GENE THERAPY

Organizers: Inder Verma and Fred Gage

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Delivery Systems

DZ 001 ADENO-ASSOCIATED VIRUS VECTORS FOR *IN VIVO* GENE TRANSFER, **B. Carter*, T. Flotte, S. Afione, W. Guggino. Targeted Genetics Corp., Seattle* & Johns Hopkins Medical School, Baltimore.**

AAV has several desirable features as a vector for gene therapy. AAV is a defective human parvovirus, is non-pathogenic, has a linear 4.7 Kb single strand DNA genome and replicates in the cell nucleus. Replication requires the AAV *rep* and *cap* genes and helper functions expressed by an adenovirus or herpesvirus. Without helper, AAV DNA integrates into the host cell genome at high efficiency. AAV has a broad host range (human, simian, rodent) and no obvious limitations of cell or tissue specificity and apparently does not require dividing cells for efficient expression.

AAV vectors require the 145 bp ITR regions in *cis* but no viral coding regions, express no viral antigens, can package a maximum of about 4.5 kb of foreign DNA and can be purified and concentrated to high titer. AAV vectors yield high transduction efficiencies in *in vitro* assays. An AAV-neo vector transduced human epthelial cells with at least 70% efficiency and an AAV-CFTR vector expressed CFTR protein in 75% of primary human nasal polyp cells from CF patients.

To assess *in vivo* delivery, and as a stringent test of the packaging capacity, we used AAV vectors with the human CFTR cDNA. These vectors complemented the cystic fibrosis chloride channel defect as measured by functional assays in excised patch-clamp of the rabbit lung using a fiberoptic bronchoscope. An *in situ* DNA PCR assay detected vector DNA in the targeted lobe. A 26 amino acid region unique to the recombinant AAV-CFTR protein was used to allow unambiguous detection of vector CFTR RNA and protein expression. Both RNA and protein expression were detected for extended periods. These results suggest that AAV vectors may be useful for *in vivo* gene therapy applications.

DZ 002 RECEPTOR-MEDIATED GENE DELIVERY. Matt Cotten[1], Ernst Wagner[1,2], Kurt Zatloukal[1], Michael Buschle[2], Susanna Chiocca[1], Christian Plank[1], Wolfgang Zauner[1], Walter Schmidt[1], and Max L. Birnstiel[1]. [1]Institute for Molecular Pathology, Dr. Bohr-gasse 7, 1030 Vienna, Austria. Fax# 43 222 798 71 53, [2]Bender &Co, 1121 Vienna, Austria.

We are making non-viral DNA delivery devices useful for gene therapy (1-4). We know that efficient DNA delivery requires condensation of the cargo DNA with a polycation (such as polylysine), inclusion of a cell binding ligand (such as transferrin) for internalization of the DNA and inclusion of an endosome disruption or membrane disruption activity (supplied by the adenovirus particle) for exit from the endosome (see figure). The endosome disruption activity is supplied by the virus capsid, therefore, the genome of the adenovirus can be destroyed with psoralen treament to improve safety and block both virus gene expression and replication. A variety of other viral and non-viral agents that trigger this egress and enhance DNA delivery have now been identified.

One application of this approach that we are pursuing is the delivery of cytokine genes to primary tumor isolates in efforts to generate a cancer vaccine. Reintroduction of cytokine expressing tumors cells triggers an immune response that can lead to the destruction of non-modified tumor cells by the host.

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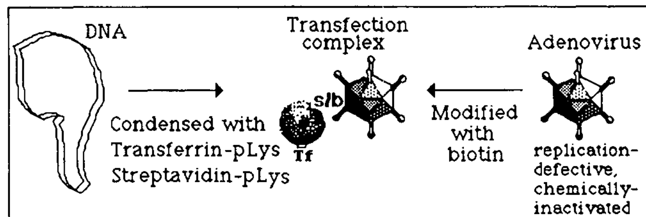

DZ 003 ADENOVIRUS-MEDIATED TRANSFER OF A HUMAN DYSTROPHIN GENE TO SKELETAL MUSCLE OF mdx MOUSE. T. Ragot [1], L.D. Stratford-Perricaudet[1], N. Vincent[2], P. Chafey[2], E. Vigne[1], H. Gilgenkrantz[2], D. Couton[3], P. Briand[3], J-C. Kaplan[2], A. Kahn[2], M. Perricaudet [1]. 1) URA 1301 CNRS, Institut Gustave Roussy 94805 Villejuif, FRANCE 2) U 129 INSERM, ICGM, 75014 Paris, FRANCE, 3) CJF 9003 INSERM, ICGM, 75014 Paris, FRANCE.

X-linked Duchenne progressive muscular dystrophy is a lethal and frequent genetic disease caused by the absence of dystrophin, a 427 kDa protein encoded by a 14 kb transcript. Approaches proposed to correct the dystrophin deficiency in muscle, such as myoblast transfer therapy and direct intramuscular injection of recombinant plasmids expressing dystrophin, are inapplicable to heart and respiratory muscles, which are not accessible to local injections.
The use of a replication-defective adenovirus carrying a gene encoding a nuclearly-targeted ß-galactosidase Ad.RSVßgal demonstrated that replication-defective adenovirus offers an efficient means to transfer a gene for extended periods of time to the liver, muscle, lung, and brain.
We have constructed a recombinant adenovirus harboring a 6.3 kb Becker-like dystrophin cDNA driven by the Rous sarcoma virus promoter. The ability of the recombinant Ad-RSVmDys to produce the minidystrophin was examined after infection of either 293 cells or C2 mouse myoblasts. A signal of the expected size was detected on immunoblots.
The ability of the recombinant virus to direct *in vivo* expression of this protein was then tested after intramuscular injection in dystrophin deficient mdx mice. Animals were injected once 5 to 9 days after birth in the biceps femoris and killed at an age of 2 to 14 weeks. Immunfluorescence studies done on cryosections of the injected muscle showed a sarcolemmal immunostaining in 5% to 50% of fibres. The expression of the transferred gene was stable for at least 6 months.
To know whether or not fibres postnatally infected by the recombinant virus are prevented from entering the degeneration process, we have injected a suspension of Ad-RSVmDys and Ad.RSVßgal in the right hindlimb (treated muscle), and a suspension of Ad.RSVßgal alone in the left hindlimb (untreated muscle) of 2 to 7 day-old mdx mice. We have compared the expression of dystrophin and ß-galactosidase in both hindlimbs, 1,2,6,13 and 24 weeks after injection. From six weeks after injection, ß-galactosidase positive fibres scarcened and were finally undetectable in the untreated muscle resulting from a progressive loss of ß-galactosidase positive fibres due to the ongoing necrosis-regeneration process. On the contrary, a high number of ß-galactosidase positive fibres was maintained in the treated muscle. All fibres expressing ß-galactosidase and dystrophin were, indeed, the only ones to be protected from degeneration.
The data demonstrate that recombinant adenoviruses are promising vectors for gene therapy of muscular diseases like muscular dystrophy.

