotein (N), from closely related bovine RSV. Vaccinated monkeys had decreased RSV in their lungs post-infection, and there was a qualitative difference in histopathology observed between vaccinated and unvaccinated animals. The combined result of safety and immunogenicity in a neonatal primate model is encouraging, suggesting the feasibility of DNA vaccines against RSV in infants.

Von Beust, B. R., W. C. Brown, et al. (1999). "Development and in vitro characterization of recombinant vaccinia viruses expressing bovine leukemia virus gp51 in combination with bovine IL4 or IL12." Vaccine **17**(4): 384.

<http://www.sciencedirect.com/science/article/B6TD4-3VGDCRH-V/2/424ba987452b80cde96b1cbf92967d1a>

Type 1 and type 2 immune responses are modulated by IL12 or IL4, respectively, at the time of lymphocyte priming. Importantly, type 1 responses have been associated with resistance to retroviral infection in mice, humans, and ruminants. Specifically, vaccination of sheep with vaccinia virus expressing bovine leukemia virus (BLV) gp51 resulted in protective immunity with the characteristics of a type 1 response, whereas vaccination of cattle resulted in a non-protective type 2 response. In order to test the hypothesis that cattle inoculated with BLV gp51 and IL12 will respond with a type 1 response, a recombinant vaccinia virus expressing BLV gp51 together with bovine IL12 was developed and characterized in vitro. For induction of type 2 responses a recombinant vaccinia virus expressing gp51 with bovine IL4 was similarly constructed and characterized. In this study recombinant cassettes were developed containing either the BLVenv gene alone or in combination with bovine IL4 or the two genes, p35 and p40, encoding bovine IL12. Correct alignment with p7.5 or p11 vaccinia promoters and orientation was confirmed by complete sequencing. Recombinant vaccinia viruses were generated by homologous recombination, selected based on large plaque formation due to reconstitution of the vp37 gene, and structurally confirmed by Southern blotting. Transcription of recombinant BLVenv, bovine IL4, p35 and p40 was demonstrated by RT-PCR. Expression of BLVenv gp51 protein and bovine IL4

was shown by immunofluorescence and immunoblotting. Biologically active bovine IL4 expressed by vaccinia virus stimulated lymphoblast proliferation, B lymphocyte proliferation in the presence of CD40L, and inhibited IFN[gamma] secretion from PHA activated PBMC in a dose dependent fashion. Finally, bovine IL12 expression and biological function was confirmed by dose dependent induction of IFN[gamma] secretion by PHA activated PBMC and the moderate enhancement of lymphoblast proliferation. In conclusion, bovine IL12 and IL4 expressed by recombinant vaccinia virus in vitro clearly exhibited type 1-type 2 modulating properties.

Wan, Y., Y. Wu, et al. (2001). "Induction of hepatitis B virus-specific cytotoxic T lymphocytes response in vivo by filamentous phage display vaccine." *Vaccine* **19**(20-22): 2918.

<http://www.sciencedirect.com/science/article/B6TD4-42NH3H1-N/2/2545758298e51d00769bfb242c467f84>

The ability of inducing MHC class I restricted cytotoxic T lymphocytes response in vivo via recombinant filamentous phage was investigated. The recombinant filamentous phage particles that displayed the Hepatitis B virus epitope S28-39 were injected into BALB/c (H-2d) mice without adjuvants. A MHC class I restricted HBs specific CTL response was found 8 days after injection. The potentiality of using the recombinant filamentous phage as anti-virus vaccine was discussed.

Xu-Amano, J., R. J. Jackson, et al. (1994). "Helper Th1 and Th2 cell responses following mucosal or systemic immunization with cholera toxin." *Vaccine* **12**(10): 903.

<http://www.sciencedirect.com/science/article/B6TD4-476MJYG-18/2/3f76d8345dcca71d6707489c69719110>

We have used the potent mucosal immunogen cholera toxin (CT) to assess antigen-specific CD4+ T-cell responses, including Th1- and Th2-type cells in mucosa-associated tissues, e.g. Peyer's patches (PP), and systemic tissue, e.g. spleen (SP), for their regulatory role in the induction of CT-specific B-cell antibody responses in the gastrointestinal (GI) tract as well as in systemic sites. The CT was given by either oral or intravenous (i.v.) routes and the mice orally immunized with CT exhibited brisk IgA anti-CT antibody responses in faecal extracts and elevated IgG anti-CT antibody responses in serum. Further, significant IgA anti-CT spot-forming cells (SFCs) were seen in lamina propria lymphocytes (LPLs) from mice orally immunized with CT. In contrast, i.v. immunization with CT induced IgM and IgG anti-CT SFC responses in SP, and serum anti-CT antibodies of these two isotypes; no anti-CT responses were induced in the GI tract after immunization by this route. The CD4+ T cells isolated from PP and SP of mice orally immunized with CT were stimulated in vitro with CT-B-coated latex microspheres for 1-6 days, and the induction of IL-2 and interferon gamma (IFN-[gamma]) (Th1-type) or IL-4 and IL-5 (Th2-type) producing SFCs were analysed by a cytokine-specific ELISPOT and cytokine-specific mRNA was detected by reverse transcriptase (RT)-PCR assays. Cultures of PP and SP CD4+ cells from orally immunized mice contained elevated numbers of IL-4- and IL-5-SFCs, significant levels of IL-4 and IL-5 mRNA, only small numbers of IFN-[gamma]- and IL-2-SFCs and low to undetectable levels of IFN-[gamma] and IL-2 mRNA. On the other hand, SP CD4+ Th-cell cultures from mice immunized i.v. with CT included both IL-4- and IL-5- as well as IFN-[gamma]- and IL-2-SFCs; no CT-specific Th cells were detectable in PP. These results show that oral immunization with CT preferentially induces CD4+ T cells which produce IL-4 and IL-5 in both PP and SP. Furthermore, the presence of elevated numbers of these Th2-type cells directly correlated with significant IgA anti-CT-B antibody responses in LPLs isolated from the GI tract.

Vascular Pharmacology (1)

Suenaga, A., T. Fujii, et al. (2004). "Up-regulation of lymphocytic cholinergic activity by ONO-4819, a selective prostaglandin EP4 receptor agonist, in MOLT-3 human leukemic T cells." Vascular Pharmacology **41**(2): 51.

<http://www.sciencedirect.com/science/article/B6X3P-4CBV1FG-1/2/78ca2e192905b534e537dca42d09bc57>

We used a selective EP4 receptor agonist, ONO-4819, and a human leukemic T cell line MOLT-3 cells, which express all four prostaglandin E2 (PGE2) receptors (EP1-EP4), to investigate whether the EP4 PGE2 receptor subtype is involved in regulating lymphocytic cholinergic activity. Phytohemagglutinin (PHA), a T cell activator, significantly enhanced the expression of EP4 receptor mRNA during the first 3-6 h of exposure, after which, expression gradually declined. Furthermore, PHA stimulation slightly but significantly up-regulated the expression of EP2 mRNA after 12 h and of EP3 mRNA after 6 h. By contrast, expression level of EP1 receptor mRNA was not affected by PHA. ONO-4819 (1 [μM]), which was added to cultures after 3 h of PHA stimulation, significantly increased cellular ACh content and release, and up-regulated ChAT mRNA expression and activity but inhibited MOLT-3 cell proliferation. These findings suggest that the activation of T lymphocytes up-regulates EP4 receptor mRNA expression and, to a lesser extent, EP2 and EP3 receptors and that PGE2 enhances nonneuronal lymphocytic cholinergic transmission in activated T cells, at least in part, via EP4 receptor-mediated pathways.

Virology (59)

Airaksinen, A., N. Pariente, et al. (2003). "Curing of foot-and-mouth disease virus from persistently infected cells by ribavirin involves enhanced mutagenesis." Virology **311**(2): 339.

<http://www.sciencedirect.com/science/article/B6WXR-48H87SC-3/2/94b0aacddb3bb5bc27e40cd6962ed1ca>

BHK-21 cells persistently infected with foot-and-mouth disease virus (FMDV) can be cured of virus by treatment with the antiviral nucleoside analogue ribavirin. To study whether the process involved an increase in the number of mutations in the mutant spectrum of the viral population, viral genomes were cloned from persistently infected cells treated or untreated with ribavirin. An increase of up to 10-fold in mutation frequencies associated with ribavirin treatment was observed in the viral genomes from the treated cultures as compared with parallel, untreated cultures. To address the possible mechanisms of enhanced mutagenesis, we investigated the mutagenic effects of ribavirin together with guanosine, and mycophenolic acid in the presence or absence of guanosine. Changes in the intracellular nucleotide concentrations were determined for all treatments. The results suggest that the increased mutation frequencies were not dependent on nucleotide pool imbalances or due to selection of preexisting genomes but they were produced by a mutagenic action of ribavirin.

Albert, J., L. Franzen, et al. (1992). "Ugandan HIV-1 V3 loop sequences closely related to the U.S./European consensus." *Virology* **190**(2): 674.

<http://www.sciencedirect.com/science/article/B6WXR-4BMTXJC-6H/2/c825f68bb4f0cbe95d0cec7040b593e2>

The third variable (V3) loop of the human immunodeficiency virus type 1 (HIV-1) envelope protein is an important determinant for virus neutralization and cell tropism. V3 loop sequences from uncultured lymphocytes obtained in 1990 from 22 Ugandan HIV-1-infected patients could, with the exception of two patients' sequences, be divided into two groups (A and B) on the basis of the V3 loop size and sequence. The V3 loop consensus sequences from both groups showed a high degree of homology to a U.S./European consensus, a characteristic also reflected by the results of peptide serology. In the case of group B the difference in sequence was only five amino acids. In contrast, the V3-flanking regions for both groups showed greater homology to an earlier (1986/1987) Ugandan consensus. The discovery of these two new Ugandan V3 loop genotypes, which are closely related to the U.S./European consensus, has implications for the understanding of the evolution of HIV-1 and for the future design of a vaccine for use in Africa.

Anwar, A., A. Chandrasekaran, et al. (2005). "West Nile premembrane-envelope genetic vaccine encoded as a chimera containing the transmembrane and cytoplasmic domains of a lysosome-associated membrane protein: increased cellular concentration of the transgene product, targeting to the MHC II compartment, and enhanced neutralizing antibody response." *Virology* **332**(1): 66.

<http://www.sciencedirect.com/science/article/B6WXR-4F0GBTC-5/2/3996e49b38859b7b1eee978ec1948cd0>

A genetic vaccine for West Nile virus (WN) has been synthesized with the WN premembrane-envelope (WN preM-E) gene sequences encoded as a chimera with the transmembrane and carboxyl terminal domains of the lysosome-associated membrane protein (LAMP). The LAMP sequences are used to direct the antigen protein to the major histocompatibility class II (MHC II) vesicular compartment of transfected professional antigen-presenting cells (APCs). Vaccine constructs encoding the native WN preM-E and WN preM-E/LAMP chimera were synthesized in pVAX1 and pITR plasmid backbones. Extracts of human fibroblast 293 and monkey kidney COS-7 cells transfected with the WN preM-E/LAMP chimera constructs contained much greater amounts of E than did the cells transfected with constructs encoding the native WN preM-E. This difference in the concentration of native E and the E/LAMP chimera in transfected cells is attributed to the secretion of native E. The amount of preM protein in cell extracts, in contrast to the E protein, and the levels of DNA and RNA transcripts, did not differ between WN preM-E- and WN preM-E/LAMP-transfected cells. Additionally, confocal and immunoelectron microscopic analyses of transfected B cells showed localization of the WN preM-E/LAMP chimera in vesicular compartments containing endogenous LAMP, MHC II, and H2-M, whereas native viral preM-E lacking the LAMP sequences was distributed within the cellular vesicular network with little LAMP or MHC II association. Mice immunized with a DNA construct expressing the WN preM-E/LAMP antigen induced significant antibody and long-term neutralization titers in contrast to the minimal and short-lived neutralization titer of mice vaccinated with a plasmid expressing the untargeted antigen. These results underscore the utility of LAMP targeting of the WN envelope to the MHC II compartments in the design of a genetic WN vaccine.

Aouacheria, A., M. Banyai, et al. (2003). "Characterization of vNr-13, the first alphaherpesvirus gene of the bcl-2 family." *Virology* **316**(2): 256.

<http://www.sciencedirect.com/science/article/B6WXR-49W1VTT-7/2/f3ea8d282db5088bd82607235df5012f>

The Bcl-2 family, including antiapoptotic and proapoptotic members, plays key regulating roles in programmed cell death. We report the characterization of a new member of the bcl-2 family, encoded by herpesvirus of turkeys (HVT). The product of this gene shares 80% homology with Nr-13, an apoptosis inhibitor, which is overexpressed in avian cells transformed by the v-src oncogene. This new gene, that we propose to call vnr-13, is the first member of the bcl-2 family to be isolated among [alpha]-herpesviruses. Results from cells expressing the HVT-vnr-13 gene product show that the encoded protein inhibits apoptosis and also reduces the rate of cellular proliferation. Contrary to all bcl-2 homologues found in [gamma]-herpesvirus, which are intronless, vnr-13 has the same organization as the cellular nr-13 gene. Hence, the HVT vnr-13 gene may have been acquired from a reverse transcriptase product of an unspliced precursor RNA, or via direct recombination with the host chromosomal DNA.

Badal, S., V. Badal, et al. (2004). "The human papillomavirus-18 genome is efficiently targeted by cellular DNA methylation." *Virology* **324**(2): 483.

<http://www.sciencedirect.com/science/article/B6WXR-4CB6542-1/2/9eb4bd3ef25a0b80b25f574982e541c6>

Human papillomaviruses (HPVs) infect epithelia, including the simple and the squamous epithelia of the cervix, where they can cause cancer and precursor lesions. The molecular events leading from asymptomatic HPV infections to neoplasia are poorly understood. There is evidence that progression is modulated by transcriptional mechanisms that control HPV gene expression. Here, we report the frequent methylation of HPV-18 genomes in cell culture and in situ. DNA methylation is generally known to lead to transcriptional repression due to chromatin changes. We investigated two cell lines derived from cervical cancers, namely, C4-1, which contains one HPV-18 genome, and different clones of HeLa, with 50 HPV-18 genomes. By restriction cleavage, we detected strong methylation of the L1 gene and absence of methylation of parts of the long control region (LCR). A 3-kb segment of the HPV-18 genomes downstream of the oncogenes was deleted in both cell lines. Bisulfite sequencing showed that in C4-1 cells and two HeLa clones, 18 of the 19 CpG residues in the 1.2-kb terminal part of the L1 gene were methylated, whereas a third HeLa clone had only eight methylated CpG groups, indicating changes of the methylation pattern after the establishment of the HeLa cell line. In the same four clones, none of the 12 CpG residues that overlapped with the enhancer and promoter was methylated. In six HPV-18 containing cancers and five smears from asymptomatic patients, most of the CpG residues in the L1 gene were methylated. There was complete or partial methylation, respectively, of the HPV enhancer in three of the cancers, and lack of methylation in the remaining eight samples. The promoter sequences were methylated in three of the six cancers and four of the six smears, and unmethylated elsewhere. Our data show that epithelial cells efficiently target HPV-18 genomes for DNA methylation, which may affect late and early gene transcription.

Bakonyi, T., E. A. Gould, et al. (2004). "Complete genome analysis and molecular characterization of Usutu virus that emerged in Austria in 2001: Comparison with the South African Strain SAAR-1776 and other flaviviruses." *Virology* **328**(2): 301.

<http://www.sciencedirect.com/science/article/B6WXR-4D8VH9G-2/2/a42a4172ec30a2bc13167a77bf0f839e>

Here we describe the complete genome sequences of two strains of Usutu virus (USUV), a mosquito-borne member of the genus *Flavivirus* in the Japanese encephalitis virus (JEV) serogroup. USUV was detected in Austria in 2001 causing a high mortality rate in blackbirds; the reference strain (SAAR-1776) was isolated in 1958 from mosquitoes in South Africa and has never been associated with avian mortality. The Austrian and South African isolates exhibited 97% nucleotide and 99% amino acid identity. Phylogenetic trees were constructed displaying the genetic relationships of USUV with other members of the genus *Flavivirus*. When comparing USUV with other JEV serogroup viruses, the closest lineage was Murray Valley encephalitis virus (nt: 73%, aa: 82%) followed by JEV (nt: 71%, aa: 81%) and West Nile virus (nt: 68%, aa: 75%). Comparison of the genomes showed that the conserved structural elements and putative enzyme motifs were homologous in the two USUV strains and the JEV serogroup. The factors that determine the severe clinical symptoms caused by the Austrian USUV strain in Eurasian blackbirds are discussed. We also offer a possible explanation for the origins and dispersal of USUV, JEV, and MVEV out of Africa.

Banham, A. H. and G. L. Smith (1992). "Vaccinia virus gene B1R encodes a 34-kDa serine/threonine protein kinase that localizes in cytoplasmic factories and is packaged into virions." *Virology* **191**(2): 803.

<http://www.sciencedirect.com/science/article/B6WXR-4BNNXTF-8J/2/99edcae5bcd6145c9d6944c76a7cfe1a>

Vaccinia virus open reading frame B1R was expressed in *E. coli* and shown to encode a serine/threonine protein kinase which phosphorylated casein and calf thymus histones in vitro. A polyclonal rabbit antiserum was raised against a TrpE-B1R bacterial fusion protein and used to characterize the B1R gene product. Immunoprecipitation and immunoblotting analyses detected a 34-kDa polypeptide that was synthesized early during vaccinia virus infection and which was apparently stable since it was easily detectable 18 hr postinfection. Immunofluorescence demonstrated that this protein localizes in cytoplasmic virus factories, the sites of virus DNA replication. Immunoblotting of vaccinia virions showed that the enzyme is packaged into virus particles.

Bergman, I., P. Whitaker-Dowling, et al. (2003). "Vesicular stomatitis virus expressing a chimeric Sindbis glycoprotein containing an Fc antibody binding domain targets to Her2/neu overexpressing breast cancer cells." *Virology* **316**(2): 337.

<http://www.sciencedirect.com/science/article/B6WXR-49W1VTT-8/2/69f060ea4efb0fc6938ddd2f2c504d44>

Vesicular stomatitis virus (VSV) is a candidate for development for cancer therapy. It is an oncolytic virus that is safe in humans. Recombinant virus can be made directly from plasmid components. We attempted to create a virus that targeted specifically to breast cancer cells. Nonreplicating and replicating pseudotype VSV were created whose only surface glycoprotein (gp) was a Sindbis gp, called Sindbis-ZZ, modified to severely reduce its native binding function and to contain the Fc-binding domain of *Staphylococcus aureus* protein A. When titrated on Her2/neu overexpressing SKBR3 human breast cancer cells, pseudotype VSV coated with Sindbis-ZZ had 5/ml. This work demonstrates the ability to easily create, directly from plasmid components, an oncolytic replicating VSV with a restricted host cell range.

Burgyan, J., T. Dalmay, et al. (1992). "The replication of cymbidium ringspot tombusvirus defective interfering-satellite RNA hybrid molecules." *Virology* **190**(2): 579.

<http://www.sciencedirect.com/science/article/B6WXR-4BMTXJC-65/2/8753a1b03e0b57e0ee6c8a1eeeac23e3>

A DNA copy of DI RNA of cymbidium ringspot tombusvirus was cloned downstream of a phage T7 promoter. In vitro-transcribed RNA replicated in *Nicotiana clevelandii* when coinoculated with full-length viral genomic RNA transcripts and protected plants from apical necrosis. Artificial deletion mutants derived from the DI RNA clone showed that most of the central sequence block is necessary for replication. Hybrid DI RNA-satRNA clones were prepared and in vitro-synthesized RNA was inoculated to plants in the presence of helper viral RNA. There was replication only of in vitro transcripts derived from hybrid clones where satRNA sequences were inserted upstream or downstream from the central block, but not of those derived from clones where satRNA sequence replaced the central block. Progeny RNA of biologically active clones was either full-length or showed deletions depending on the insertion of satRNA sequences in DI RNA. DI RNA-satRNA constructs having part of the 5' region exchanged were not replicated.

Burmeister, W.-P., R. S. Daniels, et al. (1991). "Sequence and crystallization of influenza virus b/Beijing/1/87 neuraminidase." *Virology* **180**(1): 266.

<http://www.sciencedirect.com/science/article/B6WXR-4BNJB0P-7T/2/91dc728ccc24316c2036681996395981>

Influenza B/Beijing/1/87 neuraminidase heads were isolated from virus via trypsin digestion and characterized by PAGE, N-terminal sequencing, electron microscopy, and enzyme activity. The heads were crystallized into two crystal forms; tetragonal plates, like other neuraminidase crystals described before, that diffract to medium resolution (3 Å) and a new form consisting of trigonal prisms or needles that diffract to high resolution (at least 2 Å). The gene segment coding for neuraminidase was sequenced and compared with the neuraminidase sequence of B/Lee/40. The deduced amino acid sequences for neuraminidase showed only a 7% difference, whereas those for the NB proteins differed by 20%.

Caipang, C. M. A., I. Hirono, et al. (2003). "In vitro inhibition of fish rhabdoviruses by Japanese flounder, *Paralichthys olivaceus* Mx." *Virology* **317**(2): 373.