Cancer Genetics (Joint)

DZ 004 THE MOLECULAR BASIS OF THE t(2;13) TRANSLOCATION IN THE PEDIATRIC SOLID TUMOR ALVEOLAR RHABDOMYOSARCOMA, Frederic G. Barr[1], Richard J. Davis[1], Jeannette L. Bennicelli[1], Jaclyn A. Biegel[2], Beverly S. Emanuel[2], William J. Fredericks[3], Sunil Mukhopadhyay[3], Frank J. Rauscher III[3], Giovanni Rovera[3], and Naomi Galili[3], [1]Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, [2]Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia, [3]The Wistar Institute, Philadelphia, PA 19104.

Alveolar rhabdomyosarcoma is an aggressive soft tissue tumor which occurs in the pediatric population. Histologic diagnosis is often complicated by the paucity of features of striated muscle differentiation and its similarity to a large group of pediatric solid tumors which can present as collections of poorly differentiated small round cells. Cytogenetic investigations of this tumor have identified a characteristic translocation involving chromosomes 2 and 13, t(2;13)(q35;q14). Identification of the genetic loci disrupted by the t(2;13) translocation will permit investigation of an important step in the pathogenesis of rhabdomyosarcoma and exploration of the utility of this molecular marker/target in clinical management. We developed a physical mapping strategy to localize the tumor translocation breakpoint sites on genetic maps of chromosome 2 and 13. This strategy facilitated identification of chromosome 2 and 13 probes which closely flank the breakpoint and delineation of physical intervals which contain the breakpoint. Using these reagents, we have identified the genes involved in the translocation. We have determined that PAX3 (a developmentally regulated transcription factor previously found to be mutated in Waardenburg syndrome) is the chromosome 2 locus rearranged by the t(2;13) translocation. The involved gene on chromosome 13 (FKHR) has been subsequently cloned and identified as a new member of the *fork head* domain family of transcription factors characterized by a conserved DNA binding motif. Northern blot analysis has shown that the juxtaposition of the PAX3 and FKHR genes results in a chimeric transcript consisting of the 5' PAX3 and 3' FKHR regions. PCR analysis has demonstrated that a uniform 5' PAX3-3' FKHR chimeric transcript is expressed in 8 of 8 alveolar rhabdomyosarcoma cell lines. This chimeric transcript encodes a fusion protein consisting of the intact PAX3 DNA binding domains (paired box and homeodomain), the C-terminal half of the *fork head* DNA binding domain, and C-terminal FKHR regions. This fusion protein has been detected in alveolar rhabdomyosarcoma cells by immunoprecipitation experiments with PAX3 and FKHR-specific antisera. These findings demonstrate that the t(2;13) translocation of alveolar rhabdomyosarcoma fuses functional domains from two transcription factors to generate a potentially tumorigenic hybrid transcription factor.

DZ 005 MOLECULAR GENETICS OF NEUROFIBROMATOSIS TYPE 1, Francis S. Collins[1], David H. Gutmann[2], Anna L. Mitchell[2], Steve Doran[2], Lone B. Andersen[2], Paula E. Gregory[2], Amit Hajara[2], Manju Swaroop[2], Jeffrey Cole[2], [1] National Center for Human Genome Research, National Institutes of Health, [2] University of Michigan, Ann Arbor.

Type 1 neurofibromatosis (NF1) is a common autosomal dominant disorder characterized by café-au-lait spots, neurofibromas over the skin surface, Lisch nodules of the iris, and a variety of other variable features including an increased risk of malignancy. The gene for NF1 was identified by a positional cloning strategy in 1990, allowing for the first time the opportunity to understand the disease on the molecular level. We have been investigating the genetic basis of the disease by searching for germline abnormalities in affected individuals. The large size of the gene, the fact that most mutations are subtle changes, and the fact that virtually every family appears to have a different mutation still prevent DNA diagnosis from being widely applicable. Mutations which have been identified primarily appear to be knockouts, which is consistent with a tumor suppressor gene mechanism. We have recently developed fluorescence in situ hybridization techniques to detect large deletions in the gene. In one large pedigree with apparent non-penetrance, we were able to show utilizing FISH that there are actually two independent de novo *NF1* mutations. In another NF1 patient a paracentric inversion of 17q was found to break within the *NF1* locus, providing an unusual mechanism for disease. We have also investigated somatic alterations in the *NF1* gene in tumors. The results are consistent with a tumor suppressor mechanism, wherein the malignant tumors arising in patients with NF1 appear to have sustained a second hit in the *NF1* locus. Sporadic tumors, especially melanomas and neuroblastomas, also have been found to harbor *NF1* somatic mutations, suggesting that this gene plays a larger role in malignancy. The protein product of the *NF1* gene is 2818 amino acids and encodes a protein (called neurofibromin) with homology to the GTPase activating protein (GAP) family. This protein interacts with p21-ras to catalyzesits conversion from the active GTP-bound state to the inactive GDP-bound state. We have raised antisera against neurofibromin, and recent immunofluorescence data indicates that the protein, is localized in the cytoplasm and appears to be associated with microtubules. Expression of various segments of neurofibromin in a baculovirus system for microtubule association maps to the GAP homology region. This unexpected connection between ras-mediated signal transduction and the cytoskeleton suggests that neurofibromin may play multiple roles in the regulation of cell division.

Homologous Recombination/Mouse Models (Joint)

DZ 006 THE ROLE OF DNA METHYLATION IN MAMMALIAN DEVELOPMENT, Rudolf Jaenisch, En Li, Peter Laird, Laurie Jackson-Grusby, Ruth Jüttermann, and Caroline Beard, Whitehead Institute for Biomedical Research, Cambridge, MA 02142 and Department of Biology, Massachusetts Institute of Technology, Cambridge.

We have generated a mouse strain carrying a mutated DNA methyl transferase gene. This mutant mouse strain was used to investigate whether DNA methylation is involved in the maintenance of gene inactivity of imprinted genes. For this we have measured the transcriptional activity of Igf-2 which is maternally imprinted and of H19 and Igf-R which are paternally imprinted. Contrary to expectation, homozygous mutant embryos did not express the Igf-2 or Igf-R gene but expressed, in addition to the maternal H19 allele, also the paternal H19 allele. This indicates that interference with DNA methylation activates the normally inactive (imprinted) paternal H19 allele. H19 represents, therefore, the first example of a gene whose expression *in vivo* is dependent on DNA methylation. Expression of Igf-2 may not be directly controlled by DNA methylation but might rather be inhibited in cis by an active H19 allele.

DZ 007 GENETIC ANALYSIS OF TYROSINE KINASES IN MICE, Philippe Soriano, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

To help understand the physiological role of *src* family non-receptor tyrosine kinases, we have generated mutant mice for *src*, *fyn*, and *yes* by gene targeting in ES cells. Despite widespread expression of these kinases in the embryo and the adult, mutant mice display very restricted phenotypes, such as osteopetrosis in *src* deficient mice or T cell receptor signaling and hippocampal defects in *fyn* mutant mice. The absence of more substantial phenotypes suggests functional compensation by each member of the family. In agreement with this hypothesis, crosses between the different mutant strains have failed to produce the expected number of viable double mutant mice, or double mutant *fyn yes* animals survive, but develop glomerulosclerosis.
To understand the importance of regulating kinase activity during development, we have generated mouse mutants for *csk*, a negative regulator of *src* family kinases. This mutation leads to embryonic lethality associated with defects in the neural tube and in the notochord. Cells derived from the mutant embryos exhibit an order of magnitude increase in activity of *src* and *fyn* . To help identify which kinases need to be carefuly regulated during embryogenesis, we have begun crossing the *csk* mutants with the *src*, *fyn*, and *yes* mutants. The results of these experiments will be discussed.

DZ 008 USE OF THE GENE KNOCKOUT TECHNIQUE IN THE ANALYSES OF LEARNING AND THE WHISKER-BARREL SYSTEM, Susumu Tonegawa[1], Asa Abeliovich[1], Chong Chen[1], Reha S. Erzurumlu[2], Yukiko Goda[4], Sonal Jhaveri[2], John Kim[5], Yuqing Li[1], Richard Paylor[3], Charles F. Stevens[4], Yanyan Wang[4], Jeanne M. Wehner[3], [1]Howard Hughes Medical Institute at Massachusetts Institute of Technology (MIT) Center for Cancer Research, Cambridge, Massachusetts, [2]Dept. of Brain and Cognitive Science at MIT, Cambridge, Massachusetts, [3]Institute for Behavioral Genetics, University of Colorado, Boulder, Colorado, [4]Howard Hughes Medical Institute at Salk Institute, La Jolla, California, [5]Hedco Neurosciences Bldg., University of Southern California, Los Angeles, California.
We have been applying the genetic approach based on the gene targeting technique to the analyses of learning and memory processes. The first mouse mutant produced by this technique, the α CaMKII mutant, exhibited LTP deficiency in the CA1 field of the hippocampus as well as a defect in the spatial learning in the Morris water maze. These results strengthened the notion that the hippocampal LTP is the mechanism responsible for spatial learning. In order to examine the validity of this theme further, we produced a second strain of mutant mice in which the gene encoding the γ isoform of protein kinase C is mutated. The PKC γ mutant mice exhibited at least as much deficiency in the CA1 field LTP as the α CaMKII mutant mice, but their impairment in the spatial learning in the Morris water maze was distinctly lighter than that of the α CaMKII mutant mice. In fact, the impairment in the spatial learning ability of the PKC γ mutant mice could be revealed only by the most sensitive test, namely the platform crossing test after a milder training protocol but not with other tests, such as the quadrant test or random platform test, or with any tests tried after a more intense training program. These results suggest that the CA1 LTP is probably only one of multiple synaptic mechanisms that are responsible for spatial learning. Since the hippocampal LTD is intact in the PKC γ mutants, but is defective in α CaMKII mutants or AP5-treated animals, LTD is a good candidate for one of such mechanisms.
Our second project is to apply the gene targeting technique to the analysis of the activity dependent fine-tuning of neuronal connections. In particular, we have been studying the formation of whisker barrelettes using mice lacking the NMDA receptor (NMDA-R). The mutant mice die shortly (10 to 20 hrs) after birth just around the formation of whisker-related patterns in the brainstem trigerminal complex (BTC). However, we could manage to delay the birth by one to two days. The cytochrome oxidase-stained barrelettes were present in the pups of wild type mice, while they were clearly absent in the mutant pups, despite dense staining in the nuclei. Staining with DiI indicated that the absence of the pattern in the mutant brainstem is not due to delayed growth of trigerminal ganglion axons into the BTC. These results suggest that the formation of barrelettes requires NMDA receptor activation and is activity-dependent.

Human Diseases I (Joint)

DZ 009 MOLECULAR DEFECTS IN HUMAN EXCISION REPAIR SYNDROMES, Dirk Bootsma, Geert Weeda, Wim Vermeulen, Hanneke van Vuuren, Christine Troelstra, Jan Hoeijmakers, MGC-Department of Cell Biology and Genetics, Erasmus University, Postbox 1738, 3000 DR Rotterdam, The Netherlands.

Nucleotide excision repair (NER) is a universal repair system that eliminates a wide spectrum of DNA lesions. It is of crucial importance for guarding genetic integrity and for preventing the deleterious effects of ubiquitous genotoxic agents such as cancer. In fact, two NER subpathways exist: one dealing with the rapid and efficient removal of lesions that block ongoing transcription (transcription-coupled repair), one accomplishing the more slow and less efficient repair of the bulk DNA, including the non-transcribed strand of active genes (genome overall repair).

The phenotypic consequences of a NER defect in man are apparent from 3 distinct inborn diseases characterized by hypersensitivity of the skin to sun(UV)light and a remarkable clinical and genetic heterogeneity. These are the prototype repair syndrome xeroderma pigmentosum (XP) (7 genetic complementation groups, designated XP-A to -G), Cockayne's syndrome (2 groups: CS-A and CS-B) and PIBIDS, a peculiar photosensitive form of the brittle hair disease trichothiodystrophy (TTD, at least 2 groups of which one equivalent to XP-D).