<http://www.sciencedirect.com/science/article/B6WXR-4B22XDH-1/2/c417851c048730ff700928e7bab48477>

A homologous fish cell line stably expressing the recombinant Japanese flounder Mx (JFMx) was infected with hirame rhabdovirus (HIRRV) and viral hemorrhagic septicemia virus (VHSV), both of which are negative single-stranded RNA viruses belonging to the Rhabdoviridae family. Analysis of primary transcription of the two rhabdoviruses showed that there was lower expression level and copy number of the viral nucleoprotein transcript in the JFMx-transfected cell line than the infected, control cells, although no significant difference was observed. This suggests that JFMx may not be a potent inhibitor of rhabdoviral primary transcription. Kinetics of rhabdovirus expression by RT-PCR and quantitative real-time RT-PCR showed reduced levels of the rhabdoviral glycoprotein and nucleoprotein transcripts over time, indicating the possible role of JFMx in blocking rhabdoviral replication by interfering with the transcription of the viral subgenomic mRNAs. Significant inhibition in rhabdovirus replication consequently resulted in the synthesis of fewer viral particles. This may explain why JFMx-expressing cells are less

susceptible to virus-induced cell lysis, and thus, why they would have a significantly higher survival than the infected, control cells. These results provide direct evidence that JFMx has an antiviral effect in vitro.

Calain, P., J. Curran, et al. (1992). "Molecular cloning of natural paramyxovirus copy-back defective interfering RNAs and their expression from DNA." *Virology* **191**(1): 62.

<http://www.sciencedirect.com/science/article/B6WXR-4BNVGWK-3F/2/2d6d3dfd70fbf3e1bfbabcb9d9c51ada>

Using the unique sequence organization of copy-back defective interfering (DI) RNAs of paramyxoviruses, Sendai virus (SV), and measles virus copy-back DI RNAs were PCR amplified and cloned, without having to separate them from their helper nondefective genomes. The cloning was designed so that T7 polymerase transcription of the plasmids would generate DI RNAs with the exact 5' and 3' ends. The SV DI clone, transcribed from the plasmid in BHK cells using T7 polymerase produced by a vaccinia virus recombinant, was encapsidated and replicated by the SV-L, P/C, and NP proteins expressed from cloned genes. Such experiments open the possibility of examining the cis-acting sequences involved in viral multiplication directly, without using indirect markers such as CAT activity.

Cali, S., E. Spoldi, et al. (2004). "Bacteriophage P4 Vis protein is needed for prophage excision." *Virology* **322**(1): 82.

<http://www.sciencedirect.com/science/article/B6WXR-4BXTJP7-2/2/1a6ae6668c124df6cad59fa4af2449d4>

Upon infection of its host *Escherichia coli*, satellite bacteriophage P4 can integrate its genome into the bacterial chromosome by Int-mediated site-specific recombination between the attP and the attB sites. The opposite event, excision, may either occur spontaneously or be induced by a superinfecting P2 helper phage. In this work, we demonstrate that the product of the P4 vis gene, a regulator of the P4 late promoters PLL and Psid, is needed for prophage excision. This conclusion is supported by the following evidence: (i) P4 mutants carrying either a frameshift mutation or a deletion of the vis gene were unable to excise both spontaneously or upon P2 phage superinfection; (ii) expression of the Vis protein from a plasmid induced P4 prophage excision; (iii) excision depended on a functional integrase (Int) protein, thus suggesting that Vis is involved in the formation of the excision complex, rather than in the excision recombination event per se; (iv) Vis protein bound P4 DNA in the attP region at two distinct boxes (Box I and Box II), located between the int gene and the attP core region, and caused bending of the bound DNA. Furthermore, we mapped by primer extension the 5' end of the int transcript and found that ectopic expression of Vis reduced its signal intensity, suggesting that Vis is also involved in negative regulation of the int promoter.

Chen, S.-Y., Y. Matsuoka, et al. (1991). "Golgi complex localization of the Punta Toro virus G2 protein requires its association with the G1 protein." *Virology* **183**(1): 351.

<http://www.sciencedirect.com/science/article/B6WXR-4CXDMST-BX/2/8422ff1eaac865c59723b1eb2914e033>

The glycoproteins of bunyaviruses accumulate in membranes of the Golgi complex, where virus

maturation occurs by budding. In this study we have constructed a series of full length or truncated mutants of the G2 glycoprotein of Punta Toro virus (PTV), a member of the Phlebovirus genus of the Bunyaviridae, and investigated their transport properties. The results indicate that the hydrophobic domain preceding the G2 glycoprotein can function as a translocational signal peptide, and that the hydrophobic domain near the C-terminus serves as a membrane anchor. A G2 glycoprotein construct with an extra hydrophobic sequence derived from the N-terminal NSM region was stably retained in the ER, and was unable to be transported to the Golgi complex. The full-length G2 glycoprotein, when expressed on its own, was transported out of the ER and expressed on the cell surface, whereas the G1 and G2 proteins when expressed together are retained in the Golgi complex. A truncated anchor-minus form of the G2 glycoprotein was found to be secreted into the culture medium, but was retained in the Golgi complex when coexpressed with the G1 glycoprotein. These results indicate that the G2 membrane glycoprotein is a class I membrane protein which does not contain a signal sufficient for Golgi retention, and suggest that its Golgi localization is a result of association with the G1 glycoprotein.

Chmielewicz, B., M. Goltz, et al. (2003). "A novel porcine gammaherpesvirus." *Virology* **308**(2): 317.

<http://www.sciencedirect.com/science/article/B6WXR-484SKJV-C/2/d4148bfa4022ee83deff0ecf517c988>

A novel porcine gammaherpesvirus was detected in the blood of domestic pigs by PCR. With degenerate-primer PCR and subsequent long-distance PCR approaches a 60-kbp genome stretch was amplified. Sequence analysis revealed the presence of the gammaherpesvirus ORFs 03 to 46 as well as a putative chemokine receptor and a v-bcl-2 gene. The 60-kbp sequence was compared with the corresponding sequence of the porcine lymphotropic herpesvirus 1 (PLHV-1) published recently and the sequence of PLHV-2, which was amplified from porcine tonsil. Considerable sequence differences (amino acid identities: 49-89%) were found between the novel virus and PLHV-1 as well as PLHV-2, which were very closely related to each other (amino acid identities: 85-98%). The novel virus had essentially the same genome organization as PLHV-1 and -2 and was therefore designated PLHV-3. Like PLHV-1 and -2, PLHV-3 was frequently found in the blood and in lymphoid organs of domestic and feral pigs from different geographic locations. In the blood, the PLHVs were detected predominantly in B-cells. Indication for latent as well as productive PLHV-3 infection was found in the porcine B-cell line L23. It can be concluded that the PLHVs are widespread and are likely to cause a persistent B-lymphotropic infection. Since PLHV-1 has been implicated in the development of porcine posttransplantation lymphoproliferative disease, all porcine lymphotropic gammaherpesviruses are of concern when pigs are used as donors in xenotransplantation.

Clarke, S., N. Almond, et al. (2003). "Simian immunodeficiency virus Nef gene regulates the production of 2-LTR circles in vivo." *Virology* **306**(1): 100.

<http://www.sciencedirect.com/science/article/B6WXR-47NF5P0-1/2/da2e29a5a09dc85f8f628697acc12ed8>

The replication dynamics of simian immunodeficiency virus (SIVmac32H-C8), attenuated through discrete genetic disruption of the nef gene, were compared with the wild-type parental clone (SIVmac32H-J5) using quantitative molecular methods. The primary viraemia of both infections were similar during the first week, but peaked on Day 10 at higher levels for wild-type virus. Viral RNA levels differed most markedly at Day 14. The frequency and levels of viral DNA species, detectable as gag provirus or circular 2-LTR episomes, differed depending on the virus and the lymphoid compartment sampled. 2-LTR circles persisted for prolonged periods in the peripheral blood but were never detected in any SIVmac32H C8-infected tissue, even if positive by gag

PCR. Paradoxically, the converse was observed following wild-type infection. 2-LTR circles disappeared from the peripheral blood by Day 42 postinfection but persisted in lymphoid tissues. These findings are discussed in terms of nef and the role and stability of 2-LTR circle forms in vivo.

Cook, R. F., S. J. Cook, et al. (2003). "Enhancement of equine infectious anemia virus virulence by identification and removal of suboptimal nucleotides." *Virology* **313**(2): 588.

<http://www.sciencedirect.com/science/article/B6WXR-4938MDK-2/2/970bcf15bf18cc4f0052d57bda5dc010>

Pathogenicity was reportedly restored to an avirulent molecular clone of equine infectious anemia virus (EIAV) by substitution of 3' sequences from the pathogenic variant strain (EIAVPV). However, the incidence of disease in horses/ponies was found to be significantly lower ($P = 0.016$) with the chimeric clone (EIAVUK) than with EIAVPV. This was attributable to 3' rather than 5' regions of the proviral genome, where EIAVUK differs from the consensus EIAVPV sequence by having a 68-bp duplication in the 3' LTR and arginine (R103) rather than tryptophan (W103) at position 103 in the second exon of rev. In EIAVUK recipients the duplication was rapidly eliminated and R103 replaced by W103 in the viral population. Furthermore, removal of the 3' variant sequences from EIAVUK (EIAVUK3) resulted in an equivalent ($P = 0.013$) disease potential in *Equus caballus* to EIAVPV. The 68-bp duplication and/or R103 may limit peak viral RNA accumulation during acute infection.

Crary, S. M., J. S. Towner, et al. (2003). "Analysis of the role of predicted RNA secondary structures in Ebola virus replication." *Virology* **306**(2): 210.

<http://www.sciencedirect.com/science/article/B6WXR-48065PH-3/2/b3f8e26c237066cc92741bd5166291b0>

Thermodynamic modeling of Ebola viral RNA predicts the formation of RNA stem-loop structures at the 3' and 5' termini and panhandle structures between the termini of the genomic (or antigenomic) RNAs. Sequence analysis showed a high degree of identity among Ebola Zaire, Sudan, Reston, and Cote d'Ivoire subtype viruses in their 3' and 5' termini (18 nucleotides in length) and within a second region (internal by approximately 20 nucleotides). While base pairing of the two conserved regions could lead to the formation of the base of the putative stem-loop or panhandle structures, the intervening sequence variation altered the predictions for the rest of the structures. Using an in vivo minigenome replication system, we engineered mutations designed to disrupt potential base pairing in the viral RNA termini. Analysis of these variants by screening for enhanced green fluorescent protein reporter expression and by quantitation of minigenomic RNA levels demonstrated that the upper portions of the putative panhandle and 3' genomic structures can be destabilized without affecting virus replication.

de S. Leal, E., E. C. Holmes, et al. (2004). "Distinct patterns of natural selection in the reverse transcriptase gene of HIV-1 in the presence and absence of antiretroviral therapy." *Virology* **325**(2): 181.

<http://www.sciencedirect.com/science/article/B6WXR-4CKNM2P-1/2/d10182961d826af33f861005534bf173>

The emergence of human immunodeficiency virus (HIV) drug-resistant mutations during antiretroviral therapy is explained by either the preexistence of low-frequency-resistant strains before the start of therapy or by the selection of unsuppressed resistant strains during therapy. We used pairwise and maximum likelihood analyses of the ratio of nonsynonymous to synonymous substitutions per site (dN/dS) to study the extent of positive selection in the reverse transcriptase (RT) gene of HIV-1 from multiple data sets of drug-treated (117 sequences) and drug-naïve patients (270 sequences). In the pairwise analysis, evidence for positive selection (dN/dS > 1) was only found in drug-treated individuals and in codons conferring drug resistance. By the maximum likelihood method, a positive selection at codons conferring drug resistance was only observed in patients receiving therapy, and although positive selection was detected in drug-naïve patients, this was always at codons unrelated to drug resistance. We therefore document a striking difference in the process of allele fixation in drug resistance codons (RC) between populations of HIV-1-infected individuals naïve to treatment and those receiving therapy. Furthermore, although mutations associated with drug resistance are sometimes found in drug-naïve patients, we suggest that these are fixed because of linkage to sites experiencing immune escape. Finally, we show that compensatory changes are likely to be important in the development of HIV drug resistance by counter-acting the deleterious effects normally associated with drug resistance mutations.

Diaz, L. S., M. R. Stone, et al. (2003). "Differential gene expression in CD8+ cells exhibiting noncytotoxic anti-HIV activity." *Virology* **311**(2): 400.

<http://www.sciencedirect.com/science/article/B6WXR-48R1W06-1/2/49461db4c1b6230bf6ca418f7c8a52ad>

Suppressive subtractive hybridization with polymerase chain reaction was used to identify the gene(s) associated with the CD8+ cell noncytotoxic anti-HIV response. The differences in gene expression profiles of CD8+ cells from a pair of discordant HIV-positive identical twins were studied. Forty-nine genes were identified as expressed at higher levels in the CD8+ cells from the infected twin that inhibited viral replication. The differential expression of these genes was then evaluated using Q-PCR to determine if this gene expression pattern is evident in CD8+ cells from other HIV-positive subjects showing this antiviral activity. Three genes, including one unknown, were found to have significantly increased expression in antiviral CD8+ cells.

Err, S. M. and G. L. Smith (1991). "Vaccinia virus DNA ligase is nonessential for virus replication: Recovery of plasmids from virus-infected cells." *Virology* **180**(2): 625.

<http://www.sciencedirect.com/science/article/B6WXR-4BNJB4P-9D/2/d65490e1341b6663634aac34741ba7de>

The essentiality of the vaccinia virus DNA ligase gene, SalF 15R, for virus growth was tested by insertional mutagenesis. A plasmid containing E. coli gpt inserted within a large deletion in the DNA ligase gene was transfected into vaccinia virus-infected cells and recombinant viruses selected by three cycles of plaque purification in the presence of mycophenolic acid (MPA). Surprisingly, in some isolates, which replicated in a manner indistinguishable from wild type (WT) virus, the WT gene was replaced by the gpt allele, demonstrating that the DNA ligase gene is nonessential for growth in cultured cells. In other isolates the entire plasmid was integrated into the virus genome by a single crossover event and a functional copy of the DNA ligase was retained. Southern blot analyses of the latter, drug-resistant viruses indicated extra DNA fragments, of sizes inconsistent with predicted viral structures, which represent the plasmid products of homologous recombination. Hirt extracts from cells infected with such multiply plaque purified virus isolates yielded plasmids that produced ampicillin-resistant colonies after

transformation of *E. coli*. These plasmids were of two structures, representing either the original plasmid used for transfection, or a plasmid containing the WT ligase gene rescued by recombination with the virus genome. Similarly, insertional mutagenesis of the vaccinia virus thymidine kinase (TK) gene with gpt yielded plasmids containing mutant or wild type TK alleles when recombinant viruses were selected in MPA. Such plasmids were not isolated when TK minus viruses were selected in 5-bromodeoxyuridine (BUdR).

Forslund, O., A. Antonsson, et al. (2003). "Nucleotide sequence and phylogenetic classification of candidate human papilloma virus type 92." *Virology* **312**(2): 255.

<http://www.sciencedirect.com/science/article/B6WXR-497R4GV-1/2/3ee3a1cf50db66aaa024d175320a8dcb>

From a basal cell carcinoma (BCC) the complete genome of candidate human papillomavirus (HPV) type 92 was characterized. Phylogenetically, the candidate HPV 92 was relatively distantly related to other cutaneous HPV types within the B1 group. By quantitative real time PCR, 94 viral copies were present per cell in the BCC and another BCC contained 1 viral copy per cell. Lower copy numbers were found in two solar keratoses (1 copy per 33 cells and 1 copy per 60 cells) and two squamous cell carcinomas (1 copy per 436 cells and 1 copy per 1143 cells). The high viral load of HPV 92 in two BCCs differs from the low amount of HPV DNA reported from nonmelanoma skin cancers.

Garcia-Arriaza, J., E. Domingo, et al. "A segmented form of foot-and-mouth disease virus interferes with standard virus: A link between interference and competitive fitness." *Virology In Press, Corrected Proof* <http://www.sciencedirect.com/science/article/B6WXR-4FRJKXM-1/2/eef3b3a43deafa8d80a14fc77196d280>

Serial passage of foot-and-mouth disease virus (FMDV) in BHK-21 cells at high multiplicity of infection resulted in dominance of particles containing defective RNAs that were infectious by complementation in the absence of standard viral RNA. In the present study, we show that the defective FMDV particles interfere with replication of the cognate standard virus. Coinfections of defective FMDV with standard FMDV mutants that differ up to 151-fold in relative fitness have documented that the degree of interference is higher for low fitness than for high fitness standard virus. These comparisons suggest a likely overlap between those mechanisms of intracellular competition that underlie viral interference and those expressed as fitness differences between two viruses when they coinfect the same cells. Interference may contribute to the selective pressures that help maintain dominance of segmented defective RNAs over the standard FMDV genome.

Greenblatt, R. J., T. M. Work, et al. (2004). "The *Ozobranchus* leech is a candidate mechanical vector for the fibropapilloma-associated turtle herpesvirus found latently infecting skin tumors on Hawaiian green turtles (*Chelonia mydas*)." *Virology* **321**(1): 101.

<http://www.sciencedirect.com/science/article/B6WXR-4BV4VKV-2/2/19bfbebe0dda86bdd60d7193095f4975>

Fibropapillomatosis (FP) of marine turtles is a neoplastic disease of ecological concern. A fibropapilloma-associated turtle herpesvirus (FPTHV) is consistently present, usually at loads exceeding one virus copy per tumor cell. DNA from an array of parasites of green turtles

(*Chelonia mydas*) was examined with quantitative PCR (qPCR) to determine whether any carried viral loads are sufficient to implicate them as vectors for FPTHV. Marine leeches (*Ozobranchus* spp.) were found to carry high viral DNA loads; some samples approached 10 million copies per leech. Isopycnic sucrose density gradient/qPCR analysis confirmed that some of these copies were associated with particles of the density of enveloped viruses. The data implicate the marine leech *Ozobranchus* as a mechanical vector for FPTHV. Quantitative RT-PCR analysis of FPTHV gene expression indicated that most of the FPTHV copies in a fibropapilloma have restricted DNA polymerase expression, suggestive of latent infection.

Hasegawa, H., M. Yoshida, et al. (1992). "Mutations in the envelope protein of Japanese encephalitis virus affect entry into cultured cells and virulence in mice." *Virology* **191**(1): 158.

<http://www.sciencedirect.com/science/article/B6WXR-4BNVGWK-3V/2/2f8171983d7401278b4594720968c598>

The nucleotide sequences of the envelope protein of the Kamiyama 1 strain of Japanese encephalitis (JE) virus and a passaged mutant (Kamiyama 2 strain) were determined. Two amino acid differences, Ser-Phe at residue 364 and Asn-Ile at residue 367, distinguished Kamiyama 2 from Kamiyama 1. Six neutralization-resistant variants were selected from these two strains using a JE species-specific monoclonal antibody with neutralization and hemagglutination-inhibition reactivities. All variants had a single amino acid substitution at residue 52 and significantly reduced reactivity with other JE species-specific monoclonal antibodies. The variants derived from Kamiyama 2 strain showed reduced virulence in 3-week-old mice after peripheral inoculation but were as virulent as the parent virus when inoculated intracranially. These variants also showed altered early virus-cell interaction but not replication and reproduction in Vero cells. These findings indicate that the mutations at residues 52, 364, and 367 affect early virus-cell interaction in Vero cells and virulence in mice.

Hout, D. R., M. L. Gomez, et al. (2004). "Fusion of the upstream vpu sequences to the env of simian human immunodeficiency virus (SHIVKU-1bMC33) results in the synthesis of two envelope precursor proteins, increased numbers of virus particles associated with the cell surface and is pathogenic for pig-tailed macaques." *Virology* **323**(1): 91.

<http://www.sciencedirect.com/science/article/B6WXR-4C7VXCD-6/2/8de4ae238b884e40d1141f4745b7b46d>

Previous studies have shown that the gene coding for the Vpu protein of the human immunodeficiency virus type 1 (HIV-1) is 5' to the env gene, is in a different reading frame, and overlaps the env by 90 nucleotides. In this study, we examined the processing of the Env protein as well as the maturation and infectivity of a virus (SHIVVpenv) in which a single nucleotide was removed at the vpu-env junction, fusing the first 162 bases of vpu to the env ORF. Pulse-chase analysis revealed that SHIVVpenv-infected cells gave rise to two precursor glycoprotein species (gp160 and gp175). Immune precipitation results also revealed that an anti-Vpu serum could immune precipitate the gp175 precursor, suggesting that the amino-terminal Vpu sequence was fused to the Env protein. Growth curves revealed that the SHIVVpenv-inoculated cultures released approximately three times more p27 into the culture medium than parental SHIVKU-1bMC33. Electron microscopy revealed that while both viruses matured at the cell plasma membrane, significantly higher quantities of virus particles were cell associated on SHIVVpenv-infected cells compared to cultures inoculated with parental SHIVKU-1bMC33. Furthermore, virus was observed maturing into intracellular vesicles of SHIVVpenv-infected cells. To assess the pathogenicity of SHIVVpenv, three pig-tailed macaques were inoculated with the SHIVVpenv and monitored for 6 months for CD4⁺ T cell levels, viral loads, and the stability of the deletion at the

vpu-env junction. Our results indicated that SHIVVpenv caused a severe CD4⁺ T cell loss in all three macaques within weeks of inoculation. Sequence analysis of the vpu gene analyzed from sequential PBMC samples derived from macaques revealed that this mutation was stable during the period of rapid CD4⁺ T cell loss. Sequence analysis showed that with increasing time of infection, the one base pair deletion was repaired in all three macaques inoculated with SHIVVpenv with the reversion occurring at 10 weeks in macaque CT1G and at 12 weeks in macaque CP3R and CT1R. These results indicate that fusion of the first 54 amino acids of Vpu to Env results in intracellular maturation of virus, and accumulation of virus within intracellular vesicles as well as on the cell plasma membrane. Our results indicate that while fusion of the vpu gene to env results in a virus that is still pathogenic for pig-tailed macaques, there is a selective pressure to maintain the vpu and env genes in separate reading frames.