To investigate the mechanism of NER and to resolve the molecular defect in these NER deficiency diseases we have focussed on the cloning and characterization of human DNA repair genes. One of the genes that we cloned is *ERCC3*. It specifies a chromatin binding helicase. Transfection and microinjection experiments demonstrated that mutations in *ERCC3* are responsible for XP complementation group B, a very rare form of XP that is simultaneously associated with Cockayne's syndrome (CS). In collaboration with the laboratory of J-M. Egly (Strasbourg) the ERCC3 protein was found to be part of a multiprotein complex (TFIIH) required for transcription initiation of most structural genes and for NER. This defines the additional, hitherto unknown vital function of the gene, suspected from parallels with the yeast and Drosophila *ERCC3* homologs. Part of the clinical symptoms of the corresponding disorder that were difficult to interpret on the basis of a DNA repair defect can now be fully explained as the result of a subtle impairment of transcription. It appears, that the NER system recruits for its reaction proteins engaged in replication, recombination and (as in the case of *ERCC3)* in transcription, in addition to specific NER proteins. We will summarize the present status on the biochemical characterization of several of the human NER proteins, the surprising functional relationship between transcription and repair and its implications for the clinical symptoms of the corresponding NER disorders and the use of NER genes to generate corresponding mouse models for human repair deficiencies using gene targeting and embryonal stem cell technology.

Gene Therapy: Somatic Tissue II (Joint)

DZ 010 SYSTEMIC DELIVERY OF RECOMBINANT PROTEINS BY GENETICALLY ENGINEERED MYOBLASTS, Helen M. Blau and Jyotsna Dhawan, Department of Molecular Pharmacology, Stanford University School of Medicine, Stanford, CA 94305-5332.

Studies of pattern formation during development have revealed that myoblasts are particularly advantageous for cell mediated gene transfer, because following injection they randomly fuse with all myofibers in their vicinity and become integrated into a pre-existing structure, the multinucleated myofiber[1,2]. As a result they are in contact with the circulation and sustained by neuronal activity. In a recent clinical trial, biopsies of Duchenne Muscular Dystrophy patients 1 and 6 months after implantation with myoblasts produced the missing gene product, dystrophin detected by PCR[3]. Myoblasts genetically engineered with retroviruses deliver recombinant proteins such as growth hormone to the circulation for at least 3 months[4,5]. Current studies of myoblast mediated gene transfer are directed at showing therapeutic efficacy in an animal model with a heritable defect. This approach appears to have promise both for treating myopathies and inherited and acquired nonmuscle disorders including hemophilia, cancer and heart disease.

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DZ 011 POSSIBLE ROLES FOR UTROPHIN IN GENE THERAPY OF DUCHENNE MUSCULAR DYSTROPHY, Kay E. Davies, Jon Tinsley, Derek J. Blake, Marcela Pearce, Anna Deconick and Carina Dennis, Institute of Molecular Medicine, Oxford, England.

Duchenne muscular dystrophy (DMD) is a muscle wasting disorder which results from the lack of expression in muscle of a protein, dystrophin, encoded by a very large (>2 megabases) gene at Xp21 on the human X chromosome. Although the localisation of dystrophin at the sarcolemma is well documented, its exact function remains to be defined. Several strategies are being developed for the gene therapy of DMD including myoblast transfer, retroviral infection, adenoviral infection and direct injection of plasmid DNA. Although some progress is being made in all these areas, there are considerable problems to be overcome related to the number of muscles which can be made dystrophin positive, the levels of expression and the duration of expression. The aim of our studies is to develop strategies to compensate for the loss of dystrophin in the muscle cells of patients by upregulating related genes. Our approach is to perform a detailed characterisation of a protein, utrophin, localised on human chromosome 6, which we have shown to be very closely related in sequence and genomic organisation to dystrophin. Utrophin is localised at the neuromuscular junction in normal muscle but has a localisation similar to dystrophin in DMD patients. Utrophin is also expressed at the sarcolemma in human fetal muscle before dystrophin appears. We are characterising the promoter elements of the utrophin gene in order to understand what factors determine its sarcolemmal localisation in certain circumstances and its appearance at the neuromuscular junction in others.

DZ 012 GENETICALLY MODIFIED MYOBLASTS FOR THE TREATMENT OF ERYTHROPOIETIN-RESPONSIVE ANEMIAS, Eliav Barr[1], Sandeep Tripathy[1], and Jeffrey M. Leiden[1], [1]University of Chicago, Chicago, IL 60637.

Erythropoietin is a 30 kD glycoprotein that regulates erythropoiesis in mammals. In adults, renal tubular cells synthesize and secrete erythropoietin into the systemic circulation under conditions of decreased renal oxygen tension such as occur with anemia. Erythropoietin binds to a receptor on the surface of erythroblasts stimulating both cellular proliferation and differentiation to reticulocytes. Severe anemia due to inappropriate erythropoietin secretion is a common concomitant of both end stage renal disease and symptomatic HIV infection. These erythropoietin-responsive anemias affect more than 150,000 adults in America. Erythropoietin-responsive anemias are currently treated with repeated subcutaneous or intravenous infusions of recombinant human erythropoietin. This therapy is estimated to cost more than $200 million per year. The development of a cell based system that could be used to stably deliver physiological levels of human erythropoietin to the systemic circulation would represent a significant advance in our ability to treat this disease. We have shown previously that genetically modified murine myoblasts can be used to deliver physiologic levels of human growth hormone to the systemic circulation for periods of as long as three months. In the current studies we have (i) isolated and grown primary human myoblasts, (ii) used liposome-mediated gene transfer to transfect these cells with eukaryotic human erythropoietin expression vectors, (iii) shown that these primary human myoblasts secrete high levels (16 U per day per 10^6 cells) of human erythropoietin *in vitro*, (iv) expanded clones of erythropoietin-producing primary human myoblasts to large cell numbers and implanted these cells by intramuscular injection into SCID mouse muscle *in vivo*, and (v) demonstrated that implantation of these genetically modified cells results in significant increases in the hematocrits of these SCID mice. Taken together, these studies suggest that genetically modified myoblasts represent a useful system for the gene therapy of erythropoietin-responsive anemias in humans.