Hu, F.-Q. and D. J. Pickup (1991). "Transcription of the terminal loop region of vaccinia virus DNA is initiated from the telomere sequences directing DNA resolution." *Virology* **181**(2): 716.

<http://www.sciencedirect.com/science/article/B6WXR-4CC99M9-2K/2/45192e35dfe638cda0d2362b762b215a>

The telomeres of vaccinia virus DNA are transcribed at late times after infection. Analysis of cDNAs of RNA transcripts of the terminal loop region of the viral DNA shows that both inverted and complementary forms of the terminal loop region are transcribed. These late RNAs, which contain 5' poly(A) sequences, do not appear to encode any proteins. The transcriptional start sites for most of these RNAs are within the sequences that direct the resolution of concatemeric DNA replication intermediates ([13.], *J. Virol* 63, 4354-4361). This suggests that the process of DNA resolution may involve transcription initiated from the telomere sequences required for resolution.

Hurtado, C., A. G. Granja, et al. (2004). "The C-type lectin homologue gene (EP153R) of African swine fever virus inhibits apoptosis both in virus infection and in heterologous expression." *Virology* **326**(1): 160.

<http://www.sciencedirect.com/science/article/B6WXR-4CRY5YB-2/2/c779248574a5ac4597a93a789c7c83df>

The open reading frame EP153R of African swine fever virus (ASFV) encodes a nonessential protein that has been involved in the hemadsorption process induced in virus-infected cells. By the use of a virus deletion mutant lacking the EP153R gene, we have detected, in several virus-sensitive cells, increased levels of caspase-3 and cell death as compared with those obtained after infection with the parental BA71V strain. Both transient and stable expression of the EP153R gene in Vero or COS cells resulted in a partial protection of the transfected lines from the apoptosis induced in response to virus infection or external stimuli. The presence of gene EP153R resulted in a reduction of the transactivating activity of the cellular protein p53 in Vero cell cultures in which apoptosis was induced by virus infection or staurosporine treatment. This is to our knowledge the first description of a viral C-type lectin with anti-apoptotic properties.

Junying, J., K. Herrmann, et al. (2003). "Absence of epstein-barr virus DNA in the tumor cells of european hepatocellular carcinoma." *Virology* **306**(2): 236.

<http://www.sciencedirect.com/science/article/B6WXR-48065PH->

1/2/3c7a3586600a8fafdaf2e6edfad3f4ef

The Epstein-Barr virus (EBV) has recently been associated with hepatocellular carcinoma (HCC) arising in Japanese patients. We analyzed 82 cases of HCC from Germany and the U.K. for the presence of EBV DNA and viral gene products within tumor cells. Initial screening of whole sections using quantitative (Q)-PCR detected EBV DNA in 9/58 U.K. cases and in 9/24 German cases; in positive cases viral load was very low, ranging between 1.4 and 49.1 copies of the EBV genome/1000 cell equivalents, compared to much higher values for EBV-positive Hodgkin's disease and nasopharyngeal carcinoma controls (range, 714-3259/1000 cells). EBV DNA was not detected in the tumor cells of any of the Q-PCR-positive cases either by Q-PCR of pure tumor cell populations isolated by laser capture microdissection or by isotopic in situ hybridization. Furthermore, none of the German or U.K. HCC tumors tested positive for EBER or EBNA1 expression in tumor cells. Our results provide strong evidence that HCCs from the U.K. or Germany are not associated with EBV.

Karlas, A., R. Kurth, et al. (2004). "Inhibition of porcine endogenous retroviruses by RNA interference: increasing the safety of xenotransplantation." *Virology* **325**(1): 18.

<http://www.sciencedirect.com/science/article/B6WXR-4CHRYD6-2/2/68037df1ea9e3978ab70a1bf7179f8cb>

Transplantation of porcine xenografts into human recipients is a realistic option to overcome the growing worldwide shortage of suitable allogeneic organs. However, there remains the risk of infection by porcine endogenous retroviruses (PERVs) that cannot be eliminated like that by other microorganisms by breeding pigs under specified pathogen-free conditions. To reduce the release of PERVs by porcine transplants, a new approach, RNA interference (RNAi), was applied. Here, we show significant reduction of PERV expression by synthetic short interfering RNAs (siRNAs) corresponding to different parts of the viral genes gag, pol, and env. The most inhibitory sequences were selected and expressed as short hairpin RNAs (shRNAs) by a polymerase III vector system leading to persistent suppression of PERV replication. Cells or organs from transgenic pigs producing such shRNAs should increase the safety of xenotransplantation.

Khare, P. D., A. G. Rosales, et al. (2003). "Epitope selection from an uncensored peptide library displayed on avian leukosis virus." *Virology* **315**(2): 313.

<http://www.sciencedirect.com/science/article/B6WXR-49H7027-4/2/12360a1545f59934758fb1ed7571409f>

Phage display libraries have provided an extraordinarily versatile technology to facilitate the isolation of peptides, growth factors, single chain antibodies, and enzymes with desired binding specificities or enzymatic activities. The overall diversity of peptides in phage display libraries can be significantly limited by Escherichia coli protein folding and processing machinery, which result in sequence censorship. To achieve an optimal diversity of displayed eukaryotic peptides, the library should be produced in the endoplasmic reticulum of eukaryotic cells using a eukaryotic display platform. In the accompanying article, we presented experiments that demonstrate that polypeptides of various sizes could be efficiently displayed on the envelope glycoproteins of a eukaryotic virus, avian leukosis virus (ALV), and the displayed polypeptides could efficiently attach to cognate receptors without interfering with viral attachment and entry into susceptible cells. In this study, methods were developed to construct a model library of randomized eight amino acid peptides using the ALV eukaryotic display platform and screen the library for specific

epitopes using immobilized antibodies. A virus library with approximately 2×10^6 different members was generated from a plasmid library of approximately 5×10^6 diversity. The sequences of the randomized 24 nucleotide/eight amino acid regions of representatives of the plasmid and virus libraries were analyzed. No significant sequence censorship was observed in producing the virus display library from the plasmid library. Different populations of peptide epitopes were selected from the virus library when different monoclonal antibodies were used as the target. The results of these two studies clearly demonstrate the potential of ALV as a eukaryotic platform for the display and selection of eukaryotic polypeptides libraries.

Konishi, E., S. Pincus, et al. (1992). "Mice immunized with a subviral particle containing the Japanese encephalitis virus prM/M and E proteins are protected from lethal JEV infection." *Virology* **188**(2): 714.

<http://www.sciencedirect.com/science/article/B6WXR-4BNVGYP-4J/2/b59db987cea8cd51104dac7004013f3d>

Extracellular subviral particles produced by HeLa cells infected with a recombinant vaccinia virus encoding the prM and E genes of Japanese encephalitis virus (JEV) were purified and characterized. These particles contained the JEV prM/M and E proteins embedded in a lipid bilayer, and RNA was not detected in particles using the polymerase chain reaction and primers recognizing a part of the JEV E gene. The particles were uniformly spherical with a 20-nm diameter and had 5-nm projections on their surface. Mice that received a single inoculation of the purified extracellular particles emulsified with Freund's complete adjuvant were fully protected against 4.9×10^5 LD₅₀ of JEV. Comparison of the neutralizing and hemagglutination-inhibiting antibody titers and radioimmunoprecipitation data showed that immunization with the particles induced an immune response similar to that following inoculation with the recombinant vaccinia virus.

Lane, B. R., S. R. King, et al. (2003). "The C-X-C chemokine IP-10 stimulates HIV-1 replication." *Virology* **307**(1): 122.

<http://www.sciencedirect.com/science/article/B6WXR-47NF5P0-6/2/492be838c2ac4cd24e5a0642c1241388>

Chemokines play critical roles in HIV-1 infection, serving both to modulate viral replication and to recruit target cells to sites of infection. Interferon- γ -inducible protein 10 (IP-10/CXCL10) is a C-X-C chemokine that acts specifically upon activated T cells and macrophages and attracts T cells into the cerebrospinal fluid (CSF) in HIV-associated neurological disease. We now demonstrate that IP-10 stimulates HIV-1 replication in monocyte-derived macrophages and peripheral blood lymphocytes. We further demonstrate that neutralization of endogenous IP-10 or blocking the function of its receptor, CXCR3, reduces HIV-1 replication in these same cells. Therefore, blocking the interaction between IP-10 and CXCR3 represents a possible new target for anti-retroviral therapy.

Liao, H.-J., C. C. Baker, et al. (2004). "cis-Acting and trans-acting modulation of equine infectious anemia virus alternative RNA splicing." *Virology* **323**(1): 131.

<http://www.sciencedirect.com/science/article/B6WXR-4C7W0D0-3/2/1ab717644c75e3f90794844b038ac7f1>

Equine infectious anemia virus (EIAV), a lentivirus distantly related to HIV-1, encodes regulatory proteins, EIAV Tat (ETat) and Rev (ERev), from a four-exon mRNA. Exon 3 of the tat/rev mRNA contains a 30-nucleotide purine-rich element (PRE) which binds both ERev and SF2/ASF, a member of the SR family of RNA splicing factors. To better understand the role of this element in the regulation of EIAV pre-mRNA splicing, we quantified the effects of mutation or deletion of the PRE on exon 3 splicing in vitro and on alternative splicing in vivo. We also determined the branch point elements upstream of exons 3 and 4. In vitro splicing of exon 3 to exon 4 was not affected by mutation of the PRE, and addition of purified SR proteins enhanced splicing independently of the PRE. In vitro splicing of exon 2 to exon 3 was dependent on the PRE; under conditions of excess SR proteins, either the PRE or the 5' splice site of exon 3 was sufficient to activate splicing. We applied isoform-specific primers in real-time RT-PCR reactions to quantitatively analyze alternative splicing in cells transfected with rev-minus EIAV provirus constructs. In the context of provirus with wild-type exon 3, greater than 80% of the viral mRNAs were multiply spliced, and of these, less than 1% excluded exon 3. Deletion of the PRE resulted in a decrease in the relative amount of multiply spliced mRNA to about 40% of the total and approximately 39% of the viral mRNA excluded exon 3. Ectopic expression of ERev caused a decrease in the relative amount of multiply spliced mRNA to approximately 50% of the total and increased mRNAs that excluded exon 3 to about 4%. Over-expression of SF2/ASF in cells transfected with wild-type provirus constructs inhibited splicing but did not significantly alter exon 3 skipping.

Liu, M., J. M. Wood, et al. (2003). "Preparation of a standardized, efficacious agricultural H5N3 vaccine by reverse genetics." *Virology* **314**(2): 580.

<http://www.sciencedirect.com/science/article/B6WXR-49D2CJG-2/2/217b8ef52590465770ad2774f3e12dad>

Options for the control of emerging and reemerging H5N1 influenza viruses include improvements in biosecurity and the use of inactivated vaccines. Commercially available H5N2 influenza vaccine prevents disease signs and reduces virus load but does not completely prevent virus shedding after challenge with H5N1 virus. By using reverse genetics, we prepared an H5N3 vaccine whose hemagglutinin is 99.6% homologous to that of A/CK/HK/86.3/02 (H5N1). We used the internal genes of A/PR/8/34 and the H5 of A/Goose/HK/437.4/99 (H5N1) after deletion of basic amino acids from its connecting peptide region. The resulting virus was not lethal to chicken embryos and grew to high HA titers in eggs, allowing preparation of HA protein-standardized vaccine in unconcentrated allantoic fluid. The N3 neuraminidase, derived from A/Duck/Germany/1215/73 (H2N3), permitted discrimination between vaccinated and naturally infected birds. The virus construct failed to replicate in quail and chickens. Similar to parental A/PR/8/34 (H1N1), it replicated in mice and ferrets and spread to the brains of mice; therefore, it should not be used as a live-attenuated vaccine. The H5N3 vaccine, at doses of 1.2 [μg] HA, induced HI antibodies in chickens and prevented death, signs of disease, and markedly reduced virus shedding after challenge with A/CK/HK/86.3/02 (H5N1) but did not provide sterilizing immunity. Thus, reverse genetics allows the inexpensive preparation of standardized, efficacious H5N3 poultry vaccines that may also reduce the reemergence of H5N1 genotypes.

Lo, K. M. S., M. A. Biasolo, et al. (1992). "Inhibition of replication of HIV-1 by retroviral vectors expressing tat-antisense and anti-tat ribozyme RNA." *Virology* **190**(1): 176.

<http://www.sciencedirect.com/science/article/B6WXR-4CC9B1S-91/2/9e3d3d70e88f6ceb6d86720363cf1895>

A ribozyme was constructed that specifically cleaves RNA that contains the first coding exon of the tat gene of HIV-1. This anti-tat ribozyme was incorporated into a Moloney murine leukemia

virus vector. A sequence containing only the 48-nucleotide antisense region of the ribozyme was also inserted into the retroviral vector. Human T-cell lines constitutively producing the tat-antisense and the anti-tat ribozyme RNA were created by transduction into Jurkat cells. When challenged with HIV-1, both the tat-antisense and anti-tat ribozyme-producing cells inhibited the replication of HIV-1. The antisense vector conferred a greater resistance to HIV-1 replication than did the anti-tat ribozyme vector.

Machado, A. V., N. Naffakh, et al. (2003). "Expression of a foreign gene by stable recombinant influenza viruses harboring a dicistronic genomic segment with an internal promoter." *Virology* **313**(1): 235.

<http://www.sciencedirect.com/science/article/B6WXR-491RPYR-2/2/0974c90f80c1cb48cdb4b55d253c8d71>

Based on the observation that an internally located 3' promoter sequence can be functional (R. Flick and G. Hobom, *Virology*, 1999, 262(1), 93-103), we generated transfectant influenza A viruses harboring a dicistronic segment containing the CAT gene (660 nt) or a fragment of the Mengo virus VP0 capsid gene (306 nt) under the control of a duplicated 3' promoter sequence. Despite slightly reduced NA expression, the transfectant viruses replicated efficiently and proved to be stable upon both serial passage in vitro in MDCK cells and in vivo replication in the pulmonary tissue of infected mice. Internal initiation of replication and transcription from the second, internal, 3' promoter directed the synthesis of subgenomic vRNA and mRNA and therefore permitted expression of the foreign gene product, e.g., the CAT enzyme. The design of this vector may prove particularly appropriate for the utilization of influenza virus for the expression of heterologous proteins in their native form.

Makarova, N. V., H. Ozaki, et al. (2003). "Replication and transmission of influenza viruses in Japanese quail." *Virology* **310**(1): 8.

<http://www.sciencedirect.com/science/article/B6WXR-48BTXCW-5/2/45c183e2adb7354f08d70b20631dee9e>

Quail have emerged as a potential intermediate host in the spread of avian influenza A viruses in poultry in Hong Kong. To better understand this possible role, we tested the replication and transmission in quail of influenza A viruses of all 15 HA subtypes. Quail supported the replication of at least 14 subtypes. Influenza A viruses replicated predominantly in the respiratory tract. Transmission experiments suggested that perpetuation of avian influenza viruses in quail requires adaptation. Swine influenza viruses were isolated from the respiratory tract of quail at low levels. There was no evidence of human influenza A or B virus replication. Interestingly, a human-avian recombinant containing the surface glycoprotein genes of a quail virus and the internal genes of a human virus replicated and transmitted readily in quail; therefore, quail could function as amplifiers of influenza virus reassortants that have the potential to infect humans and/or other mammalian species.

Matsumoto, Y., Y. Momoi, et al. (1992). "Detection of enhancer repeats in the long terminal repeats of feline leukemia viruses from cats with spontaneous neoplastic and nonneoplastic diseases." *Virology* **189**(2): 745.

<http://www.sciencedirect.com/science/article/B6WXR-4BNVHMJ-GJ/2/c5e57e09f04f354ecf3c317f70993670>

Enhancer duplication in the long terminal repeat of feline leukemia virus (FeLV) was examined in primary cells from naturally FeLV-infected cats with various neoplastic and nonneoplastic diseases using the polymerase chain reaction. In all cases, a 170-bp band, corresponding to a standard exogenous FeLV with one copy of enhancer, was detected. Repeated enhancer sequences were found in all 8 cases of thymic-form lymphosarcoma, in some cases of lymphosarcoma of other forms (3/8) and myeloid tumors (2/3), and in only 1 of 6 cases with nonneoplastic diseases. The copy number of FeLV proviruses with a repeated enhancer seemed higher than that of those with one copy of enhancer in 3 cases of thymic form lymphosarcoma. In 5 cases of thymic form lymphosarcoma and in 1 case of erythroleukemia, coexistent FeLVs with double and triple enhancers of different sizes were found. Of the enhancer elements, only the SV40 core binding site was found in all the enhancer direct repeats of these FeLVs. All the provirus clones with single and duplicated enhancer sequences from a single tumor showed mutations or deletions characteristic to that tumor, indicating that enhancer repeats may arise in individual animals after infection with a single virus clone. The present findings indicate that FeLV with enhancer repeats generated in the cat is associated with the induction of neoplastic diseases in natural conditions.

Milavetz, B. (2004). "Hyperacetylation and differential deacetylation of histones H4 and H3 define two distinct classes of acetylated SV40 chromosomes early in infection." *Virology* **319**(2): 324.

<http://www.sciencedirect.com/science/article/B6WXR-4BFVHGX-1/2/8edbdf09a46559a5faeee51dfd88dd61>

SV40 chromosomes undergoing encapsidation late in infection and SV40 chromatin in virions are hyperacetylated on histones H4 and H3. However, the fate of the SV40 chromosomes containing hyperacetylated histones in a subsequent round of infection has not been determined. In order to determine if SV40 chromosomes undergo changes in the extent of histone acetylation during early infection, we have analyzed SV40 chromosomes isolated 30 min and 3 h postinfection by quantitative ChIP assays, depletion ChIP assays, competitive ChIP assays, and ChIP assays combined with restriction endonuclease sensitivity using antibodies to hyperacetylated histones H4 and H3. We have shown that at 30 min postinfection, the hyperacetylated histones are associated with two distinct classes of SV40 chromosomes. One form is hyperacetylated specifically on histone H4 while a second form is hyperacetylated on both H4 and H3. Both forms of chromosomes appear to contain a nucleosome-free promoter region. Over the course of the next few hours of infection, the class of SV40 chromosomes hyperacetylated on only H4 is reduced or completely eliminated through deacetylation.

Morris, B. A. M., K. A. Richardson, et al. (1992). "The nucleotide sequence of the infectious cloned dna component of tobacco yellow dwarf virus reveals features of geminiviruses infecting monocotyledonous plants." *Virology* **187**(2): 633.

<http://www.sciencedirect.com/science/article/B6WXR-4BNVHHH-D4/2/5a25cc1ce816b81022fa65f8a4e69ad3>

An infectious clone of the Australian geminivirus tobacco yellow dwarf virus (TobYDV) was constructed from virus-specific double-stranded DNA isolated from infected tobacco and used to demonstrate a single-component genome. The nucleotide sequence of TobYDV DNA comprises 2580 nucleotides. TobYDV DNA has three coding regions, two in the virion sense and one in the complementary sense, homologous to those identified for other geminiviruses, particularly those infecting monocotyledonous (monocot) plants. The complementary sense coding region is comprised of two overlapping reading frames, with an intron of 86 nucleotides. Efficient splicing of the mRNA for this coding region was observed in the infected dicotyledonous (dicot) hosts bean

and tobacco despite the intron having an A + U content (57%) more typical of geminiviruses of monocot plants. TobYDV encapsidates a small oligonucleotide able to prime synthesis of the complementary DNA strand in vitro. The TobYDV genome organization, low A + U intron, and encapsidated oligonucleotide primer resemble those of the monocot-infecting geminiviruses. These results strongly suggest that TobYDV is a monocot geminivirus which has become adapted to dicot hosts.

Naidu, R. A., G. B. Collins, et al. (1992). "Peanut stunt virus satellite RNA: Analysis of sequences that affect symptom attenuation in tobacco." *Virology* **189**(2): 668.

<http://www.sciencedirect.com/science/article/B6WXR-4BNVHMJ-G8/2/c68492ddf7df7f6bad3c913a7fb16503>

The V-satellite RNA (V-satRNA) of peanut stunt virus (PSV) has no effect on symptoms produced in tobacco by PSV. In contrast, the G-satRNA induced complete or nearly complete suppression of systemic symptom development. Because G-satRNA differs from V-satRNA in only five nucleotide positions, these two satRNAs provide excellent material for investigating the molecular basis of satRNA-mediated symptom attenuation. For this purpose, we constructed transcription vectors containing full-length cDNA clones from which infectious RNA transcripts can be synthesized in vitro, and produced chimeric and mutant satRNA molecules. Although an A → C substitution at position 362 of the V-satRNA molecule delayed systemic symptom development and reduced symptom severity, changes at both nucleotide positions 226 (C → U) and 362 (A → C) of V-satRNA were required for suppression of systemic symptom development. Our results are consistent with the idea that PSV satRNAs are noncoding molecules that exert their biological activities by directly interacting with host/viral components.

Neilan, J. G., L. Zsak, et al. (2004). "Neutralizing antibodies to African swine fever virus proteins p30, p54, and p72 are not sufficient for antibody-mediated protection." *Virology* **319**(2): 337.