DZ 013 ß-GLUCURONIDASE GENE TRANSFER IN ANIMAL MODELS OF MUCOPOLYSACCHARIDOSIS TYPE VII John Wolfe[1], Rosanne Taylor[1], Mark Sands[1], Margret Casal[1], Noam Harel[1], Gil-Hong Park[1], Michael Parente[1], John Fyfe[1], Mark Haskins[1], Don Patterson[1], and Evan Snyder[2] [1]University of Pennsylvania School of Veterinary Medicine, Philadelphia PA and [2]Harvard Medical School, Boston MA.

Mucopolysaccharidosis (MPS) type VII (Sly disease) is caused by an inherited deficiency of ß-glucuronidase (GUSB) resulting in failure to degrade glycosaminoglycans (GAGs). The storage of undegraded substrates in lysosomes of many cell types leads to progressive degeneration of multiple organ systems, mental retardation, and early death. Mouse, dog, and cat models of MPS VII have essentially the same pathology and clinical features as human patients. Treatment strategies for lysosomal storage diseases are based on the observation that lysosomal enzymes can be transported from normal cells to mutant cells ("cross-correction") via receptor-mediated uptake or cell-to-cell contact. Thus normal enzyme can potentially be delivered to patients by transplantation of heterologous normal cells, infusion of purified enzyme, or gene transfer to autologous somatic cells. Bone marrow transplantation can produce substantial clinical improvement in the visceral and skeletal disease in MPS VII mice and dogs, especially when performed at a young age, but is not effective in delivering therapeutic levels of GUSB to the brain. Intravenous injection of GUSB results in widespread delivery to the body, including the brain if treatment is begun at birth, but lysosomal storage recurs if treatment is stopped. Retroviral vector-mediated gene transfer in vitro corrects the GUSB deficiency of MPS VII cells, restoring substrate degradation to normal, and the transferred enzyme is exported and cross-corrects untreated target cells. In vivo, retroviral vector-mediated gene transfer to MPS VII hematopoietic stem cells or fibroblasts has shown that long-term expression of only low levels of normal GUSB can produce clinically significant improvements in pathology in liver and spleen, but therapeutic levels of GUSB do not reach the brain. A herpesvirus vector transferred GUSB directly into non-mitotic neurons and long-term expression was achieved using the promoter of the HSV latency associated transcript (LAT) gene, but only a low number of cells were corrected. To attempt to transfer therapeutic amounts of GUSB into the brain of MPS VII animals we are currently using retroviral vectors to transduce autologous cells ex vivo and transplant them by stereotaxic injection into the brain to circumvent the blood-brain barrier. To increase the amount of cross-corrective enzyme delivered, the cells were corrected with a double-copy vector that releases 5-fold higher than normal levels of GUSB, acting as an "enzyme pump." Engrafted cells survive in the brain for more than 6 months, but GUSB expression declines over time, although small numbers of positive cells can be detected long-term. We are also using a neuronal progenitor cell line (C17.2) which may be useful as a general delivery vehicle to transfer foreign genes into the brain. These cells differentiate into neurons, astrocytes, and oligodendrocytes, can be transplanted across MHC barriers, and appear to be non-tumorigenic. Transplantation into the brains of newborns produced widespread engraftment and cytoarchitecturally appropriate differentiation, resulting in GUSB expression for at least 8 months.

Human Diseases II (Joint)

DZ 014 MOLECULAR GENETICS OF CHARCOT-MARIE-TOOTH DISEASE, Benjamin B. Roa[1], Nacer Abbas[1], Carol A. Wise[1], Kent Anderson[1], Pragna I. Patel[2], and James R. Lupski[1,3,4], Institute for Human Genetics[1], Department of Neurology[2], Human Genome Center[3], and Department of Pediatrics[4], Baylor College of Medicine, Houston, Texas 77030.

Charcot-Marie-Tooth disease (CMT) is the most common inherited peripheral neuropathy. CMT type 1 (CMT1) is characterized by distal muscle atrophy, decreased motor nerve conduction velocity (NCV) and peripheral nerve demyelination. The predominant subtype, autosomal dominant CMT1A, exhibits linkage of the disease locus to DNA markers on proximal 17p. The majority of CMT1A cases are associated with a submicroscopic DNA duplication on 17p11.2-p12. The 3.0-Mb CMT1A duplication consists of a tandem duplication of a 1.5-Mb region. Evidence of a CMT1 phenotype in patients with cytogenetically visible dup(17p) supports a gene dosage mechanism by which the CMT1A duplication leads to the disease. Flanking the 1.5-Mb monomer unit are large (>17kb) homologous sequences (CMT1A-REP) that appear to provide an intrinsic mechanism for recombination leading to the CMT1A duplication of the same size arising in *de novo* CMT1A patients and inherited in CMT1A families of different ethnic origins. In addition, CMT1A-REP appears to be involved in generating the apparent reciprocal deletion of 1.5 Mb associated with hereditary neuropathy with liability to pressure palsies (HNPP), which is clinically distinct from CMT1A. The *PMP22* gene encoding a 22-kDa peripheral nerve myelin protein has been mapped within the CMT1A duplication region and shown to be highly expressed in peripheral nerve. The role of *PMP22* in the demyelinating disease process of CMT1A has been confirmed by the identification of several *PMP22* point mutations in CMT1A patients. These mutations predict single amino acid substitutions in *PMP22*, and include autosomal dominant alleles, as well as an apparent recessive *PMP22* allele contributing to CMT1A. The collective data indicates that Charcot-Marie-Tooth disease type 1A can be caused by two alternative molecular mechanisms: point mutation in the *PMP22* gene, and increased gene dosage due to DNA duplication of the region containing *PMP22*.

Gene Therapy: Somatic Tissue III (Joint)

DZ 015 DIRECT GENE TRANSFER AND NONVIRAL VECTORS FOR HUMAN CANCER AND AIDS, Gary J. Nabel[1], Bernard A. Fox, Ph.D.[1], Gregory E. Plautz[1], Bei-Yue Wu[1], X. Gao[2], L. Huang[2], Alfred E. Chang[1], Elizabeth G. Nabel[1], [1]University of Michigan, Ann Arbor, MI; [2]University of Pittsburgh, Pittsburgh, PA.