<http://www.sciencedirect.com/science/article/B6WXR-4BFXSHX-5/2/3827aabe53024a72505f4e0ce6196bbe>

Although antibody-mediated immune mechanisms have been shown to be important in immunity to ASF, it remains unclear what role virus neutralizing antibodies play in the protective response. Virus neutralizing epitopes have been identified on three viral proteins, p30, p54, and p72. To evaluate the role(s) of these proteins in protective immunity, pigs were immunized with baculovirus-expressed p30, p54, p72, and p22 from the pathogenic African swine fever virus (ASFV) isolate Pr4. ASFV specific neutralizing antibodies were detected in test group animals. Following immunization, animals were challenged with 10⁴ TCID₅₀ of Pr4 virus. In comparison to the control group, test group animals exhibited a 2-day delay to onset of clinical disease and reduced viremia levels at 2 days postinfection (DPI); however, by 4 DPI, there was no significant difference between the two groups and all animals in both groups died between 7 and 10 DPI. These results indicate that neutralizing antibodies to these ASFV proteins are not sufficient for antibody-mediated protection.

Noesch, J. P. F., S. F. Cotmore, et al. (1992). "Expression of functional parvoviral NS1 from recombinant vaccinia virus: Effects of mutations in the nucleotide-binding motif." *Virology* **191**(1): 406.

<http://www.sciencedirect.com/science/article/B6WXR-4BNVGWK->

4R/2/db2cea1fd2bced28db1ff6bc5af69681

The gene encoding the major replicative protein, NS1, of minute virus of mice (MVM) was transferred into a recombinant vaccinia virus vector in place of the vaccinia thymidine kinase gene. The NS1 gene was placed under control of a bacteriophage T7 promoter and expressed in cells coinfecting with another recombinant vaccinia virus, vTF7-3, which encodes the T7 RNA polymerase. Expression of NS1 was further enhanced by the presence of a 5' untranslated region, derived from encephalomyocarditis virus, which allows efficient cap-independent translation. This system was used to produce and analyze wild-type NS1 and two mutant forms of the protein, NS1 K405R and NS1 K405M, in which the highly conserved lysine codon located in the putative purine triphosphate binding site of NS1 was changed to arginine and methionine, respectively. Full-length NS1 was expressed efficiently in both human and mouse cells infected with each of the three recombinant viruses, and in each case the NS1 was rapidly and efficiently translocated into the nucleus. Wild-type NS1 expressed in this way was biologically active. It was able to trans-activate an MVM P38 promoter located in a host chromosomal site, whereas the two mutant forms of NS1 showed no significant activity in this assay, and it was capable of resolving palindromic junction fragments cloned from multimeric MVM replicative form DNA molecules. These substrates, representing MVM genomic left-end:left-end and right-end:right-end fusions, were resolved in a DNA synthesis-dependent *in vitro* reaction supplemented with nuclear extracts containing recombinant wild-type NS1. Neither of the two mutant forms of the polypeptide had any detectable activity in this assay.

Oh, S. T., J. S. Seo, et al. (2004). "A naturally derived gastric cancer cell line shows latency I Epstein-Barr virus infection closely resembling EBV-associated gastric cancer." *Virology* **320**(2): 330.

<http://www.sciencedirect.com/science/article/B6WXR-4BN0GV4-1/2/6dc326e0ac9b65841a2f1dde5a5f0841>

In a process seeking out a good model cell line for Epstein-Barr virus (EBV)-associated gastric cancer, we found that one previously established gastric adenocarcinoma cell line is infected with type 1 EBV. This SNU-719 cell line from a Korean patient expressed cytokeratin without CD19 or CD21 expression. In SNU-719, EBNA1 and LMP2A were expressed, while LMP1 and EBNA2 were not. None of the tested lytic EBV proteins were detected in this cell line unless stimulated with phorbol ester. EBV infection was also shown in the original carcinoma tissue of SNU-719 cell line. Our results support the possibility of a CD21-independent EBV infection of gastric epithelial cells *in vivo*. As the latent EBV gene expression pattern of SNU-719 closely resembles that of the EBV-associated gastric cancer, this naturally derived cell line may serve as a valuable model system to clarify the precise role of EBV in gastric carcinogenesis.

Owens, R. A., S. M. Thompson, et al. (1991). "Effects of random mutagenesis upon potato spindle tuber viroid replication and symptom expression." *Virology* **185**(1): 18.

<http://www.sciencedirect.com/science/article/B6WXR-4BSDY28-D4/2/42c7dec15d4365c4011eab17719eed7c>

A combination of random chemical mutagenesis plus temperature gradient gel electrophoresis was used to isolate a collection of 57 potato spindle tuber viroid (PSTV) cDNAs containing mutations distributed throughout the entire 359 nucleotide genome. Although the presence of multiple mutations was often associated with a loss of cDNA infectivity, infectious PSTV cDNAs containing as many as four unlinked alterations could be isolated. Several mutations in the pathogenicity domain and left terminal loop were stably maintained in the resulting progeny, but

those which affect base pairing in secondary hairpins I and II were either lethal or rapidly reverted to wild-type. One stable C → U substitution which may promote significant structural rearrangement within the right side of the pathogenicity domain had no detectable effect upon symptom expression. The variable domains of several noninfectious mutants contained an A → G substitution which is likely to inhibit the in vitro formation of secondary hairpin II via stabilization of the native structure, and the lethal nature of this mutation was confirmed by oligonucleotide-directed mutagenesis. Several lines of evidence now point toward an essential role for secondary hairpin II in the replication of PSTV and related viroids.

Reis, J. K. P., J. K. Craig, et al. (2003). "Characterization of EIAV LTR variability and compartmentalization in various reservoir tissues of long-term inapparent carrier ponies." *Virology* **311**(1): 169.

<http://www.sciencedirect.com/science/article/B6WXR-48NBY5W-2/2/4ebced3d11c340dfe1cbe2ed0be5b325>

Dynamic genomic variation resulting in changes in envelope antigenicity has been established as a fundamental mechanism of persistence by equine infectious anemia virus (EIAV), as observed with other lentiviruses, including HIV-1. In addition to the reported changes in envelope sequences, however, certain studies indicate the viral LTR as a second variable EIAV gene, with the enhancer region being designated as hypervariable. These observations have led to the suggestion that LTR variation may alter viral replication properties to optimize to the microenvironment of particular tissue reservoirs. To test this hypothesis directly, we examined the population of LTR quasispecies contained in various tissues of two inapparent carrier ponies experimentally infected with a reference EIAV biological clone for 18 months. The results of these studies demonstrated that the EIAV LTR is in fact highly conserved with respect to the infecting LTR species after 1.5 years of persistent infection and regardless of the tissue reservoir. Thus, these comprehensive analyses demonstrate for the first time that the EIAV LTR is highly conserved during long-term persistent infection and that the observed variations in viral LTR are associated more with in vitro adaptation to replication in cultured cells rather than in vivo replication in natural target cells.

Rodahl, E. and J. G. Stevens (1992). "Differential accumulation of herpes simplex virus type 1 latency-associated transcripts in sensory and autonomic ganglia." *Virology* **189**(1): 385.

<http://www.sciencedirect.com/science/article/B6WXR-4BNVK2T-139/2/0ed1fbaea65578e07c1e0b4e660b346d>

We have analyzed the capacity of sensory and autonomic ganglia to demonstrate latency-associated transcripts (LATs) following inoculation of the anterior chamber of the mouse eye with Herpes simplex virus type 1 (HSV-1). In autonomic ganglia, the number of LAT-containing neurons decreased 50-fold or more from the acute to the latent phase, while in the trigeminal ganglion, the decrease was less than 2-fold. The decrease in autonomic ganglia could not be related to destruction of neurons expressing LATs, since these ganglia harbored substantial amounts of viral DNA. The data demonstrate that during the latent phase of the infection, accumulation of LATs varies depending on the type of infected neuron and suggest that some neurons may harbor a latent infection in the absence of LAT expression.

Santin, A. D., F. Zhan, et al. (2005). "Gene expression profiles of primary HPV16- and HPV18-infected

early stage cervical cancers and normal cervical epithelium: identification of novel candidate molecular markers for cervical cancer diagnosis and therapy." *Virology* **331**(2): 269.

<http://www.sciencedirect.com/science/article/B6WXR-4DVBHP7-3/2/ea8d19b0f585ff8fa712d3a765aeab10>

With the goal of identifying genes with a differential pattern of expression between invasive cervical carcinomas (CVX) and normal cervical keratinocytes (NCK), we used oligonucleotide microarrays to interrogate the expression of 14,500 known genes in 11 primary HPV16 and HPV18-infected stage IB-IIA cervical cancers and four primary normal cervical keratinocyte cultures. Hierarchical cluster analysis of gene expression data identified 240 and 265 genes that exhibited greater than twofold up-regulation and down-regulation, respectively, in primary CVX when compared to NCK. Cyclin-dependent kinase inhibitor 2A (CDKN2A/p16), mesoderm-specific transcript, forkhead box M1, v-myb myeloblastosis viral oncogene homolog (avian)-like2 (v-Myb), minichromosome maintenance proteins 2, 4, and 5, cyclin B1, prostaglandin E synthase (PTGES), topoisomerase II alpha (TOP2A), ubiquitin-conjugating enzyme E2C, CD97 antigen, E2F transcription factor 1, and dUTP pyrophosphatase were among the most highly overexpressed genes in CVX when compared to NCK. Down-regulated genes in CVX included transforming growth factor beta 1, transforming growth factor alpha, CFLAR, serine proteinase inhibitors (SERPING1 and SERPINF1), cadherin 13, protease inhibitor 3, keratin 16, and tissue factor pathway inhibitor-2 (TFPI-2). Differential expression of some of these genes including CDKN2A/p16, v-Myb, PTGES, and TOP2A was validated by quantitative real-time PCR. Flow cytometry on primary CVX and NCK and immunohistochemical staining of formalin fixed paraffin-embedded tumor specimens from which primary CVX cultures were derived as well as from a separate set of invasive cervical cancers confirmed differential expression of the CDKN2A/p16 and PTGES markers on CVX versus NCK. These results identify several genes that are coordinately dysregulated in cervical cancer, likely representing common signaling pathways triggered by HPV transformation. Moreover, these data obtained with highly purified primary tumor cultures highlight novel molecular features of human cervical cancer and provide a foundation for the development of new type-specific diagnostic and therapeutic strategies for this disease.

Scarpa, M., D. Cournoyer, et al. (1991). "Characterization of recombinant helper retroviruses from moloney-based vectors in ecotropic and amphotropic packaging cell lines." *Virology* **180**(2): 849.

<http://www.sciencedirect.com/science/article/B6WXR-4BNJB4P-BF/2/e3bb950cda8aa222374eb0b803dde254>

We have characterized the recombinant replication-competent retrovirus (RCV) arising from p[Delta]N2-derived vectors in the packaging cell lines [Psi]2 (ecotropic) and PA317 (amphotropic). Detailed restriction patterns and sequence of the envelope region of these RCVs has indicated that they arose from recombination events between the virus plasmids used to create the packaging cell line and the vectors. There was no evidence of recombination involving endogenous murine retroviral sequences in the packaging cell line or in transduced hematopoietic cells. In addition, we have confirmed that the mutation of the start codon of the pXM5(N2) derivatives gag+ sequence drastically decreased the occurrence of RCV production. These results offer encouragement that the risk of RCV production can be adequately decreased in gene therapy applications of defective retrovirus vectors.

Serafini, M., L. Naldini, et al. (2004). "Molecular evidence of inefficient transduction of proliferating human B lymphocytes by VSV-pseudotyped HIV-1-derived lentivectors." *Virology* **325**(2): 413.

<http://www.sciencedirect.com/science/article/B6WXR-4CMYM43-2/2/958832d3fc12ca733fe5e5fde07abbd>

Lentiviral vectors are attractive tools to transduce dividing and nondividing cells. Human tonsillar B lymphocytes have been purified and induced to proliferate by the addition of anti-CD40 + IL-4 or anti-CD40 + anti-[mu] signals and transduced at high MOI with a VSV pseudotyped lentivector carrying the eGFP gene under the control of the PGK promoter. Parallel cultures of PHA-stimulated T lymphocytes containing a comparable amount of cycling cells during the infection reached over 70% eGFP transduction. By contrast, only less than 3% B lymphocytes became eGFP positive after 7 days from transduction. Molecular analysis of the viral life cycle shows that cytoplasmic retrotranscribed cDNA and nuclear 2LTR circles are detectable at lower levels and for a shorter period of time in proliferating B cells with respect to proliferating T lymphocytes. Moreover, FACS-sorted eGFP-positive and negative B cell populations were both positive for the presence of retrotranscribed cDNA and 2LTR circles nuclear forms. By contrast, nested Alu-LTR PCR allowed us to detect an integrated provirus in FACS-sorted eGFP-positive cells only. Together with the demonstration that infection in saturation conditions led to an increase in the percentage of transduced cells (reaching 9%), these findings suggest that in proliferating B lymphocytes, lentiviral transduction is an inefficient process blocked at the early steps of the viral life cycle possibly involving partially saturable restriction factors.

Singh, D. K., D. M. Griffin, et al. (2003). "The presence of the casein kinase II phosphorylation sites of Vpu enhances the CD4+ T cell loss caused by the simian-human immunodeficiency virus SHIVKU-1bMC33 in pig-tailed macaques." *Virology* **313**(2): 435.

<http://www.sciencedirect.com/science/article/B6WXR-493HN1N-C/2/24be09dbc5f1d0b6ba941c0f41cdadb9>

The simian-human immunodeficiency virus (SHIV)/ macaque model for human immunodeficiency virus type 1 has become a useful tool to assess the role of Vpu in lentivirus pathogenesis. In this report, we have mutated the two phosphorylated serine residues of the HIV-1 Vpu to glycine residues and have reconstructed a SHIV expressing this nonphosphorylated Vpu (SHIVS52,56G). Expression studies revealed that this protein was localized to the same intracellular compartment as wild-type Vpu. To determine if this virus was pathogenic, four pig-tailed macaques were inoculated with SHIVS52,56G and virus burdens and circulating CD4+ T cells monitored up to 1 year. Our results indicate that SHIVS52,56G caused rapid loss in the circulating CD4+ T cells within 3 weeks of inoculation in one macaque (CC8X), while the other three macaques developed no or gradual numbers of CD4+ T cells and a wasting syndrome. Histological examination of tissues revealed that macaque CC8X had lesions in lymphoid tissues (spleen, lymph nodes, and thymus) that were typical for macaques inoculated with pathogenic parental SHIVKU-1bMC33 and had no lesions within the CNS. To rule out that macaque CC8X had selected for a virus in which there was reversion of the glycine residues at positions 52 and 56 to serine residues and/or compensating mutations occurred in other genes associated with CD4 down-regulation, sequence analysis was performed on amplified vpu sequences isolated from PBMC and from several lymphoid tissues at necropsy. Sequence analysis revealed a reversion of the glycine residues back to serine residues in this macaque. The other macaques maintained low virus burdens, with one macaque (P003) developing a wasting syndrome between months 9 and 11. Histological examination of tissues from this macaque revealed a thymus with severe atrophy that was similar to that of a previously reported macaque inoculated with a SHIV lacking vpu (*Virology* 293, 2002, 252). Sequence analysis revealed no reversion of the glycine residues in the vpu sequences isolated from this macaque. These results contrast with those from four macaques inoculated with the parental pathogenic SHIVKU-1bMC33, all of which developed severe CD4+ T cell loss within 1 month after inoculation. Taken together, these results indicate that casein kinase II phosphorylation sites of Vpu contributes to the pathogenicity of the SHIVKU-1bMC33 and suggest that the SHIVKU-1bMC33/pig-tailed macaque model will be

useful in analyzing amino acids/domains of Vpu that contribute to the pathogenesis of HIV-1.

Steinthorsdottir, V. and V. Mautner (1991). "Enteric adenovirus type 40: E1B transcription map and identification of novel E1A-E1B cotranscripts in lytically infected cells." *Virology* **181**(1): 139.

<http://www.sciencedirect.com/science/article/B6WXR-4BMTXD3-4J2/36126bc3fe0478a777fd4bf4ab4ddd21>

Adenovirus 40 (Ad40) is defective for growth in tissue culture but is complemented when the Ad2/5 or Ad12 E1B 55K protein is supplied in trans. Ad40 E1B mRNA has not been detected in E1-transformed cells, or at early times in lytically infected cells. In cells constitutively expressing the E1B region of Ad2, Ad40 E1B mRNAs are detected at late times in infection, after the onset of DNA replication. We have determined the Ad40 E1B transcription map from RNA produced at late times in infected KB16 cells, using S1 nuclease, primer extension, PCR-cDNA analysis, and Northern blotting. E1B transcripts corresponding to Ad2 14 S, 22 S, and 9 S mRNAs were identified but no 13 S mRNA equivalent was detected, a pattern similar to that seen in the Ad12 transcription map. The coding potential for E1B 19K, 55K, and 15K proteins and for pIX is retained in the Ad40 transcripts. In addition we find novel E1A-E1B cotranscript counterparts of the 14 S and 22 S mRNAs. These contain the first 40 codons of the E1A first exon linked to a site 4-5 nt downstream of the E1B cap site, retaining all the coding potential of the E1B mRNAs. No new open reading frames are created by the junction, and the E1A ORF terminates with one codon added after the junction. Each E1A-E1B cotranscript is present in abundance comparable to that of its authentic E1B counterpart. The E1A-E1B junction is unusual in that it does not conform to splice consensus sequences and thus may not be generated by a conventional splicing mechanism.

Taylor, E. R. and I. M. Morgan (2003). "A novel technique with enhanced detection and quantitation of HPV-16 E1- and E2-mediated DNA replication." *Virology* **315**(1): 103.

<http://www.sciencedirect.com/science/article/B6WXR-49M6PHH-6/2/c3e2ce4c11b0b5126c54dc5c4b5434fb>

Transient DNA replication assays to detect papillomavirus E1/E2-mediated DNA replication have depended upon Southern blotting. This technique is hazardous (radioactive), labour intensive, semiquantitative, and physically limited in the number of samples that can be processed at any one time. We have overcome these problems by developing a real-time PCR protocol for the detection of E1/E2-mediated transient DNA replication. The results demonstrate detection of replication at levels not seen using Southern blotting demonstrating enhanced sensitivity. This technique is also, by definition, highly quantitative. Therefore, the real-time PCR technique is the optimal method for the detection of E1/E2-mediated DNA replication.

Torres, A. N., C. K. Mathiason, et al. (2005). "Re-examination of feline leukemia virus: host relationships using real-time PCR." *Virology* **332**(1): 272.

<http://www.sciencedirect.com/science/article/B6WXR-4F31PRD-4/2/a279ee363e68f42f10890bd1534ac134>

The mechanisms responsible for effective vs. ineffective viral containment are central to immunoprevention and therapies of retroviral infections. Feline leukemia virus (FeLV) infection is

unique as a naturally occurring, diametric example of effective vs. ineffective retroviral containment by the host. We developed a sensitive quantitative real-time DNA PCR assay specific for exogenous FeLV to further explore the FeLV-host relationship. By assaying p27 capsid antigen in blood and FeLV DNA in blood and tissues of successfully vaccinated, unsuccessfully vaccinated, and unvaccinated pathogen-free cats, we defined four statistically separable classes of FeLV infection, provisionally designated as abortive, regressive, latent, and progressive. These host-virus relationships were established by 8 weeks post-challenge and could be maintained for years. Real-time PCR methods offer promise in gaining deeper insight into the mechanisms of FeLV infection and immunity.

Vergne, L., A. Bourgeois, et al. (2003). "Biological and genetic characteristics of HIV infections in Cameroon reveals dual group M and O infections and a correlation between SI-inducing phenotype of the predominant CRF02_AG variant and disease stage." *Virology* **310**(2): 254.

<http://www.sciencedirect.com/science/article/B6WXR-48D38S7-G/2/9408366d850549ccf39292d1939a8176>

In Yaounde, Cameroon, HIV-1 group-specific V3 serology on 1469 HIV-positive samples collected between 1996 and 2001 revealed that group O infections remained constant around 1% for 6 years. Only one group N sample was identified and 4.3% reacted with group M and O peptides. Although the sensitivity of the group-specific polymerase chain reaction (PCR) in two genomic regions was not optimal, we confirmed, in at least 6 of 49 (12.2%) dual O/M seropositive samples and in 1 of 9 group O samples, dual infection with group O and M viruses (n = 4) or with group O or M virus and an intergroup recombinant virus (n = 3). Partial env (V3-V5) sequences on a subset of 295 samples showed that at least eight subtypes and five circulating recombinant forms (CRFs) of HIV-1 group M co-circulate; more than 60% were CRF02_AG and 11% had discordant subtype/CRF designations between env and gag. Similarly as for subtype B, the proportion of syncytium-inducing strains increased when CD4 counts were low in CRF02_AG-infected patients. The V3-loop charge was significantly lower for non-syncytium-inducing strains than for syncytium-inducing strains but cannot be used as an individual marker to predict phenotype. The two predominant HIV-1 variants in Africa, CRF02_AG and subtype C, thus have different biological characteristics.

Vieth, S., A. E. Torda, et al. (2004). "Sequence analysis of L RNA of Lassa virus." *Virology* **318**(1): 153.

<http://www.sciencedirect.com/science/article/B6WXR-4B6CP2M-1/2/0f6d6f13f5ec15bd1d0764d235955de6>

The L RNA of three Lassa virus strains originating from Nigeria, Ghana/Ivory Coast, and Sierra Leone was sequenced and the data subjected to structure predictions and phylogenetic analyses. The L gene products had 2218-2221 residues, diverged by 18% at the amino acid level, and contained several conserved regions. Only one region of 504 residues (positions 1043-1546) could be assigned a function, namely that of an RNA polymerase. Secondary structure predictions suggest that this domain is very similar to RNA-dependent RNA polymerases of known structure encoded by plus-strand RNA viruses, permitting a model to be built. Outside the polymerase region, there is little structural data, except for regions of strong alpha-helical content and probably a coiled-coil domain at the N terminus. No evidence for reassortment or recombination during Lassa virus evolution was found. The secondary structure-assisted alignment of the RNA polymerase region permitted a reliable reconstruction of the phylogeny of all negative-strand RNA viruses, indicating that Arenaviridae are most closely related to Nairoviruses. In conclusion, the data provide a basis for structural and functional characterization of the Lassa virus L protein and reveal new insights into the phylogeny of negative-strand RNA

viruses.

Watanabe, M., B.-J. Lee, et al. (2003). "Borna disease virus induces acute fatal neurological disorders in neonatal gerbils without virus- and immune-mediated cell destructions." *Virology* **310**(2): 245.

<http://www.sciencedirect.com/science/article/B6WXR-48D38S7-T/2/7b7f41334f5ea6a0f23a48809f050205>

Borna disease virus (BDV) is a noncytolytic, neurotropic RNA virus that is known to cause neurological disturbances in various animal species. Our previous experiment demonstrated that neonate gerbils develop an acute fatal neurological disease following infection with BDV (Watanabe et al., 2001, *Virology* 282, 65-76). The study suggested that BDV directly causes functional damage of neuronal cells resulting in the lethal disorder in neonatal gerbils. To extend this finding, we examined whether BDV can induce neurological diseases in the absence of virus- and immune-mediated cell destruction, by using cyclosporine A (CsA)-treated neonatal gerbils. Although CsA completely suppressed specific antibody production and brain inflammation in the infected gerbil brains, the fatal neurological disorder was not inhibited by the treatment. Furthermore, we demonstrated that CsA treatment significantly decreased brain levels of cytokines, except interleukin (IL)-1[β], in the infected gerbils. These results suggested that BDV replication, as well as brain cytokines, at least IL-1[β], rapidly induces fatal disturbances in gerbil brain. We demonstrate here that BDV exhibits a unique neuropathogenesis in neonatal gerbil that may be pathologically and immunologically different from those in two other established rodent models, rats and mice. With this novel rodent model of virus infection it should be possible not only to examine acute neurological disturbances without severe neuroanatomical and immunopathological alterations but also to analyze molecular and cellular damage by virus replication in the central nervous system.

Yu, H. J., D. Lim, et al. (2003). "Characterization of a novel single-stranded RNA mycovirus in *pleurotus ostreatus*." *Virology* **314**(1): 9.

<http://www.sciencedirect.com/science/article/B6WXR-497HB2X-1/2/1940aa611d0fe689cbfd9b60131711bf>

A mycovirus, named oyster mushroom spherical virus (OMSV), was isolated from cultivated oyster mushrooms with a severe epidemic of oyster mushroom Die-back disease. OMSV was a 27-nm spherical virus encapsidating a single-stranded RNA (ssRNA) of 5.784 kb with a coat protein of approximately 28.5 kDa. The nucleotide sequence of the virus revealed that its genomic RNA was positive strand, containing 5784 bases with seven open reading frames (ORF). ORF1 had the motifs of RNA-dependent RNA polymerases (RdRp) and helicase. ORF2 encoded a coat protein. ORF3 to 7 could encode putative polypeptides of approximately 12, 12.5, 21, 14.5, and 23 kDa, respectively, but none of them showed significant similarity to any other known polypeptides. The 5' end of the viral RNA was uncapped and the 3' end was polyadenylated with 74 bases. Genomic structure and organization and the derived amino acid sequence of RdRp and helicase domain were similar to those of tymoviruses, a plant virus group.

Ahne, W., W. N. Batts, et al. (1999). "Comparative sequence analyses of sixteen reptilian paramyxoviruses." Virus Research **63**(1-2): 65.

<http://www.sciencedirect.com/science/article/B6T32-3X9YRG1-9/2/a5409eb149d626a83fd21c11ab184c82>

Viral genomic RNA of Fer-de-Lance virus (FDLV), a paramyxovirus highly pathogenic for reptiles, was reverse transcribed and cloned. Plasmids with significant sequence similarities to the hemagglutinin-neuraminidase (HN) and polymerase (L) genes of mammalian paramyxoviruses were identified by BLAST search. Partial sequences of the FDLV genes were used to design primers for amplification by nested polymerase chain reaction (PCR) and sequencing of 518-bp L gene and 352-bp HN gene fragments from a collection of 15 previously uncharacterized reptilian paramyxoviruses. Phylogenetic analyses of the partial L and HN sequences produced similar trees in which there were two distinct subgroups of isolates that were supported with maximum bootstrap values, and several intermediate isolates. Within each subgroup the nucleotide divergence values were less than 2.5%, while the divergence between the two subgroups was 20-22%. This indicated that the two subgroups represent distinct virus species containing multiple virus strains. The five intermediate isolates had nucleotide divergence values of 11-20% and may represent additional distinct species. In addition to establishing diversity among reptilian paramyxoviruses, the phylogenetic groupings showed some correlation with geographic location, and clearly demonstrated a low level of host species-specificity within these viruses.

Black, J. B., D. A. Burns, et al. (1997). "Biologic properties of human herpesvirus 7 Strain SB." Virus Research **52**(1): 25.

<http://www.sciencedirect.com/science/article/B6T32-3RSN41S-3/2/ea8c1ba745d2a2d5cdb9993bd6fd5a04>

The growth characteristics of human herpesvirus 7 strain SB (HHV-7 (SB)) were studied in human umbilical cord blood lymphocyte (CBL) cultures. The virus has approximately a 4-day growth cycle, as measured by immunofluorescence analysis, quantitation of the relative viral DNA concentration, and examination of infected cells by electron microscopy on consecutive days post-infection. By systematically varying the culture media components, improved culturing conditions were established. Activated lymphocytes were required for virus growth. HHV-7(SB) grew best in phytohemagglutinin-stimulated CBL cultured in media containing 0.01 mg/ml hydrocortisone. Addition of recombinant human interleukin 2 (IL-2) at concentrations exceeding 1-10 U/ml inhibited virus growth in most CBL cultures. Addition of exogenous IL-2 to the culture media had no effect on viral DNA production. However, the percentage of virus antigen-positive cells was highest when 0.1-1 U/ml was added to the media. Differences in the ability of individual CBL cultures to replicate HHV-7(SB) was not explained by differing CD4+ cell concentrations. However, individual cultures varied in the level of endogenous IL-2 production, which may contribute to the virus growth variability in CBL. HHV-7(SB) grew in the CD4-positive T-cell line SupT1, but not in a variety of other lymphocyte, fibroblast, or epithelial cell lines. Nine compounds were tested for antiviral activity against HHV-7 in vitro. Phosphonoformic acid inhibited virus growth with a 50% effective concentration of 4.8 [μM]. Ganciclovir (200 [μM]) and phosphonoacetic acid (100 [μM]) inhibited more than 90% of virus production. None of the compounds were cytotoxic at concentrations which inhibited the virus. A generalized increase in host cell protein synthesis was also observed in virus-infected cells similar to that seen in CBL infected with human herpesvirus 6.

Blackburn, R. V., V. R. Racaniello, et al. (1992). "Construction of an infectious cDNA clone of echovirus 6." Virus Research **22**(1): 71.

<http://www.sciencedirect.com/science/article/B6T32-476NFS5-2R/2/87e33d321998347b25f931a1d9a567ce>

A complete cDNA copy of the echovirus 6 genome was constructed. Complementary DNA was reversed transcribed from viral RNA. Subgenomic cDNAs were obtained by direct cloning and polymerase chain reactions. Full length cDNA was constructed into the Bluescript II vector (pBSII) using unique, overlapping, restriction sites of four clones. The cDNA was infectious and produced echovirus 6 particles that behaved in the same manner as the parental virus.

Britton, P., K. L. Mawditt, et al. (1991). "The cloning and sequencing of the virion protein genes from a British isolate of porcine respiratory coronavirus: comparison with transmissible gastroenteritis virus genes." Virus Research **21**(3): 181.

<http://www.sciencedirect.com/science/article/B6T32-476RHMC-H3/2/03febcbfa6ad877ddcea1edb33770685>

Previous analysis of porcine respiratory coronavirus (PRCV) mRNA species showed that mRNAs 2 and 3 were smaller than the corresponding transmissible gastroenteritis virus (TGEV) mRNA species (Page et al. (1991) J. Gen. Virol. 72, 579-587). Sequence analysis showed that mRNA 3 was smaller due to the presence of a new putative RNA-leader binding site upstream of the PRCV ORF-3 gene. However, this observation did not explain the deletion observed in PRCV mRNA 2. Polymerase chain reaction (PCR) was used to generate cDNA from the 3' coding region of the putative polymerase gene to the poly (A) tail of PRCV for comparison to the equivalent region from TGEV. The PRCV S protein was found to consist of 1225 amino acids, which had 98% similarity to the TGEV S protein. However, the PRCV S gene contained a 672 nucleotide deletion, corresponding to 224 amino acids (residues 21 to 245 in TGEV S protein), 59 nucleotides downstream of the S gene initiation codon. The PRCV genome from the ORF-3 gene to the poly (A) tail was sequenced for comparison to TGEV in order to identify other potential differences between the two viruses. Four ORFs were identified that showed 98% similarity to the TGEV ORF-4, M, N and ORF-7 genes. No other deletions or any PRCV specific sequences were identified.

Brown, E. G. and J. E. Bailly (1999). "Genetic analysis of mouse-adapted influenza A virus identifies roles for the NA, PB1, and PB2 genes in virulence." Virus Research **61**(1): 63.

<http://www.sciencedirect.com/science/article/B6T32-3WP2PH3-7/2/111d95b360524ee6c02bea1f0cd274dd>

Adaption of the prototype A/FM/1/47 H1N1 strain to mice resulted in selection of the A/FM/1/47-MA variant with increased virulence. Earlier analysis identified mutations in the HA and M1 genes that increase virulence in the mouse. Complete sequence analysis identified mutations in the PB1, PB2, HA, NA, and M1 genes. Reassortants were produced between the parental FM and FM-MA strains to obtain viruses that differ due to combinations of mutant genes. To assess the relationship between virulence and replication, the median lethal dose was determined for mice and growth properties were assessed in mouse lung, MDCK cells and chicken embryo. Not only were all five mutations shown to control virulence but also the replicative capacity in the mouse. The HA, NA and M1 mutations increased yield in all three hosts whereas in combination the PB1 and PB2 mutations were host restrictive changing the virus to a mouse specific strain. For the NA

and M1 mutations the increase in growth in mouse lung was proportional to a 2-fold (log₁₀) increase in virulence however the HA mutation increased virulence largely independent of increased growth indicating a change in pathological properties that damage the host. Thus mutations that affect virulence can be classified according to host-dependent and independent ability to increase growth as well as changes in pathological properties. Each of the PB1, PB2, NA, HA, and M1 genes acquired gain-of-function mutations for mouse infection that involve structural motifs that may serve as markers for virulence or targets for antiviral therapy.

Casper, C., C. Leib-Mosch, et al. (1998). "Mapping of a mouse mammary tumor virus integration site by retroviral LTR--arbitrary polymerase chain reaction." Virus Research **54**(2): 207.

<http://www.sciencedirect.com/science/article/B6T32-3T3JWH9-8/2/ada35dde9992b29a3427d4094c3e79b7>

The de novo integration of retroviral genomes within the mammalian genome is believed to contribute to the tumorigenic process. Integration may result in the disruption or inappropriate transcription of key regulatory genes. We describe the application of an arbitrarily primed PCR method for the mapping and cloning of genomic integration sites of the mouse mammary tumor virus (MMTV). We have amplified DNA sequences between a selected retroviral MMTV-LTR and random sites in the 3' flanking DNA. Using this technique we were able to visualize several proviral integration sites present in a MMTV-induced mammary tumor derived cell line that were absent from the germ line. Cloning and sequencing of the PCR product corresponding to one site established its identification as a unique 3' flanking sequence.

Cheng, S.-M., R. Vainionpää, et al. (2004). "Detection of influenza B in clinical specimens: comparison of high throughput RT-PCR and culture confirmation." Virus Research **103**(1-2): 85.

<http://www.sciencedirect.com/science/article/B6T32-4C47KNC-4/2/56ce21e76e987bc17594da635d27dd0d>

Influenza virus is one of the major causes of worldwide respiratory tract infections during the winter season. Here we describe a high throughput (HTP) protocol for rapid diagnosis of influenza B that combines automated viral RNA extraction with detection and quantification by TaqMan-based PCR. Using this methodology, we tested 4176 nasal swabs collected from children enrolled in a European influenza vaccine trial during the winter of 2000 to compare our HTP PCR method to culture confirmation for detection of influenza B. Among these, 37 were positive by culture and 169 were positive by PCR irrespective of virus copy number. However, when specimens with fewer than 20 copies of the viral genome were disregarded, a good correlation between two methods was observed. At this cut-off, 34 specimens were positive and 4106 were negative by both methods. Statistical analysis of the data using culture confirmation as the standard indicated that the sensitivity of HTP RT-PCR for influenza B was 92% (95% CI, 0.83-1), and the specificity was 99% (95% CI, 0.99-1). In summary, HTP RT-PCR was proved to be more rapid and sensitive than culture confirmation, and it correlated significantly with culture confirmation for specimens containing more than 20 copies of the viral genome.

Drebot, M. A., I. Gavrilovskaya, et al. (2001). "Genetic and serotypic characterization of Sin Nombre-like viruses in Canadian *Peromyscus maniculatus* mice." Virus Research **75**(1): 75.

<http://www.sciencedirect.com/science/article/B6T32-42SPNFG->

8/2/28701da1c1250a1f5d57e5400ac2b01c

In Canada, hantavirus infected deer mice (*Peromyscus maniculatus*) have been collected from British Columbia to Newfoundland. Partial sequencing of G1 and N protein encoding regions from Canadian *Peromyscus maniculatus*-borne hantaviruses demonstrated the existence of significant genotypic divergence among strains. Phylogenetic analysis showed that Sin Nombre (SN)-like viruses from eastern and western Canadian deer mice can be divided into at least two broad-based genogroups. Sequencing of mitochondrial DNA from infected deer mice originating from various eastern and western provinces showed that SN-like virus genogroups appeared to be associated with distinct haplotypes of mice. Sera from deer mice infected with eastern and western viral genotypes neutralized the Sin Nombre virus strain, Convict Creek 107, but not the New York 1 hantavirus. Despite the genetic heterogeneity of Canadian SN-like strains these hantaviruses do not appear to define unique hantavirus serotypes.

Fogdell-Hahn, A., S. S. Soldan, et al. "Co-purification of soluble membrane cofactor protein (CD46) and human herpesvirus 6 variant A genome in serum from multiple sclerosis patients." Virus Research **In Press, Corrected Proof** <http://www.sciencedirect.com/science/article/B6T32-4FGXX7N-1/2/7a8d4bc9070f27987c5ac4cac2d7fbb2>

The association of human herpesvirus 6 (HHV-6) and multiple sclerosis (MS) has been supported by several immunological and molecular studies. Recently, membrane cofactor protein (CD46) has been identified as the cellular receptor for the A and B variants of HHV-6. Elevated levels of soluble CD46 (sCD46) have been reported in the serum and CSF of MS patients. The aim of this study was to investigate a possible correlation between elevated levels of soluble CD46 and the presence of serum HHV-6 DNA in MS patients. An immunoaffinity column comprised of immobilized monoclonal antibodies to CD46 was developed to isolate sCD46 from cell free body fluids of MS patients and controls. After immunoaffinity purification, DNA was extracted from anti-CD46 column eluates and subjected to PCR amplification. Of the 42 MS samples tested, 4 serum samples were HHV-6 positive, 3 of which were typed as HHV-6A. The co-purification of sCD46 and HHV-6 DNA from MS sera indicates that HHV-6 is tightly connected to its receptor, CD46, in the serum of MS patients.

Gundersen-Rindal, D. and E. M. Dougherty (2000). "Evidence for integration of *Glyptapanteles indiensis* polydnavirus DNA into the chromosome of *Lymantria dispar* in vitro." Virus Research **66**(1): 27.

<http://www.sciencedirect.com/science/article/B6T32-3YF3WFB-3/2/3b5451068a2a8831c148f40d5654e16f>

Polydnaviruses replicate within calyx cells of the female ovaries of certain species of parasitic wasps and are required for the successful parasitization of lepidopteran hosts. These viruses, which have unusual double-stranded circular DNA segmented genomes, are integrated as proviruses into the genomes of their associated wasp hosts and are believed to be transmitted vertically through germline tissue. Here, by combined Southern hybridization, polymerase chain reaction (PCR) assays and viral sequence analyses we provide evidence that DNA originating from two distinct double-stranded circular segments of the polydnavirus genome from the braconid *Glyptapanteles indiensis* (GiPDV) integrates in vitro into the genome of cells derived from the natural host, *Lymantria dispar*. The *G. indiensis* polydnavirus DNA, as a result of its unique ability to be integrated in part into the chromosome of cells derived from its lepidopteran host, has potential to be developed as an in vitro cell transformation system.

Hall, W. S., R. Goto-Mandeville, et al. (1997). "Molecular analysis of episomal human papillomavirus type 16 DNA in a cervical carcinoma cell line." Virus Research **51**(2): 183.

<http://www.sciencedirect.com/science/article/B6T32-497C7DM-14/2/7ba2ebb3f33161cf193f3bf23c359aaf>

Integration of human papillomavirus type 16 DNA sequences into host DNA is a frequent event in cervical carcinogenesis. However, recent studies showing that HPV16 is present exclusively in an episomal form in many primary cervical cancers suggest that HPV16 can transform target cells by mechanisms that do not require viral integration. We have established a cervical carcinoma cell line that harbors episomal copies of HPV16 DNA of approximately 10 kb. Restriction enzyme and two-dimensional gel analysis confirmed that HPV16 DNA was extrachromosomal with both monomeric and multimeric forms present. HPV16 was maintained as episomes with passage both in culture and after subcutaneous growth in nude mice. The 10 kb viral genome, consisting of a full-length copy of HPV16 and a partial duplication of the long control region and the L1 open reading frame, exhibited transforming activity comparable to prototype HPV16. This cell line should provide a useful model system for studying the biological significance of the physical state of the HPV16 genome in cervical carcinoma cells.

He, X., H. Yoshida, et al. (1995). "Analysis of the role of CD4+ T-cells during murine cytomegalovirus infection in different strains of mice." Virus Research **36**(2-3): 233.

<http://www.sciencedirect.com/science/article/B6T32-3YN9F8H-9/2/9ebc2acc5abbaedc1911d5dc61880bf2>

We examined the role of CD4+ T-cells in peritoneal exudate cells (PECs) during the course of acute murine cytomegalovirus (MCMV) infection in two strains of mice. Cell counts of PECs and cytofluorometric analysis showed that C57BL/6, a resistant strain, had more CD4+ T-cells than BALB/c, a susceptible strain, after intraperitoneal infection of 3 x 10³ PFU of the Smith strain of MCMV, though both strains had an equivalent number of CD8+ T-cells. CD4+ T-cells of both strains expressed mRNA of IFN-[gamma], IL-2, and IL-4 on days 5 and 7 after infection, with much higher expression of these cytokines in C57BL/6 than in BALB/c. At the same time point after infection, macrophages were shown to express mRNA of IL-1[alpha] and TNF-[alpha] with higher expression of IL-1[alpha] in C57BL/6 than in BALB/c. Production of nitric oxide, recently shown to be one of the antiviral effector mechanisms of macrophages, by macrophages of both strains was examined showing more production of nitric oxide on day 7 after infection in C57BL/6 than in BALB/c. From these findings, we suggest the possibility that CD4+ T-cells contribute to the protection against MCMV infection via the secretion of cytokines and the resultant activation of macrophages to produce nitric oxide.

Hernandez-Crespo, P., J. C. Veyrunes, et al. (2000). "The spheroidin of an Entomopoxvirus isolated from the grasshopper *Anacridium aegyptium* (AaEPV) shares low homology with spheroidins from lepidopteran or coleopteran EPVs." Virus Research **67**(2): 203.