The expression of recombinant genes in living organisms provides potential alternative approaches to the treatment of human diseases. Such approaches can be used, for example, to confer protection against HIV infection by stimulation of the immune system or by preventing infection of otherwise susceptible cells. We have prepared retroviral vectors containing a Rev gene encoding a dominant negative inhibitor. Expression of this gene product confers significant resistance to HIV infection without alteration of normal T cell function. This genetic intervention may provide therapeutic benefits in HIV infection, and a human clinical protocol has recently received NIH regulatory approval to address this question. We have also described a method to stimulate immune responses by expressing a foreign MHC gene in malignant tumors in vivo. By direct gene transfer, a murine class I H-2K^s gene was introduced in vivo into subcutaneous tumors from the CT26 mouse colon adenocarcinoma (H-2^d). Expression of this foreign MHC gene within tumors induced a cytotoxic T cell response to H-2K^s and, more importantly, to other antigens present on unmodified tumor cells. This immune response attenuated tumor growth or caused complete tumor regression in several animals. The results of a clinical trial which utilizes direct gene transfer of a foreign histocompatibility gene for human melanoma will be summarized.

DZ 016 GENE THERAPY FOR METABOLIC DISORDERS, Savio L.C. Woo, Ph.D., Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas.

The liver is the major organ for metabolism and there are dozens of known metabolic disorders secondary to a variety of hepatic deficiencies in man. The development of technologies to deliver functional genes into hepatocytes in vivo would permit gene therapy for these disorders in the future. There are two general strategies for hepatic gene delivery: ex vivo and in vivo. For the ex vivo approach, we have demonstrated that genetically modified hepatocytes injected directly into the portal vein or the spleen of mice migrated to the liver, survived and continued to function as hepatocytes for the life of the recipients. More recently, we have also directly delivered therapeutic genes into mouse hepatocytes with recombinant viral vectors in vivo. Using recombinant retroviral vectors to deliver the canine Factor IX gene into the liver of Hemophilia B dogs, we were able to achieve sustained partial correction of the bleeding phenotype in vivo. In addition, we have investigated the suitability of recombinant adenoviral vectors for hepatic gene delivery. We observed that mouse hepatocytes can be transduced quantitatively in vivo without apparent liver pathology by direct infusion of viral particles into the portal vein at a virus to hepatocytes ratio of 100. We have applied these gene delivery technologies to two animal models of metabolic deficiencies. Hemophilia B is caused by a deficiency of plasma coagulation Factor IX which is synthesized in the liver. Under appropriate experimental conditions, complete restoration of clotting time to normal was achieved in the Hemophilia B dogs, although the transduced gene did not persist beyond 2-3 months in vivo. Similar results were obtained for treatment of phenylketonuria in a phenylalanine hydroxylase-deficient mouse model. Patients with phenylketonuria have highly elevated levels of serum phenylalanine that causes severe mental retardation. Serum phenylalanine was completely restored to normal in deficient mice within one week of treatment using a recombinant adenoviral vector containing the human phenylalanine hydroxylase gene. Finally, DNA based vectors can be delivered to hepatocytes by receptor mediated endocystosis, and the transgene activity can be enchanced by 3 orders of magnitude with the use of an endosomal lysis agent. Taken together, these results suggest that direct gene delivery strategies can be applied to the future treatment of a variety of metabolic disorders in man.

Gene Transfer in CNS

DZ 017 HERPES SIMPLEX VIRUS AND GENE TRANSFER TO THE NERVOUS SYSTEM, J.C. Glorioso[1], N. DeLuca[1], M.A. Bender[1], W.F. Goins[1], D.J. Fink[2], [1]Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA, 15261 [2] Department of Neurology, University of Michigan Medical School, Ann Arbor, MI

Herpes simplex virus type 1 (HSV-1) naturally establishes latency in neurons of the peripheral nervous system with the concommitant expression of the latency associated transcripts (LATS) and the loss in lytic gene functions. We have attempted to exploit these features to develop HSV vectors for gene transfer to brain. However two impediments to using this virus for gene delivery have to be overcome: namely viral cytotoxicity and the design of promoter/regulatory systems to maintain foreign gene expression during latency in CNS neurons. To address the first problem, virus mutants deleted for the essential immediate early (IE) genes (ICP4 and ICP27) responsible for initiating the lytic gene program were constructed and propagated on specifically engineered complementing cell lines. Also in progress are the elimination of nonessential genes involved in increasing lytic gene transcription (ICP0) and the virus-encoded host shut off function (UL41). Mutant viruses tested so far are completely defective. To attack the second problem, a variety of viral, cellular and recombinant promoters was recombined into defective HSV vectors and tested for their ability to continuously express reporter gene RNA and protein in rat hippocampus following stereotaxic inoculation. These included both polII and polIII promoter constructs, the natural HSV-1 latency promoters LAP1 and LAP2 and a recombinant autocatalytic promoter driving expression of a GAL4-VP16 transactivator. While transient gene expression was observed for a variety of viral (e.g.. the IE promoter of HCMV and the RSV LTR) and cellular promoters (NSE and NF), sustained expression was not found. However, at least one polIII promoter, VA1 of adenovirus, continued to express in the absence of viral lytic genes and was detectable in brain during latency. The HSV-1 LAPs also remained active in brain but at reduced levels compared with their ability to express reporter genes in the peripheral nervous system. A detailed genetic analysis of these promoters has been carried out in order to identify latency-specific enhancer elements which might be exploited for maintenance of strong promoter activities during latency. Among the elements identified were sequences potentially involved in altering chromatin structure. Finally, a vector carrying a recombinant yeast GAL4:VP16 fusion gene was capable of sustained activation by transactivation of its own promoter. This recombinant promoter is being exploited to express the rat tyrosine hydroxylase gene from a recombinant vector and will be tested in animals for production of dopamine in nondopaminergic neurons following stereotaxic inoculation of caudate and raphe nucleus in a rat model of Parkinson's disease. Success in these studies should provide an effective gene delivery system for adult animal brains, a requirement for developing potentially useful gene therapeutic approaches in the treatment of human neurodegenerative diseases.

DZ 018 STEPS ON THE PATH TO PRECURSOR CELL TRANSPLANTATION FOR TISSUE REPAIR, Mark Noble, Ludwig Institute for Cancer Research, 91 Riding House Street, London W1P 8BT, U.K.

Our studies on the biology of glial precursor cells of the central nervous system (CNS) will be discussed as a paradigm for the demonstrating some of the overlaps between the fields of developmental biology, regeneration research and oncology. In addition, I will discuss new tools which may greatly facilitate the study of precursors cell function in other tissues.

Our studies on the CNS have been focused largely on oligodendrocyte-type-2 astrocyte (O-2A) progenitor cell of the rat optic nerve. In vitro, this cell can be induced to give rise to oligodendrocytes or type-2 astrocytes. Astrocytes of a separate glial lineage modulate division and differentiation of O-2A progenitor cells through secretion of platelet-derived growth factor (PDGF).

Growth of O-2A progenitors in the presence of both PDGF and basic fibroblast growth factor (bFGF) induces continuous self-renewal in the absence of differentiation. Thus, cooperation between growth factors can "conditionally immortalize" these precursor cells as effectively as expression of activated oncogenes. The ability to grow enriched populations of O-2A progenitors in the absence of differentiation has allowed us to repair demyelinating lesions transplantation of purified and expanded O-2A progenitor populations .

Optic nerves of adult rats contain a different cell type, the O-2A*adult*progenitor, with properties specialized for the physiological requirements of the adult nervous system. In particular O-2A*adult*progenitors have many of the features of stem cells. O-2A*adult*progenitors are derived from a subset of O-2A*perinatal* progenitors, thus indicating that the O-2A*perinatal* progenitor population is tripotential, rather than bipotential. These observations offer novel insights into the possible origin of self-renewing stem cells and also into the role that generation of stem cells may play in helping to terminate the explosive growth of embryogenesis. Exposure of these cells to a combination of PDGF and bFGF causes cells to go through a transient period of accelerated division, as might be required during repair of demyelinating lesions in vivo. The properties of O-2A*adult* progenitor cells are strikingly consistent with the failure of successful myelin repair in multiple sclerosis.

In addition, we have developed transgenic mice that harbour a temperature-sensitive version of the SV40 large T antigen under the transcriptional control of the H2-K^b promotor. Growth of cells derived from these animals in tissue culture at 33°C in the presence of interferon-gamma produces conditional immortalization of a variety of cell types. These animals present a new tool which may obviate the need for retroviral- or transfection mediated gene insertion in the creation of novel cell lines.

Tissue Specific Gene Expression (Joint)

DZ 019 MECHANISMS CONTROLLING KERATINOCYTE-SPECIFIC GENE EXPRESSION: IMPLICATIONS FOR GENE THERAPY AND FOR ELUCIDATING THE GENETIC BASIS OF SKIN DISEASES. Fuchs, E., Aneskievich, B., Byrne, C., Chan, Y.-M., Cheng, J., Faus, I., Hsu, H., Hutton, M.E., Syder, A., Turksen, K., Yang, Z., Zhou, P. and Zinkel, S. Howard Hughes Medical Institute, Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL 60637.

Keratins are the major structural proteins of keratinocytes. They are coexpressed as specific pairs that form obligatory heteropolymers. When either protein of a pair is mutated within certain domains critical for 10 nm filament assembly, degeneration of the keratinocytes can occur, and this results in genetic disease which is transmitted in an autosomal dominant fashion. Since keratin pairs are differentially expressed during differentiation and development in stratified squamous epithelia, different diseases arise depending upon where the keratin pair is expressed. In addition to their role in degenerative diseases, keratins provide useful biochemical markers to study how complex differentiation programs in epithelia are controlled. A knowledge of the sequences and factors involved in regulating keratin genes should lead to insights into how particular epithelial differentiation fates are controlled during embryonic development. In addition, this information should be valuable in optimizing the promoters-/enhancers of these genes to provide valuable tools for targetting expression of regulatory and structural genes (1) to specific epithelial cells of transgenic mice, for the purpose of generating animal models for human disease, and (2) to keratinocytes in culture, for the purpose of gene therapy. In this talk, I will review what we currently know about genetic disorders of keratin and about keratinocyte-specific gene expression, and discuss how this information can be applied to issues of gene therapy.

DZ 020MECHANISMS REGULATING MUSCLE CELL FATE DURING EMBRYOGENESIS, Eric N. Olson, The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

Transcription of muscle-specific genes during myogenesis is regulated by combinatorial interactions among cell-type specific and widely expressed transcription factors, and is influenced by extracellular signals that activate complex intracellular pathways of signal transduction. Our laboratory has focused on the mechanisms through which the myogenic helix-loop-helix (HLH) protein myogenin regulates muscle-specific transcription as well as on the mechanisms that regulate transcription of the *myogenin* gene itself. In collaboration with J. Merlie and colleagues (St. Louis), we have generated transgenic mice harboring lacZ transgenes linked to the *myogenin* 5' flanking region. These transgenes recapitulate the complete temporal and spatial pattern of myogenin expression throughout development. The pattern of expression of mutant *myogenin-lacZ* transgenes reveals the existence of heterogeneous myogenic precursor cells that can be distinguished by their endogenous activators of the *myogenin* locus and they suggest that myogenic HLH proteins and the muscle enhancer factor, MEF-2, participate in separate regulatory circuits leading to *myogenin* transcription in the somites and limb buds. In collaboration with P. Hasty, W. Klein and A. Bradley (Houston), the mouse *myogenin* gene has been inactivated by homologous recombination. Mice homozygous for a targeted mutation in the *myogenin* gene survive fetal development but die immediately after birth and exhibit severe reduction of all skeletal muscle. We conclude that myogenin is an essential component of the regulatory pathway leading to skeletal muscle formation during mouse embryogenesis.

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AIDS Gene Therapy

DZ 021RETROVIRAL VECTORS AS DIRECTLY ADMINISTERED THERAPEUTICS FOR HIV INFECTION, Douglas J. Jolly, John F. Warner, Bruce Merchant, Steve Mento, and the Viagene HIV Project Team, Viagene, Inc., 11075 Roselle Street, San Diego, California 92121.