<http://www.sciencedirect.com/science/article/B6T32-40J7B63-B/2/2f79a9c0d4eca8afd339b81fc410f8da>

Based on virion morphology, the current virus taxonomy groups entomopoxviruses (EPVs) (Poxvirus: Entomopoxvirinae) from coleopteran and dipteran hosts in separated genera, while it keeps viruses infecting either lepidopteran or orthopteran hosts in the same genus. In contrast to the morphological criteria, the few data available from recent studies at the genetic level have

suggested that EPVs infecting different insect orders are phylogenetically distant. In order to elucidate EPVs phylogeny we have cloned and sequence the highly conserved/highly expressed spheroidin gene of *Anacridium aegyptium* entomopoxvirus (AaEPV). This gene and its promoter is of interest for the development of genetic engineering on EPVs. The spheroidin gene was located in the AaEPV genome by Southern blot and hybridisation with specific degenerated oligonucleotides probes synthesised after partial sequencing of the purified spheroidin protein. A total of 3489 bp were sequenced. This sequence included the coding and promoter region of 969 residues 108.8 kDa protein identified as spheroidin. AaEPV spheroidin contains 21 cysteine residues (2.2%) and 14 N-glycosylation putative sites distributed along the sequence. The cysteine residues are particularly abundant at the C-terminal end of the protein, with 11 residues in the last 118 aa. Our results confirm that the spheroidin is highly conserved only between EPVs isolated from the same insect order. Polyclonal antibodies raised against AaEPV spherules specifically revealed spheroidin in Western Blots failing to cross-react with MmEPV or AmEPV spheroidins or MmEPV fusolin. Comparison of spheroidins at the aa level demonstrate that AaEPV spheroidin shares only 22.2 and 21.9% identity with the lepidopteran AmEPV and the coleopteran MmEPV spheroidins, respectively, but 82.8% identity with the orthopteran MsEPV spheroidin. Only two highly conserved domains containing the sequence (V/Y)NADTG(C/L) and LFAR(I/A) have been identified in all known spheroidins. The phylogenetic tree constructed according to the analysis program revealed that EPVs are clearly separated in three groups -- lepidopteran, coleopteran and orthopteran -- according to the insect order of the virus hosts. In base to our results, the split of the genus Entomopoxvirus B dissociating lepidopteran and orthopteran EPVs into two different genera is suggested.

Hurtado, A., A. L. Garcia-Perez, et al. (2003). "Genetic diversity of ruminant pestiviruses from Spain." Virus Research **92**(1): 67.

<http://www.sciencedirect.com/science/article/B6T32-47CYB10-3/2/43a4a82cc7299eaef4cfff0fcf139afa>

The genetic diversity of ruminant pestiviruses from Spain was investigated by sequencing analysis of the 5' non-coding region (5'NCR) from 10 ovine and 41 bovine samples collected along 4 years (1999-2002) from different farms. The 5'NCR amplicons generated by a one-tube RT-PCR using primers 324/326 were sequenced and phylogenetically analyzed. When compared with strains from GenBank database, Spanish viruses clustered into three genotypes: BVDV 1, BDV and CSFV. No BVDV 2 strains were identified. The 41 bovine samples were all BVDV 1 genotype, but they were further subdivided into subgroups 1b (35) and 1e (6). There was no apparent association between the genotype and clinical symptoms, or the geographic area of origin. However, subgroup 1e has been recently described for isolates from France and, interestingly, subgroup 1e viruses were collected from areas close to the French border. Nine of the ten ovine samples formed a tight cluster within BDV type but clearly differed from BDV subgroups A and B sustaining the need to define a new subgroup as BDV-C in which these ovine strains would be included. Finally, although further studies are needed, the grouping of an ovine sample with CSFV strains would be the first description of a CSFV genotype for ovine pestiviruses.

Jang, H.-K., M. Niikura, et al. (1997). "Characterization and expression of the Marek's disease virus serotype 2 glycoprotein E in recombinant baculovirus-infected cells: initial analysis of its DNA sequence and antigenic properties." Virus Research **48**(2): 111.

<http://www.sciencedirect.com/science/article/B6T32-3RSFX0S-1/2/012d1f2521fc87d384ba75d3f347ddf9>

In Marek's disease virus (MDV) serotype 2 (MDV2) genome, a gene equivalent to the glycoprotein E (gE) of other alphaherpesviruses was identified and sequenced. The primary translation product comprises 488 amino acids with a Mr of 54.3 kDa. The predicted amino acid sequence possesses several characteristics typical of membrane glycoproteins, including a N-terminal hydrophobic signal sequence, C-terminal transmembrane and cytoplasmic domains, and extra-cellular region containing four potential N-linked glycosylation sites. Compared with other MDV serotypes, MDV2 gE showed 47.3% identity with MDV1 gE, and 38.9% identity with HVT gE at the amino acid level. In transcriptional analyses, a 2.0 kb mRNA which starts between 65 and 86 bps upstream of the potential translational initiation codon of gE was identified as the gE-specific transcript. By a recombinant baculovirus, this potential gE coding region was expressed as several specific products from 66 to 72 kDa. These products were susceptible to tunicamycin treatment, indicating that they were glycoprotein in nature. Further, the expressed gE reacted with all chicken-antisera raised to each of the three serotypes of MDV (strains GA, SB-1, and FC126), suggesting that gE is expressed by all three serotypes of MDV in infected cells and conserves common antigenic epitope(s) beyond those that are serotype specific.

Johansson, P., M. Olsson, et al. (2004). "Complete gene sequence of a human Puumala hantavirus isolate, Puumala Umea/hu: sequence comparison and characterisation of encoded gene products." Virus Research **105**(2): 147.

<http://www.sciencedirect.com/science/article/B6T32-4CYNSKD-1/2/f9f8ba5a1750df9dc517a1f7ce7600fa>

Puumala virus is a member of the hantavirus genus in the Bunyaviridae family, and the major causative agent of haemorrhagic fever with renal syndrome in Europe. This study was conducted with a human Puumala virus isolate (PUUV Umea/hu), and contains the determination of the first complete PUUV sequence from a human source. When the relationship to other Puumala viruses was analysed, a possible RNA segment exchange between two local strains of PUUV was noticed. Furthermore, the coding regions of PUUV Umea/hu S- and M-segments were cloned, and a large set of gene products were expressed in mammalian cells. In addition, postulated N- and O-linked glycosylation sites in the two envelope proteins (Gn and Gc) were investigated individually by site-directed mutagenesis followed by gel-shift analysis. Our data demonstrate that N-linked glycosylation occurs at three sites in Gn (N142, N357 and N409), and at one site in Gc (N937). Also, one possible O-glycosylation site was identified in Gc (T985). We conclude that the diversity between different Puumala virus isolates is high, and consequently characterization of local PUUV isolates is important for clinical diagnostic work. Finally, the obtained results concerning the encoded gene products are of great importance for the design of new vaccines.

Kariwa, H., Y. Isegawa, et al. (1994). "Comparison of nucleotide sequences of M genome segments among Seoul virus strains isolated from Eastern Asia." Virus Research **33**(1): 27.

<http://www.sciencedirect.com/science/article/B6T32-476M3G4-67/2/72da252499873c2618084d3db26051d1>

The nucleotide sequences of the M genome segments of three Seoul virus strains (KI strains) which were isolated from urban rats inhabiting the same enzootic focus between 1983 and 1988 were compared. The viral cDNAs were amplified by PCR and were directly sequenced. The nucleotide sequences of KI strains were extremely homologous regardless of isolation year (less than 10 substitutions in 3651 nucleotides, less than 4 substitutions in 1133 amino acids). In addition, the nucleotide sequence of the KI strain isolated in 1983 (KI-83-262) was also quite similar to that of other Seoul viruses, which were isolated from laboratory rats in Japan (strain SR-11, 98.1% and B-1 strain, 96.5%), from an urban rat in Korea (Seoul 80-39, 96.5%) and from

an urban rat in China (R22 strain, 93.4%). All possible N-glycosylation sites in the deduced amino acid sequences were conserved among all Seoul viruses examined. The nucleotide and amino acid sequences of Seoul virus strains were highly conserved although they were isolated from various districts of eastern Asia. These results indicate the genetic stability of Seoul virus strains maintained under a natural environment and the homology of Seoul viruses isolated from various districts of eastern Asia. The relationship among Seoul virus strains isolated from eastern Asia was compared by phylogenetic analysis.

Kariwa, H., K. Yoshimatsu, et al. (1999). "Genetic diversities of hantaviruses among rodents in Hokkaido, Japan and Far East Russia." Virus Research **59**(2): 219.

<http://www.sciencedirect.com/science/article/B6T32-3VS2KGV-9/2/b8dff1269111120e5469eccc528f9bb1>

Seroepizootiologic surveys among wild rodents were carried out in Japan and Far East Russia in 1995 and 1996. Seropositive animals were only identified in *Clethrionomys rufocanus* (23/134) in Hokkaido, Japan. On the other hand, seropositives were identified in *C. rufocanus* (1/8), *Apodemus agrarius* (2/66), *Apodemus* spp. (2/26) and *Microtus fortis* (3/22) in Vladivostok, Far East Russia. Total RNA was isolated from lungs of seropositive animals and the S genome segments were amplified by PCR, cloned and sequenced. The S and M genomes of hantavirus, derived from Japanese *C. rufocanus* (Tobetsu genotype), were most closely related with Puumala viruses (76-79% nucleotide and 95% amino acid identities for S genome, 70-78% nucleotide and 87-92% amino acid identities for M genome). The recombinant nucleocapsid protein of Tobetsu genotype was antigenically quite similar with that of Sotkamo. These suggest that the virus endemic in Japanese *C. rufocanus* belongs to Puumala virus. Phylogenetic analysis indicates that the genotype forms a distinct lineage within Puumala viruses. Partial S segment (1-1251 nt), derived from seropositive *M. fortis* in Vladivostok, was sequenced and analyzed. The S genome segment, which was designated Vladivostok genotype, was most closely related with Khabarovsk virus (79% nucleotide and 90% amino acid identities) which was isolated from *M. fortis*.

Kim, O., H. Li, et al. (2003). "Demonstration of sheep-associated malignant catarrhal fever virions in sheep nasal secretions." Virus Research **98**(2): 117.

<http://www.sciencedirect.com/science/article/B6T32-49W36PY-4/2/28e348518137f700d6ff9c2edaa9ac0f>

Ovine herpesvirus-2 (OvHV-2) is the causative agent for sheep-associated malignant catarrhal fever, which has never been propagated *in vitro*. Previous studies from this laboratory demonstrated significantly high levels of OvHV-2 DNA in sheep nasal secretions, suggesting a likely avenue of transmission. In the present study, real-time PCR was used to identify sheep experiencing an episode of intense OvHV-2 DNA shedding in their nasal secretions. A nuclease-resistance assay was used to examine the secretions for the presence of intact cell-free enveloped OvHV-2 virions. The results revealed that all nasal secretion samples from five selected individuals experiencing intensive shedding events contained cell-free OvHV-2 virions. Virions could not be identified in secretion samples from 11 OvHV-2 infected sheep that were not experiencing a shedding event. This is the first unequivocal demonstration of cell-free OvHV-2 virions. These results suggest that OvHV-2 lytic infection occurs in the epithelium of certain tissues in the upper respiratory tract of the natural host.

Kosuge, H., Y. Isegawa, et al. (1997). "Nucleotide sequence analysis of a 30-kilobase-pair region of human herpesvirus-6B (HHV-6B) genome and strain-specific variations in major immediate-early genes." *Virus Research* **52**(1): 1.

<http://www.sciencedirect.com/science/article/B6T32-3RSN41S-1/2/1bc9f21a031b8c164f98087c7c3fd093>

Human herpesvirus 6 (HHV-6) is now classified into two distinct variants such as HHV-6 variant A(HHV-6A) and B(HHV-6B) (Ablashi et al., Arch. Virol. 129, 1993, 1-4) and the DNA of HHV-6A strain U1102 was completely sequenced (Gompels et al., Virology 209, 1995, 29-51). We have sequenced a 30-kilobase pair (kbp) (genomic positions around 111-141 kb) of HHV-6B strain HST, and a sequence of this region was compared with that of HHV-6A strain U1102. Dodecameric repeats, G/T and Kpn repeat elements, putative major immediate early 1 (MIE1) and major immediate early 2 (MIE2) genes were found in this region. The DNA sequences of HHV-6A (U1102) and HHV-6B (HST) were markedly different in the MIE1 region, Kpn repeat elements and the putative MIE2 region. Dodecameric repeat element was located in the putative MIE2 locus of HHV-6. When primers covering dodecameric repeat region were used to amplify HHV-6 DNA of clinical isolates from patients with exanthem subitum (ES) by polymerase chain reaction (PCR), variations in size of PCR products in each isolate were found, indicating strain-specific features. Furthermore, the results of molecular biological analysis by PCR using DNA samples in a family suggest that HHV-6 infects within a family.

Lili, K., C. Zongyu, et al. (1992). "Lactate dehydrogenase-elevating virus (LDV): subgenomic mRNAs, mRNA leader and comparison of 3'-terminal sequences of two LDV isolates." *Virus Research* **23**(1-2): 55.

<http://www.sciencedirect.com/science/article/B6T32-476RJMJ-V1/2/4f53634b304e1927f6f2c1b1a0d6b31c>

The 3'-terminal 1314 nucleotides of the genome of one isolate of lactate dehydrogenase-elevating virus, LDV-P, has been derived by sequence analyses of cDNAs from several genomic libraries and compared to that of another LDV isolate, LDV-C (Godeny et al. (1990) Virol. 177, 768-771). The 3'-non-coding segment of 80 nucleotides of the two LDV genomes is identical, whereas marked, but varying nucleotide and amino acid divergence is apparent in the three upstream overlapping open reading frames (ORF). The third ORF from the 3'-end exhibits only 82% nucleotide and 90% amino acid identity, whereas the 3'-terminal ORF, which encodes the nucleocapsid protein, exhibits approximately 99% amino acid identity. The second 3'-terminal ORF encodes an 18.8 kDa protein which lacks N-glycosylation sites but possesses 2 or 3 potential transmembrane helices in the N-terminal half of the molecule. A similar membrane organization is observed for the corresponding protein of equine arteritis virus and the M protein of mouse hepatitis virus. The sequence analyses combined with Northern hybridization analyses of RNA from LDV-infected macrophages and spleens of LDV-infected mice indicate that the three ORFs encoded by the 3'-terminal end of the LDV genome are expressed via the three smallest mRNAs (mRNAs 6-8) of the seven subgenomic mRNAs of LDV (mRNAs 2-8), which range in size from about 0.8 to 3.6 kb. All mRNAs have been shown to carry poly(A)-tracts and a common leader sequence. The seven mRNAs were produced in infected macrophage cultures concomitantly with genomic LDV RNA. Maximum LDV RNA synthesis was observed between 6 and 8 h post-infection. The same seven subgenomic mRNAs were detected in macrophages infected with three different isolates of LDV, but different relative amounts of some of the mRNAs were produced. The relative proportions of molecules of mRNAs 1-8 present in 6 h LDV-P-infected macrophages were about 13, 5, 5, 8, 6, 11, 11 and 27% of the total, respectively.

Locke, D. P., H. S. Sellers, et al. (2000). "Newcastle disease virus phosphoprotein gene analysis and transcriptional editing in avian cells." Virus Research **69**(1): 55.

<http://www.sciencedirect.com/science/article/B6T32-4164TSC-7/2/5671f811f6bcf7c7cb696703bba06908>

Nucleotide sequence was determined for the phosphoprotein (P) gene from 23 Newcastle disease virus (NDV) isolates representing all defined pathotypes with different chronological and geographic origins. Sequence variation, with synonymous substitutions dominating, occurred throughout the P gene. An exception was a conserved central region containing the transcriptional editing site. Four G nucleotide additions were detected in NDV P gene mRNA potentially creating alternative open reading frames. However, only one in-frame stop codon exists with a single G addition among all isolates that would allow for a potential V protein. A second potential stop codon does not exist in the P gene consensus sequence among all isolates with more than one G nucleotide addition at the editing site. This precludes a possible W protein in these isolates. A second potential alternative in-frame start site exists among all isolates that could encode a predicted X protein for NDV. Comparison of the P gene editing sites among the Paramyxovirinae and predicted P gene usage demonstrates that NDV more closely resembles the respiroviruses and morbilliviruses. Phylogenetic analysis of P gene sequences among NDV isolates demonstrates there are two clades of these viruses. One group includes viruses isolated in the US prior to 1970, while a second cluster includes virulent viruses circulating worldwide.

Lopez, N., P. Padula, et al. (1997). "Genetic characterization and phylogeny of Andes virus and variants from Argentina and Chile." Virus Research **50**(1): 77.

<http://www.sciencedirect.com/science/article/B6T32-3PM7H3K-8/2/9728723e4f07f95e3199b7890d3e9b7b>

Andes virus, one of five hantaviruses known to cause hantavirus pulmonary syndrome (HPS), emerged in 1995 in southwestern Argentina (Lopez et al. (1996) *Virology* 220, 223-226). The complete nucleotide sequence of Andes virus S genome segment was determined and compared with sequences of viral RNAs in autopsy tissues of more recently reported HPS cases from southwestern Argentina and south of Chile (cases ESQ H-1/96 and CH H-1/96). Andes virus S segment was found to be 1876 nucleotides in length and to encode the nucleocapsid protein (N), 428 amino acids in length. S segment analysis also revealed a long 5' non-coding region (547 nucleotides) which displays three copies of an octanucleotide sequence repeat. Comparisons of S segment sequences of ESQ H-1/96 and CH H-1/96 (82% of the entire genome sequence) with the corresponding sequences of Andes virus revealed identities of 97.2% and 98.5%, respectively. Sequence motifs identical and in the same positions as exhibited in Andes virus 5' non-coding region were found in both, ESQ H-1/96 and CH H-1/96 sequences. Three genome fragments of the M segment sequence of the viruses (representing approximately 34% of the entire sequence) were also analyzed. Comparisons of S and M segment sequences of Andes virus with the corresponding sequences of ESQ H-1/96 showed S and M segment identities which differ by less than 1.4%. Andes virus and CH H-1/96 have S segments that differ by 1.5% from one another while their M segment fragments differ by 5.5-8.2%. Phylogenetic analysis showed that Andes virus along with ESQ H-1/96 and CH H-1/96 form a distinct lineage within the clade containing Bayou and Black Creek Canal viruses. It also showed that Andes virus branch of trees derived from comparisons of S or M sequences differed. It is concluded that Andes virus variants causing HPS circulate east and west of the Andes mountains.

Mankertz, A., M. Domingo, et al. (2000). "Characterisation of PCV-2 isolates from Spain, Germany and France." Virus Research **66**(1): 65.

<http://www.sciencedirect.com/science/article/B6T32-3YF3WFB-6/2/3c9e6a6a3df551791a314d07a77d0273>

The new isolated circovirus variant PCV-2 is discussed to be the etiological agent of a new emerging swine disease with a variable morbidity and high lethality, postweaning multisystemic wasting syndrome (PMWS). PMWS has been diagnosed in North America and West Europe. Clinical signs include dyspnea, loss of weight, lymph node enlargement and lymphocyte depletion in lymphoid tissues. This report describes the characterisation of PCV-2 isolates from animals affected with PMWS from Germany, Spain and France. We could demonstrate the presence of circovirus by electron microscope, in situ hybridisation and PCR. PCR revealed incidence of PCV-2 in many tissues of one infected animal with the exception of heart and nervous system. The phylogenetic analysis of all PCV-2 isolates yet published in the database, showed relationship of isolates from Spain, Germany and France, with three sequences from Canada determined recently and two isolates from Taiwan, while other North American sequences display a separate cluster. PCR screening of randomly collected organ samples from German pigs not affected with PMWS, revealed a rate of infection with PCV-1 of 5% and with PCV-2 of 26.8%, while blood samples showed a lower incidence.

Meissner, J. D., J. O. Mecham, et al. (2001). "Verification of bluetongue virus S9 segment nucleotide sequences." Virus Research **81**(1-2): 93.

<http://www.sciencedirect.com/science/article/B6T32-448YJMP-9/2/5b3f0efc3ca39541aa8e1e04c2549719>

During the course of our bluetongue virus (BTV) nucleic acid sequence investigations, conflicts among United States (US) prototype BTV S9 genome segment sequences deposited in GenBank were noted. In order to rectify these inter-laboratory discrepancies, the S9 segments of Arthropod-borne Animal Diseases Research Laboratory (ABADRL)-stored US prototype BTV 2, BTV 10, BTV 11, BTV 13, and BTV 17 isolates were resequenced. Our S9 sequences, determined by direct sequencing of full-length reverse transcriptase-polymerase chain reaction (RT-PCR) generated amplicons, shared 99% or greater nucleotide identity with one or more respective S9 sequences previously reported. Possible sources of remaining unsupported US prototype BTV S9 sequences were evaluated by amplifying and sequencing the S9 segments of BTV 2 Ona A strain, South African (SA) prototype BTV 1, BTV 2, and BTV 4 strains, and the North American (NA) prototype epizootic hemorrhagic disease virus (EHDV) serotype 2 (Alberta) strain. Comparative analysis using these S9 sequences, as well as sequences of US BTV 2 field isolates, identified potential contributors to inter-laboratory sequence disagreements.

Mizutani, T., M. Ikeda, et al. (1998). "Detection of negative-stranded hepatitis C virus RNA using a novel strand-specific reverse transcription-polymerase chain reaction." Virus Research **53**(2): 209.

<http://www.sciencedirect.com/science/article/B6T32-3SBVWB5-R/2/46af4b9439c792336f004caa147052fd>

We developed a novel single-tube reverse transcription-polymerase chain reaction (RT-PCR) for the specific detection of negative-stranded hepatitis C virus (HCV) RNA. By using in vitro synthesized positive- and negative-stranded HCV RNAs, it was demonstrated that as few as 50 copies of negative-stranded HCV RNA could be specifically detected with a set of primers that amplify a 232-base pair sequence unique to the 5'-non-coding region of HCV RNA, while 108 copies of positive-stranded HCV RNA were not detected. In addition, we demonstrated that this method allows the detection as few as 100 copies of negative-stranded HCV RNA even with the

coexistence of a 100-fold excess of positive-stranded HCV RNA. Furthermore, with this method, negative-stranded HCV RNA was detected in RNAs from liver biopsy specimens obtained from patients with chronic hepatitis C, but not in RNAs from HCV-positive sera.

Okoye, A., P. Cordano, et al. (2005). "Human papillomavirus 16 L2 inhibits the transcriptional activation function, but not the DNA replication function, of HPV-16 E2." Virus Research **108**(1-2): 1.

<http://www.sciencedirect.com/science/article/B6T32-4D63968-1/2/60a453a8bdd5d601b5c5b773e501822a>

In this study we analysed the outcome of the interaction between HPV-16 L2 and E2 on the transactivation and DNA replication functions of E2. When E2 was expressed on its own, it transactivated a number of E2-responsive promoters but co-expression of L2 led to the down-regulation of the transcription transactivation activity of the E2 protein. This repression is not mediated by an increased degradation of the E2 protein. In contrast, the expression of L2 had no effect on the ability of E2 to activate DNA replication in association with the viral replication factor E1. Deletion mutagenesis identified L2 domains responsible for binding to E2 (first 50 N-terminus amino acid residues) and down-regulating its transactivation function (residues 301-400). The results demonstrate that L2 selectively inhibits the transcriptional activation property of E2 and that there is a direct interaction between the two proteins, although this is not sufficient to mediate the transcriptional repression. The consequences of the L2-E2 interaction for the viral life cycle are discussed.

Parkin, N. T., P. Chiu, et al. (1996). "Temperature sensitive mutants of influenza A virus generated by reverse genetics and clustered charged to alanine mutagenesis." Virus Research **46**(1-2): 31.

<http://www.sciencedirect.com/science/article/B6T32-3TT632H-4/2/e0c75d30897207e1367e8a50fe5fccc>

Temperature sensitive (ts) mutants of influenza A virus have the potential to serve as live attenuated (att) virus vaccines. Previously, ts mutants were isolated by chemical mutagenesis or arose spontaneously, and most likely contained point mutations in one or more genes. While sufficiently attenuated, even the most genetically stable of these viruses was found to revert to a more virulent form in a seronegative vaccinee. Recently developed technology, however, allows the introduction of engineered mutations into the genome of influenza A and B viruses, permitting the rational design of attenuated mutants with the potential for increased genetic stability. To accomplish this goal, we have introduced ts mutations into the PB2 gene of A/Los Angeles/2/87 (H3N2) and rescued the mutated genes into infectious viruses. We have used clustered charged to alanine mutagenesis (substitution of alanine for charged amino acid residues which are present in clusters) of the PB2 gene to generate novel ts mutants. Viruses containing such ts PB2 genes were attenuated in mice and ferrets. This approach has thus yielded several vaccine candidates with ts and attenuated characteristics in animal models. Combination of these mutations with each other or with other ts mutations may lead to a high level of genetic stability.

Parks, G. D., C. D. Ward, et al. (1992). "Molecular cloning of the NP and L genes of simian virus 5: identification of highly conserved domains in paramyxovirus NP and L proteins." Virus Research **22**(3): 259.

<http://www.sciencedirect.com/science/article/B6T32-476RGT0->

3K/2/628c1aa93f80a77ef41c727eef670bde

We have molecularly cloned and determined the nucleotide sequence of the 3' and 5' regions of the genomic RNA of the paramyxovirus simian virus 5 (SV5), including the 3' leader sequence, nucleocapsid protein (NP) gene, large (L) protein gene, and 5' anti-genomic leader (trailer) sequence. The vRNA 3' proximal leader sequence contains 55 nucleotides. The NP gene is 1725 nucleotides in length and encodes a negatively charged protein consisting of 509 residues (MW 56,534). A comparison of the amino acid sequences of 10 paramyxovirus NP proteins indicates a region of high sequence identity near the middle of the protein, and a C-terminal region which is enriched in negatively charged residues. Overall, the SV5 NP protein showed the highest degree of sequence identity with the NP proteins of parainfluenza type 2 virus (58%) and mumps virus (56%). The L gene extends 6804 nucleotides and encodes a positively charged protein consisting of 2255 residues (MW 255,923). The 5' proximal region of the vRNA consists of a 31 nucleotide trailer RNA. The SV5 L protein sequence showed 62% overall identity with the parainfluenza type 2 L protein. Although little overall sequence identity was found between the SV5 and other paramyxovirus L protein sequences, short stretches of extensive amino acid identity were found near the middle of each of the known paramyxovirus L protein sequences, and these common regions may represent sites important for enzymatic activity.

Peerenboom, E., V. Jacobi, et al. (1997). "A large duplication in the 3'-untranslated region of a subpopulation of RNA2 of the UK-M isolate of barley mild mosaic bymovirus." Virus Research **47**(1): 1.

<http://www.sciencedirect.com/science/article/B6T32-3TT632H-W/2/511207c2b2648193aa78f93820fea7a1>

The UK-M isolate of the bipartite barley mild mosaic bymovirus (BaMMV UK-M) cannot be fungally transmitted, and has previously been shown to have a 1092 nt deletion in the coding region of RNA2. We now report, using sequence and reverse transcriptase-polymerase chain reaction (RT-PCR) data, that a subpopulation of BaMMV UK-M RNA2 contains a direct imperfect sequence repeat of 552 nt in the 3' untranslated region. The secondary structure of the 3' end of RNA2, and its possible effects on replication of the virus, are also discussed.

Pletneva, M. A., S. V. Sosnovtsev, et al. (1998). "Characterization of a recombinant human calicivirus capsid protein expressed in mammalian cells." Virus Research **55**(2): 129.

<http://www.sciencedirect.com/science/article/B6T32-3T8W814-2/2/6820b095817b29e46cbffb5cd8e05486>

The capsid protein of the Hawaii strain of human calicivirus was expressed in the transient MVA/bacteriophage T7 polymerase hybrid expression system in order to examine its processing in mammalian cells. Selected amino acid modifications (an insertion, deletion, and substitution) at the predicted amino terminus of the capsid protein as well as the presence or absence of the ORF3 gene were examined for their effect on capsid expression. The protein was expressed efficiently in cell lines derived from three different species, with most of the expressed protein remaining localized within the cells. There was no evidence for N-linked glycosylation or myristylation of the 57 kDa capsid protein. Hawaii virus-like particles (HV VLPs), efficiently produced in the baculovirus expression system, were not observed in this expression system under the conditions in this study.

Pumphrey, C. Y. and W. L. Gray (1995). "DNA sequence of the simian varicella virus (SVV) gH gene and analysis of the SVV and varicella zoster virus gH transcripts." Virus Research **38**(1): 55.

<http://www.sciencedirect.com/science/article/B6T32-3YN9F2R-122/25041d62788aa44f82e352bb3e71931d>

The varicella zoster virus (VZV) glycoprotein H (gH) stimulates VZV-specific immune responses and may be involved in virus penetration. This study reports the genomic map position and the DNA sequence of a simian varicella virus (SVV) homologue of the VZV gH gene. A 32P-labeled VZV gH-specific DNA probe hybridized to the HindIII B subclone of the SVV BamHI B restriction endonuclease (RE) fragment. The DNA sequence of the SVV HindIII B subclone was determined and analysis indicated a SVV open reading frame (ORF) homologous to several herpesvirus gH genes. The SVV gH ORF is 2559 base pairs in size and encodes a 852-amino acid protein. The SVV gH contains characteristics of a transmembrane glycoprotein including: 9 consensus N-linked glycosylation sites, a potential amino terminal signal sequence, and a predicted transmembrane segment located near the carboxyl terminus. The SVV and VZV gH genes exhibit 60.0% identity and the predicted polypeptides exhibit 54.5% identity. The SVV and VZV gH transcripts were analyzed and the promoter regions were compared. 32P-labeled SVV and VZV gH-specific DNA probes each hybridized to a single 2.9 kilobase transcript. The mRNA start sites of the SVV and VZV gH genes were determined by primer extension analysis, and alignment of the promoter regions indicated similar content and arrangement. The extensive conservation of SVV and VZV genes and predicted polypeptides further supports the use of SVV infection of non-human primates as a model of VZV infection of humans.

Qiao, M., E. J. Gowans, et al. (1990). "Serological analysis of duck hepatitis B virus infection." Virus Research **17**(1): 3.

<http://www.sciencedirect.com/science/article/B6T32-476RKCH-122/2/692f892c6d5382c0987c8885059e4304>

A radioimmunoassay was developed to detect duck hepatitis B virus surface antigen and antibody; viraemia (DHBV DNA or DHBsAg) was detected in all ducks inoculated within 3 weeks post-hatch, and persistent infection developed in 93% of birds in this group. In contrast, only 80% and 60% of ducks inoculated 4-and 6-weeks post-hatch respectively developed viraemia, and approximately 70% of the viraemic ducks became carriers. Markers of viraemia were undetected in ducks inoculated 8 weeks post-hatch and in uninfected controls. A typical anti-DHBs seroconversion developed subsequently in 2 of 4 birds that showed transient viraemia, and antibody also developed in 3 of 7 ducks inoculated 4-8 weeks post-hatch that showed no viraemia. However, gene amplification by the polymerase chain reaction demonstrated DHBV DNA in ducks from the latter group suggesting that the antibody did not result from passive vaccination. Thus, increased resistance to infection develops with increasing age that may be related to several factors including host immunity. This model may help elucidate similar age-related features of human hepatitis B virus infections.

Sarcinella, E., M. Brown, et al. (2004). "Detection of RNA in purified cytomegalovirus virions." Virus Research **104**(2): 129.

<http://www.sciencedirect.com/science/article/B6T32-4CDJHJG-2/2/68122e7a0dfd00050e42ffd2e3fa4ccc>

Five viral RNA transcripts have recently been detected in purified virions of human

cytomegalovirus (HCMV) strain AD169, a well-characterized member of the family Herpesviridae [Science 288 (2000) 2373]. While the function of these transcripts and/or the proteins they encode remains to be elucidated, it is not known whether these transcripts are unique to strain AD169 or are present in other HCMV strains. The objective of this study was to determine if these RNAs are present in other HCMV laboratory strains (Towne and Davis), and a low passage clinical isolate (CL203). These strains of CMV were purified by sequential ultracentrifugation through 20% -sorbitol and glycerol-potassium tartarate gradients and the morphology and infectivity of the virions confirmed by electron microscopy and inoculation into cell culture. When RNA extracted from the purified virions was amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) the UL 21.5 and TRL/IRL 2-5 transcripts were detected in virions of HCMV strains AD169, Davis, Towne and CL203. The presence of the UL 21.5 and TRL/IRL 2-5 RNA transcripts in all strains tested demonstrates that the packaged transcripts occurs in all strains of HCMV suggesting that they may have a relevant role in the biology of this virus.

Seal, B. S. (1994). "Analysis of capsid protein gene variation among divergent isolates of feline calicivirus." Virus Research **33**(1): 39.

<http://www.sciencedirect.com/science/article/B6T32-476M3G4-68/2/aa5990c5eaf1e2ac19d739c03bfd66a0>

Genomic variability within the capsid protein gene of feline calicivirus (FCV) was evaluated among different isolates using hybridization analysis and enzymatic viral nucleic acid amplification. Total infected cell RNA was first hybridized with cDNA clones generated to the capsid gene of the FCV isolates CFI/68, 255, LLK, NADC and KCD. Field isolates of FCV were categorized by hybridization with capsid gene cDNA from the reference strains. Isolates that did not hybridize were positive by Western blot using a cross-reactive cat polyclonal FCV CFI/68 capsid protein antiserum. Using previously published sequence information, oligonucleotide primers were generated based on conserved sequences surrounding the hypervariable capsid protein gene regions. Analysis of the FCV capsid protein gene hypervariable regions was completed by sequencing products of FCV nucleic acid amplified by reverse transcription and polymerase chain reaction. From these data, amino acid substitutions in the hypervariable regions of the capsid protein were identified for those isolates that did not hybridize with the original cDNA clones. An association between phylogenetic relationships and serum neutralization was established among FCV isolates examined.

Seal, B. S., D. J. King, et al. (2000). "Molecular evolution of the Newcastle disease virus matrix protein gene and phylogenetic relationships among the paramyxoviridae." Virus Research **66**(1): 1.

<http://www.sciencedirect.com/science/article/B6T32-3YF3WFB-1/2/fcda134146b810036a495a6ac276e8f0>

Matrix (M) gene sequences for recent field isolates and older reference Newcastle disease viruses (NDV) were examined to determine phylogenetic relationships and population trends among these viruses. Overall, the M gene has a majority of synonymous nucleotide sequence substitutions occurring among NDV isolates. However, several predicted amino acid changes in the M protein of specific NDV isolates have occurred that correlate to phylogenetic relationships. Nucleotide substitutions in these codons have a greater number of nonsynonymous base changes. The NDV isolates arising since the 1970s belong to a population of viruses that expanded worldwide at an exponential rate. These viruses may have their origins in free-living birds, are present worldwide, and continue to circulate causing disease in poultry. A specific NDV lineage composed of virulent isolates obtained in the US prior to 1970 appears to no longer exists among free-living birds or commercial poultry. However, "vaccine-like" viruses are common in the

US and continue to circulate among commercial poultry. Based on M protein amino acid sequences, NDV separates as a clade most closely related to morbilliviruses and not with their current designated category, the rubulaviruses among the Paramyxoviridae. Consequently, avian paramyxoviruses should have their own taxonomic subfamily among the Paramyxovirinae.

Seal, B. S., C. Lutze-Wallace, et al. (1995). "Isolation of caliciviruses from skunks that are antigenically and genotypically related to San Miguel sea lion virus." Virus Research **37**(1): 1.

<http://www.sciencedirect.com/science/article/B6T32-3YS2B9B-M/2/d77942186828a68bd81ca0e120d66b3c>

Caliciviruses were isolated from feces of skunks imported from the north central United States to Canada. Virus isolation was accomplished using adenovirus-transformed human kidney (293) cells, swine testes and Vero cells. Plaque size variants were present, but there was no apparent difference in virus morphology by negative stain or immune electron microscopy. Pigs infected with skunk calicivirus had a slightly elevated body temperature at 3 days postinfection. Although the infected animals seroconverted, no overt clinical signs were observed. Purified infectious genomic skunk calicivirus RNA behaved exactly as San Miguel sea lion virus (SMSV) 1 and 4 genomic RNA in cell culture transfection studies. Of the cell types examined, only primary porcine kidney, 293 and Vero cells supported viral replication. No viral replication was detected in cells of bovine, equine, ovine, caprine or feline origin. The skunk caliciviruses contained a single capsid protein with a relative mobility similar to SMSV virus 1 and 4 capsid proteins. The capsid protein was positive by Western blot analysis with SMSV and vesicular exanthema of swine virus (VESV) antisera. Purified RNA from skunk calicivirus infected cells was subjected to reverse transcription followed by polymerase chain reaction. Nucleotide sequences were identified that had greater than 85% similarity to the 2C and RNA polymerase gene regions of SMSV 1 and 4 and VESV A48. Predicted amino acid sequences of these regions were greater than 95% similar and the partial coding sequence of the polymerase gene contained the YGDD sequence common to positive-strand RNA virus polymerases.

Sullivan, D. G. and R. K. Akkina (1995). "A nested polymerase chain reaction assay to differentiate pestiviruses." Virus Research **38**(2-3): 231.

<http://www.sciencedirect.com/science/article/B6T32-3YN9F1W-B/2/f0590863768770619bcf713885009f9c>

Viruses that comprise the Pestivirus genus cause significant losses to the livestock industry. Based on sequence analysis, currently 4 distinct genotypes are identified of which 3 infect cattle and sheep. Distinguishing between bovine and ovine isolates by serological tests has often been difficult because of a high degree of cross reactivity. In this study, a nested polymerase chain reaction (PCR) assay was developed to identify and distinguish between bovine viral diarrhea virus (BVDV) type I, BVDV type II, as well as border disease virus (BDV) genotypes. Consensus oligonucleotide primers were designed to amplify a 826-bp product from any of the 3 pestivirus types in a reverse transcription-PCR (R-PCR). This product was subjected to a second round of nested PCR with type-specific primers which yielded DNA products of unique size characteristic for each pestivirus genotype. Using this assay, we were able to rapidly characterize several viral isolates and determine that all 3 genotypes can be found among ovine isolates.

Sullivan, D. G., G.-J. Chang, et al. (1997). "Genetic characterization of ruminant pestiviruses: sequence

analysis of viral genotypes isolated from sheep." Virus Research **47**(1): 19.

<http://www.sciencedirect.com/science/article/B6T32-3TT632H-Y/2/aa2c93d6e5ee9d3ed9300ee024624925>

Historically, the genus pestivirus was believed to contain three species of viruses; bovine viral diarrhea virus (BVDV), border disease virus (BDV) and classical swine fever virus (CSFV). However, based on limited sequence analysis of a small number of pestiviral isolates from domestic livestock, evidence has recently emerged indicating that at least four distinct genotypes exist. In an attempt to gain a better understanding of the degree of viral variation among ruminant pestiviruses, the entire structural gene coding region of an ovine pestivirus, BD31, genome encompassing 3358 nucleotides was cloned and sequenced. Sequence analysis revealed that BD31 shares less than 71% nucleotide similarity with other pestiviruses, suggesting that BD31 is distinct from BVDV, CSFV as well as other ovine and bovine pestiviruses currently referred to as BVDV type II. Based on this data, BD31 is the first North American pestivirus isolate that falls under the category 'true BDV'. Results from the analysis of the nucleotide sequence of the E0-E1 coding region of six additional ruminant pestiviruses identified the existence of three distinct virus genotypes in North America. Thus, among ruminant pestiviruses, bovine isolates can be grouped into two genotypes, namely types 1 and 4, whereas ovine isolates fall into genotypes 1, 3 and 4.

Sullivan, D. G., G.-J. Chang, et al. (1994). "Nucleotide sequence analysis of the structural gene coding region of the pestivirus border disease virus." Virus Research **33**(3): 219.

<http://www.sciencedirect.com/science/article/B6T32-476RJ85-T1/2/b638dde4cee34be6a9a15c02591934d9>

Border disease virus (BDV) of sheep, an important ovine pathogen, is serologically related to the two other well characterized members of the Pestivirus genus of the Flaviviridae family, namely bovine viral diarrhea virus (BVDV) and hog cholera virus (HoCV). To determine its genetic relationship to BVDV and HoCV, the genome of BDV strain, BD-78 encompassing the 5' untranslated region (UTR) and structural gene coding region was molecularly cloned and the nucleotide sequence determined. The sequenced region of 3,567 nucleotides contained one open reading frame encoding 1063 amino acids. The nucleotide and amino acid sequences of BD-78 were compared with those of two BVDV strains NADL and SD-1, and the Alfort and Brescia strains of HoCV. The overall nucleotide sequence homologies of the region sequenced of BD-78 are 68.3% with BVDV-NADL, 67.8% with BVDV-SD-1, 69.0% with HoCV-Brescia, and 65.8% with HoCV-Alfort. The overall amino acid sequence homologies of BD-78 are 76.1% with NADL, 76.5% with SD-1, 74.2% with Brescia, and 72.9% with Alfort. The most conserved nucleotide and amino acid sequences between BD-78 and the other pestiviruses are in the 5' UTR and the capsid protein coding region (p14), whereas the most divergent sequences are in the E2 coding region. These findings suggest that BDV is a unique virus in the Pestivirus genus.

Tetsu, M., I. Yuji, et al. (1995). "Identification of the major capsid protein gene of human herpesvirus 7." Virus Research **37**(1): 55.

<http://www.sciencedirect.com/science/article/B6T32-3YS2B9B-T/2/0d2bde30b3146ea58bfa98aa05368eef>

Two human herpesvirus 6 (HHV-6) open reading frames were identified with significant amino acid similarity to UL86 (major capsid protein, MCP) and UL85 of human cytomegalovirus (HCMV) in human herpesvirus 7 (HHV-7) genes. The predicted lengths of the complete HHV-7 MCP and

the HCMV-UL85 open reading frames were 1344 and 293 amino acids with estimated molecular weights of approximately 153 and 33 kDa, respectively. Computer analysis showed that the amino acid of HHV-7 MCP was 61% identical to the MCP of HHV-6 variants A and B, 28% to HCMV. These results suggest that HHV-7 is more closely related to HHV-6 than to HCMV.

Thiery, R., J.-C. Raymond, et al. (1999). "Natural outbreak of viral encephalopathy and retinopathy in juvenile sea bass, *Dicentrarchus labrax*: study by nested reverse transcriptase-polymerase chain reaction." Virus Research **63**(1-2): 11.

<http://www.sciencedirect.com/science/article/B6T32-3X9YRG1-3/2/8b3e221fb8b64418cf34a750f92363f1>

In order to improve the sensitivity of the diagnosis of viral encephalopathy and retinopathy (VER) in sea bass, a nested reverse transcriptase-polymerase chain reaction (RT-PCR) detection method was developed. The reverse transcription step and the first stage PCR were performed using outer primers specific for the coat protein gene, whereas a new primer set was used as inner primers for the second stage PCR. Fish were collected just before, during and after a VER outbreak occurring in a mediterranean fish farm. For each time point, ten different fish were analysed individually by nested RT-PCR, single step PCR and virus cultivation. The results showed that the frequency of positive samples was always higher using the nested RT-PCR assay. In particular, it was possible to detect nodavirus specific signals 1 month before the appearance of the first mortalities, but only by nested RT-PCR. Altogether these results showed that the sensitivity of nodavirus detection is greatly improved using a nested RT-PCR method. In particular, it was possible to monitor the presence of viral genome in asymptomatic carrier fish using this method.

Truong, M.-J., V. Delsart, et al. (2004). "Differentially expressed genes in HIV-1-infected macrophages following treatment with the virus-suppressive immunomodulator murabutide." Virus Research **99**(1): 25.

<http://www.sciencedirect.com/science/article/B6T32-4B50499-2/2/37d173da5cb2b63de9fd7c8795e826bd>

The synthetic immunomodulator murabutide has been found to suppress human immunodeficiency virus type-1 (HIV-1) replication, in macrophages, through a regulated expression of cellular factors needed at different steps in the virus replication cycle. To identify cellular genes implicated in the murabutide-induced virus inhibition, we have carried out a differential display analysis on HIV-1-infected macrophages that were treated, or not, with murabutide. Sequencing of the differentially regulated cDNA bands and verification of the reproducibility of the murabutide effects, by reverse transcription-polymerase chain reaction or by Northern blotting, revealed an up-regulated expression of 21 genes and a down-regulation of seven others. The murabutide-regulated genes encoded proteins implicated in DNA binding, regulation of transcription, oxidative stress, metal binding, and other physiological functions. Six of the genes corresponded to unassigned/expressed sequence tags with yet unknown function. Among the genes which were up-regulated by murabutide and with established effects on inhibiting virus transcription, was the octamer binding factor 1 (Oct-1). We demonstrate the ability of murabutide to induce enhanced Oct-1 protein expression and DNA-binding activity in macrophages. Furthermore, our findings suggest the potential implication of additional transcription factors and metal-binding proteins in mediating the inhibitory effect of murabutide on virus transcription.

Webby, R. J., K. Rossow, et al. (2004). "Multiple lineages of antigenically and genetically diverse influenza A virus co-circulate in the United States swine population." Virus Research **103**(1-2): 67.

<http://www.sciencedirect.com/science/article/B6T32-4C7DGH7-3/2/9acc9d50a2cb720bc53c8a837da6c0fc>

Before the isolation of H3N2 viruses in 1998, swine influenza in the United States was an endemic disease caused exclusively by classical-swine H1N1 viruses. In this study we determined the antigenic and phylogenetic composition of a selection of currently circulating strains and revealed that, in contrast to the situation pre-1998, the swine population in the United States is now a dynamic viral reservoir containing multiple viral lineages. H3N2 viruses still circulate and representatives of each of two previously identified phylogenetic groups were isolated. H1N1 and H1N2 viruses were also identified. In addition to the genotypic diversity present, there was also considerable antigenic diversity seen. At least three antigenic profiles of H1 viruses were noted and all of the recent H3N2 viruses reacted poorly, if at all, to the index A/swine/Texas/4199-2/98 H3N2 antiserum in hemagglutination inhibition assays. The influenza reservoir in the United States swine population has thus gone from a stable single viral lineage to one where genetically and antigenically heterogeneous viruses co-circulate. The growing complexity of influenza at this animal-human interface and the presence of viruses with a seemingly high affinity for reassortment makes the United States swine population an increasingly important reservoir of viruses with human pandemic potential.

Weli, S. C., O. Nilssen, et al. (2005). "Avipoxvirus multiplication in a mammalian cell line." Virus Research **109**(1): 39.

<http://www.sciencedirect.com/science/article/B6T32-4DWH5WB-1/2/5a04295a4ff999e071920be2cc57f8f8>

Avipoxviruses have many advantages and are being increasingly employed as recombinant vaccine vectors. One attractive feature is that while inserted transgenes are expressed in immunologically favourable ways, avipoxvirus infections of mammalian cells are believed to be abortive. The experimental evidence supporting this belief is, however, based on a limited number of mammalian cell-types and a few avipoxvirus species. We evaluated two avian and eight mammalian cell lines for permissivity to three avipoxvirus strains, one reference fowlpoxvirus and two newly isolated strains from sparrow and pigeon, respectively. Both avian cell lines were, as expected, permissive for all three avipoxvirus strains. However, by multiplication assays, we found to our surprise that Syrian baby hamster kidney (BHK-21) cells were equally permissive to all virus strains. Results from electron microscopy of infected BHK-21 cells revealed viral morphogenesis proceeding to various forms of infectious viruses. These results were supported by the demonstration of avipoxvirus specific late gene expression and avipoxvirus specific DNA restriction pattern in BHK-21 infected cells.

Wise, A. G., S. S. Monroe, et al. (2004). "Molecular characterization of noroviruses detected in diarrheic stools of Michigan and Wisconsin dairy calves: circulation of two distinct subgroups." Virus Research **100**(2): 165.

<http://www.sciencedirect.com/science/article/B6T32-4BDCBKS-1/2/3994cb9be313dc8073267e5924ab7988>

Noroviruses have emerged as the leading worldwide cause of acute non-bacterial gastroenteritis in humans. The presence of noroviruses in diarrheic stool samples from calves on Michigan and Wisconsin dairy farms was investigated by RT-PCR. Norovirus-positive samples were found on all eight farms studied in Michigan and on 2 out of 14 farms in Wisconsin. Phylogenetic analyses of partial polymerase and capsid sequences, derived for a subset of these bovine noroviruses, showed that these strains formed a group which is genetically distinct from the human noroviruses, but more closely related to genogroup I than to genogroup II human noroviruses. Examination of 2 full and 10 additional partial capsid (ORF2) sequences of these bovine strains revealed the presence of two genetic subgroups or clusters of bovine noroviruses circulating on Michigan and Wisconsin farms. One subgroup is "Jena-like", the other "Newbury agent-2-like".

Yokoyama, M., Y. Nakao, et al. (1995). "Alterations in physical state and expression of human papillomavirus type 18 DNA following crisis and establishment of immortalized ectocervical cells." Virus Research **37**(2): 139.

<http://www.sciencedirect.com/science/article/B6T32-3YS2B8X-H/2/7bf44bb789bbfa3b8b5709e02ec48f79>

Integration of episomal human papillomavirus (HPV) DNA in infected cervical lesions during malignant progression is frequently observed, but the importance of integration is poorly understood. We have studied immortalization by HPV-18 of human cervical cells as an in vitro model system. Here, the status and expression of HPV-18 DNA in precrisis ectocervical keratinocytes was compared with that in the same cells after crisis and establishment of immortalization. Southern blots reserved, and two-dimensional gel analysis confirmed, that the precrisis culture contained more than 100 copies/cell of episomal HPV-18 DNA and no detectable integrated viral DNA. In contrast, the postcrisis cells contained a low copy number of only integrated viral genome. The Northern blot patterns of E6-E7 and E2/E4 RNA expression were also different. Analysis of RNA by RT-PCR indicated that neither culture expressed the unspliced HPV-18 E6 oncogene present in tumor cell lines and that the precrisis, but not postcrisis, culture expressed the full-length E2 repressor. The two cultures displayed a similar keratinocyte morphology in vitro and a similar low grade dysplasia in vivo and both were non-tumorigenic. These results suggest that, although insufficient for complete malignant conversion, viral DNA integration during crisis is associated with the establishment of an immortalized phenotype in which HPV-18 DNA is integrated and HPV-18 RNA expression is altered.

Yuan, S., C. J. Nelsen, et al. (1999). "Recombination between North American strains of porcine reproductive and respiratory syndrome virus." Virus Research **61**(1): 87.

<http://www.sciencedirect.com/science/article/B6T32-3WP2PH3-9/2/614042da577760b5801de7c752d8733f>

Porcine reproductive and respiratory syndrome virus (PRRSV), a recently discovered arterivirus swine pathogen, was shown to undergo homologous recombination. Co-infection of MA-104 cells with two culture-adapted North American PRRSV strains resulted in recombinant viral particles containing chimeric ORF 3 and ORF 4 proteins. Nucleotide sequence analysis of cloned recombinant PCR products, encompassing 1182 bases of the 15.4 kb viral genome, revealed six independent recombination events. Recombinant products persisted in culture for at least three passages, indicating continuous formation of recombinant viruses, growth of recombinant viruses in competition with parental viruses, or both. The frequency of recombination was estimated from <2% up to 10% in the 1182 b fragment analyzed, which is similar to recombination frequencies observed in coronaviruses. An apparent example of natural ORF 5 recombination between naturally occurring wild type viruses was also found, indicating that recombination is likely an

important genetic mechanism contributing to PRRSV evolution.

Zongyu, C., K. S. Faaberg, et al. (1994). "Detection of negative-stranded subgenomic RNAs but not of free leader in LDV-infected macrophages." Virus Research **34**(2): 167.

<http://www.sciencedirect.com/science/article/B6T32-476TXW9-6/2/ac06f19134ab354869758366085a30b1>

The mechanism of synthesis of the seven subgenomic mRNAs of lactate dehydrogenase-elevating virus (LDV) was explored. One proposed mechanism, leader-primed transcription, predicts the formation of free 5'-leader in infected cells which then primes reinitiation of transcription at specific complementary sites on the antigenomic template. No free LDV 5'-leader of 156 nucleotides was detected in LDV-infected macrophages. Another mechanism, independent replication of the subgenomic mRNAs, predicts the presence of negative complements to all subgenomic mRNAs in infected cells which might be generated from subgenomic mRNAs in virions. Full-length antigenomic RNA was detected in LDV-infected macrophages by Northern hybridization at a level of < 1% of that of genomic RNA, but no negative polarity subgenomic RNAs. Negative complements to all subgenomic mRNAs, however, were detected by reverse transcription of total RNA from infected macrophages using as primer an oligonucleotide complementary to the antileader followed by polymerase chain reaction amplification using this sense primer in combination with various oligonucleotide primers complementary to a segment downstream of the junction between the 5' leader and the body of each subgenomic RNA. It is unclear whether these minute amounts of negative subgenomic RNAs function in the replication of the subgenomic mRNAs. They could also be by-products of the RNA replication process. Finally, no subgenomic mRNAs were detected in LDV virions.

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Balding, S. D., S. A. Sjöberg, et al. (1998). "Pigment gene expression in protan color vision defects." Vision Research **38**(21): 3359.

<http://www.sciencedirect.com/science/article/B6T0W-43N89PX-V/2/02287de9b55b8ec81f6bda5c84fdadd8>

We screened 150 male eye donors and identified four who did not have or express L pigment genes, consistent with each of them having a congenital protan color vision defect. One donor was identified as a protanope because he had and expressed a single X-chromosome photopigment gene that encoded an M pigment. Three were categorized as protanomalous because each expressed significant levels of genes specifying two spectrally different M pigments. The first gene in each of the protanomalous arrays was expressed the most and encoded an M pigment that differed in amino acid sequence from M pigments in color normal men.

Farjo, R., J. Yu, et al. (2002). "Mouse eye gene microarrays for investigating ocular development and disease." Vision Research **42**(4): 463.

<http://www.sciencedirect.com/science/article/B6T0W-454J5RW-8/2/546095c9d28979c46b82c4a0cd99f3af>

Funkenstein, B. and S. B. Jakowlew (1997). "Piscine (*Sparus aurata*) [α] subunit of the G-protein transducin is homologous to mammalian cone and rod transducin." Vision Research **37**(18): 2487.

<http://www.sciencedirect.com/science/article/B6T0W-3YVVBWX-2/2/a3a1386f42d51b340927bb5db8740607>

A novel cDNA encoding [α] subunit of the GTP-binding protein, transducin, has been cloned from a marine fish, *Sparus aurata*. The cDNA contains an open reading frame of 1050 nt (encoding 350 amino acid residues). A high degree of identity was found with known mammalian transducin proteins of cones (Gt2[α]) or rods (Gt1[α]): human Gt2[α] (80.2%), bovine Gt2[α] (79.3%), mouse Gt1[α] (78.2%), mouse Gt2[α] (78%) and bovine Gt1[α] (77.9%). Northern blot analysis of different tissues revealed a transcript of about 2.5 kb, which is expressed only in the fish eye and not in other tissues from adult fish, supporting its identification as transducin. Ontogeny of transducin mRNA expression during early development of *Sparus aurata*, determined by Northern blot analysis, showed very low levels in larvae 3 days after hatching but not earlier. Levels increased 3- and 6-fold on days 4 and 6 (respectively) compared with those on day 3 and remained essentially unchanged thereafter, until day 21 after hatching (the last day studied). Our results suggest that in fish only one [α] subunit of transducin is found, which shows similar identity with cone and rod [α] subunits of mammals.

Kainz, P. M., M. Neitz, et al. (1998). "Molecular genetic detection of female carriers of protan defects." Vision Research **38**(21): 3365.

<http://www.sciencedirect.com/science/article/B6T0W-43N89PX-W/2/5cf6d66a7d142b3c688cd2bcf986e60e>

Females heterozygous for congenital colour vision defects are of interest because they are believed to have cone photoreceptor ratios and cone photopigments that differ from normal. We describe a molecular genetic method to identify protan carriers that involves characterizing the genes that occur in the most upstream position in each of the X-chromosome photopigment gene arrays.

Kraft, T. W., J. Neitz, et al. (1998). "Spectra of human L cones." Vision Research **38**(23): 3663.

<http://www.sciencedirect.com/science/article/B6T0W-3TGVGKR-1/2/320bc635a4622e7a2f97906a495e5679>

Variations in the amino acid sequences of the human cone opsins give rise to spectrally variant subtypes of L and M cone pigments even in the population with normal color vision. In vitro mutagenesis studies have shown that a limited number of amino acid substitutions produce shifts in the wavelength sensitivity. Presented here are results comparing electrophysiological measurements of single human cones with the expressed cone pigment gene sequences from the same retina. In a sample of eight long-wavelength sensitive cone (L cone) spectra obtained from five donors the precise spectral sensitivities, measured in situ, of the two most commonly occurring spectral variants were determined. The peak sensitivity of the Lser180 cone was 563

nm while that of the Lala180 cone was 559 nm.

Muradov, H., K. K. Boyd, et al. "Structural determinants of the PDE6 GAF A domain for binding the inhibitory [gamma]-subunit and noncatalytic cGMP." Vision Research **In Press, Corrected Proof**
<http://www.sciencedirect.com/science/article/B6T0W-4CVWR61-1/2/ce02b44937aa7b723acb086736798cd5>

Photoreceptor cGMP phosphodiesterases (PDE6 family) are modular enzymes with each catalytic subunit containing two N-terminal regulatory GAF domains, GAF A and GAF B. The GAF A domains contribute to dimerization of the PDE6 catalytic subunits and to binding of the inhibitory P[gamma] subunits, and represent candidate sites for noncatalytic binding of cGMP. We performed a mutational analysis of selected residues from the GAF A domain of cone PDE[alpha] to identify the cGMP-binding pocket and delineate the P[gamma]-binding surface. Results of this analysis establish the noncatalytic cGMP-binding site within the PDE6 GAF A domain and suggest that occupation of the pocket by cGMP is required for high-affinity binding of P[gamma] to the proximate contact surface.

Muradov, H., K. K. Boyd, et al. (2004). "Structural determinants of the PDE6 GAF A domain for binding the inhibitory [gamma]-subunit and noncatalytic cGMP." Vision Research **44**(21): 2437.

<http://www.sciencedirect.com/science/article/B6T0W-4CX0S4Y-1/2/93ac035ef300ef22df1024ae946c14dd>

Photoreceptor cGMP phosphodiesterases (PDE6 family) are modular enzymes with each catalytic subunit containing two N-terminal regulatory GAF domains, GAF A and GAF B. The GAF A domains contribute to dimerization of the PDE6 catalytic subunits and to binding of the inhibitory P[gamma] subunits, and represent candidate sites for noncatalytic binding of cGMP. We performed a mutational analysis of selected residues from the GAF A domain of cone PDE[alpha] to identify the cGMP-binding pocket and delineate the P[gamma]-binding surface. Results of this analysis establish the noncatalytic cGMP-binding site within the PDE6 GAF A domain and suggest that occupation of the pocket by cGMP is required for high-affinity binding of P[gamma] to the proximate contact surface.

Neitz, M., T. W. Kraft, et al. (1998). "Expression of L cone pigment gene subtypes in females." Vision Research **38**(21): 3221.

<http://www.sciencedirect.com/science/article/B6T0W-43N89PX-4/2/a71c68824c468ac8eefd6d4003a35a4e>

Spectral subtypes of L pigment are produced by a serine/alanine dimorphism at amino acid position 180. X-chromosomes that carry genes for the different subtypes occur with about equal frequency in normal men. Females have two X-chromosomes; thus, about 50% of women will inherit genes for both L pigment subtypes, although on different X-chromosomes. In these women, X-inactivation is expected to produce about equal numbers of LS180 and LA180 cones in addition to middle (M) and short (S) wavelength-sensitive cones to total four spectrally distinct cone types. Consistent with this expectation we found nearly equal expression of genes for two spectrally distinct subtypes of L pigment in five of nine female retinas examined.

Osborne, N. N., R. Safa, et al. (1999). "Photoreceptors are preferentially affected in the rat retina following permanent occlusion of the carotid arteries." Vision Research **39**(24): 3995.

<http://www.sciencedirect.com/science/article/B6T0W-3XD3H6N-3/2/c39b907ec0d63957bafa23a1c5a91a85>

Carotid artery occlusion (two vessel occlusion; 2-VO) for 3 or 9 months causes a suppression of the electroretinogram. However, after 3 months the retinal morphology appears unaffected judging from the localisation of GABA, ChAT, [alpha]PKC, Thy-1 and GFAP immunoreactivities. Moreover, no difference in NMDA-R1, opsin or Thy-1 mRNA levels were detected. In contrast, after 9 months 2-VO photoreceptor degeneration occurred as indicated by thinning of the outer nuclear layer and reduced Ret-P1 immunoreactivity. All other immunoreactivities appeared normal. These findings were supported by analysis of retinal mRNA levels. We conclude that the major effect of prolonged 2-VO is photoreceptor degeneration.

Sjoberg, S. A., M. Neitz, et al. (1998). "L-cone pigment genes expressed in normal colour vision." Vision Research **38**(21): 3213.

<http://www.sciencedirect.com/science/article/B6T0W-43N89PX-3/2/c28ebef78b26eab0aef85f1ffa8620f4>

To directly test the hypothesis that only two pigment genes are expressed from the X-chromosome array, we examined expressed M and L pigment gene sequences from >100 male eye donors. In this sample, there were eight men who expressed high levels of more than one L pigment gene in addition to M pigment genes. The fact that these eyes expressed both L and M pigment genes at significant levels suggests they were from men with normal colour vision. We reject the hypothesis that only two pigment genes from one X-chromosome array can be expressed.

Terao, K., A. Mikami, et al. (2005). "Identification of a protanomalous chimpanzee by molecular genetic and electroretinogram analyses." Vision Research **45**(10): 1225.

<http://www.sciencedirect.com/science/article/B6T0W-4F1SVBD-9/2/0330fcde256125cb6d904166c842d52e>

We determined the structures of long (L)-wavelength-sensitive and middle (M)-wavelength-sensitive opsin gene array of 58 male chimpanzees and we investigated relative sensitivity to red and green lights by electroretinogram flicker photometry. One subject had protanomalous color vision, while others had normal color vision. Unlike in humans, a polymorphic difference in the copy number of the genes and a polymorphic base substitution at amino acid position 180 were not frequently observed in chimpanzees.

Vardi, N., K. Morigiwa, et al. (1998). "Neurochemistry of the mammalian cone 'synaptic complex'." Vision Research **38**(10): 1359.

<http://www.sciencedirect.com/science/article/B6T0W-3SY2TSW-7/2/b9532a1bb68999789b85f37b87d44d42>

The cone 'synaptic complex' is a unique structure in which a single presynaptic axon secretes

glutamate onto processes of bipolar cells (both ON and OFF) and horizontal cells. In turn, the horizontal cell processes antagonize cone and bipolar responses to glutamate (probably by GABA). What still remains largely unknown is the molecular identity of the postsynaptic receptors and their exact locations. We identified several subunits of the glutamate receptor and the GABAA receptor expressed at the cone synaptic complex and localized them ultrastructurally. Glutamate receptors: (i) Invaginating (probably ON) bipolar dendrites in the monkey and rat express the metabotropic glutamate receptor, mGluR6. The stain is intense on the dendritic membrane where it first enters the invagination, and weak at the tip nearest to the ribbon. The cone membrane is electron-dense where it apposes the intense stain for mGluR6. Surprisingly, invaginating bipolar dendrites in the cat also express the AMPA receptor subunits, GluR2/3 and GluR4. (ii) Dendrites forming basal contacts in the cat (probably OFF) express the AMPA subunits GluR2/3, GluR4, and also the kainate subunit, GluR6/7. The stain is especially intense at the dendritic tips in apposition to electron-dense regions of cone membrane. (iii) Horizontal cells in the cat express the AMPA subunits GluR2/3, GluR4 and the kainate subunit, GluR6/7. The stain is strongest in the cytosol of somas and primary dendrites, but is also present in the invaginating terminals where it localizes to the membrane subjacent to the ribbon. GABAA receptors: (i) ON and OFF bipolar dendrites in the monkey express the [alpha]1 and [beta]2/3 subunits. The stain is localized to the bipolar cell membrane in apposition to horizontal cell processes. (ii) Cones did not express the GABAA subunits tested by immunocytochemistry, but [beta]3 mRNA was amplified by RT-PCR from rat photoreceptors. Conclusions: (i) mGluR6 receptors concentrate on dendrites at the base of the invagination rather than at the apex. This implies that receptors at both 'invaginating' and 'basal' contacts lie roughly equidistant from the release sites and should therefore receive similar spatiotemporal concentrations of glutamate. (ii) The 'cone' membrane is electron-dense opposite to the receptor sites on both ON and OFF bipolar cells. This suggests a special role for this region in synaptic transmission. Possibly, these densities signify a transporter that would regulate glutamate concentration at sites remote (>200 nm) from the locus of vesicle release.