Retroviral vectors can be used in animal models to elicit cytotoxic T lymphocyte (CTL) and antibody responses against the protein product of the gene(s) they carry. Such responses can be elicited by both *ex vivo* (implantation of vector transduced cells) or *in vivo* (direct intramuscular injection of vector preparations) administration. Responses are seen with a variety of different disease linked antigens (e.g., HIV, HBV, HSV, HPV), and the vectors can be used to construct permanent target cell lines for CTL assays. Retroviral vectors can also be manufactured in large scale with relative simplicity and can be quality controlled to give product suitable for clinical trials. Finally, *in vitro* and animal testing studies show no overt toxicity or tumorigenic potential for these retroviral vectors. On this basis, we have begun Phase I clinical safety trials in HIV infected individuals using both the *ex vivo* and *in vivo* administration routes to stimulate immune responses to HIV-1 env and rev gene products. The rationale for this approach is that induction of augmented CTL responses to HIV envelope or rev antigens may eliminate HIV-1 infected cells, reduce viral burden, and hence produce a therapeutic effect. A summary of interpretable results, to date, from this project will be presented.

DZ 022 GENE THERAPY TO HIV-1 INFECTION: RNA-DECOYS AND COMBINATION APPROACHES, Julianna Lisziewicz[1], Daisy Sun[1], Jason Smythe[1,2], Franco Lori[1], Bo Peng[1], Bruce Trapnell[3], John Rossi[4], and Robert C. Gallo[1], [1]Laboratory of Tumor Cell Biology, National Cancer Institute, NIH, Bethesda, MD 20892, [2]Johnson & Johnson, Sydney, Rushcutters Bay, Australia, [3]Genetic Therapy Inc., Gaithersburg, MD 20877, [4]Beckman Research Institute, City of Hope, CA

Our antiviral gene therapy is aimed at genetically modifying HIV-1 permissive cells by introducing exogenous genes that will render them permanently resistant to HIV-1 replication. In addition, regulated expression of the exogenous inhibitory gene is considered essential to avoid problems, such as toxicity, that can be associated with long-term constitutive intracellular expression of any foreign gene. We have developed inhibitory genes that are only expressed in infected cells in the presence of the HIV-1 regulatory proteins Tat and Rev. One of the most important targets in antiviral gene therapy is the HIV-1 Tat protein, because it is not only a potent transactivator of HIV-1 gene expression, but it also demonstrates extracellular functions. Our approach is to block the intracellular and extracellular functions of Tat by expressing a polymeric-TAR RNA decoy (1) and antisense-Tat RNA to sequester the Tat protein and inhibit translation of the Tat mRNA, respectively (LTR-25TARαTat construct). Another target for inhibiting HIV-1 replication is the viral Gag protein, the function of which can be inhibited by expression of a *trans*-dominant Gag mutant protein and by a Gag mRNA specific ribozyme. A combination therapy based on these inhibitory genes would also be expected to reduce the probability of viral escape mutants being produced. T-lymphocytes were transduced with the antiviral constructs and pooled cells selected with the neomycin analogue G418. Once selected, the T-lymphocyte cell lines were challenged with HIV-1(IIIB) and maintained in culture. The T-lymphocytes transduced with the amphotropic viruses containing antiviral constructs produced at least 90% less HIV-1(IIIB) than T-lymphocytes transduced with the retroviral control vector (2,3). These results were confirmed using another gene delivery system (see Abstract Lori *et al.*). In addition, we have demonstrated that the polymeric-TAR RNA decoy and the *trans*-dominant Gag mutant protein can inhibit SIV replication, suggesting that both antiviral genes may have activity against a number of primate lentiviruses. We have chosen the LTR-25TARαTat construct as a candidate for Phase I clinical trails because it has proven to be the most potent inhibitor of HIV-1 gene expression in T-cell lines. Moreover, this construct also demonstrates significant anti-HIV activity when transduced into human peripheral blood lymphocytes. Our goal is to develop additional combination constructs designed to inhibit HIV-1 replication via different mechanisms.

(1.) Lisziewicz *et al. New Biol* 3:82, 1991; (2.) Lisziewicz *et al. PNAS*, 90:8000, 1993; (3.) Smythe *et al. PNAS*, in press.

DZ 023 PROMOTER FOR EXPRESSION OF TRANSDUCED ANTI-HIV AND SIV RIBOZYMES. John J. Rossi[1], Cecile Carbonnelle[1], Shirley Li[2], Saswati Chatterjee[2], John A. Zaia[2], Garry Larson[1], and Edouard Bertrand[1], [1]Department of Molecular Genetics, Beckman Research Institute of the City of Hope, Duarte, CA 91010 and [2]Division of Pediatrics, City of Hope National Medical Center, Duarte, CA 91010.

We have been pursuing the development of a ribozyme strategy for the treatment of HIV-1 infection in man. In parallel with the development of anti-HIV-1 ribozymes, we are developing ribozymes to various target sites in SIV-1 with the intent of evaluating the efficacy of our ribozymes, expression and delivery system in a simian model for AIDS. Our ribozyme strategies are varied, but include the use of hammerhead type ribozymes, expressed in a variety of transcriptional motifs to optimize ribozyme-substrate interactions. Several different promoter systems are being examined for expression and intracellular localization of the ribozyme transcripts. These include three different constructs which utilize Pol III promoters (human $tRNA^{Met}$, human U6 +1 and +19 which differ by the amount of U6 information present), and four different Pol II promoter systems, including three versions of the human U1 gene and the RSV LTR promoter. Each of these promoters has been cloned into an AAV vector system, and several into a moloney based retroviral system. Following transduction or transfection, the relative strengths of each promoter have been assessed, and the intracellular localization of the ribozyme bearing transcripts have been analyzed. Our results demonstrate that the $tRNA^{Met}$, U6 +19, and U1 construct (which includes the majority of the U1 mature coding sequence) generate the most RNA. Each of these expression systems is being evaluated for anti-viral activity in cell culture as well. Finally, a $tRNA^{Lys3}$-anti-HIV-1 ribozyme construct, which produces a product which competes with $tRNA^{Lys3}$ for interaction with HIV-1 reverse transcriptase, produces a Pol III transcript which maintains the entire tRNA body as well as the ribozyme. This is being evaluated for its anti-viral activity, and as a novel mechanism for targeting a ribozyme to the viral capsid.

DZ 024 DEVELOPMENT OF RIBOZYME GENE THERAPY AGAINST HIV Flossie Wong-Staal, Mang Yu, Osamu Yamada, Mark Leavitt, Midori Maruyama, Anthony Ho et al. Departments of Biology and Medicine, UCSD, La Jolla, CA 92093-0665

Ribozymes are RNA molecules that contain anti-sense sequences for specific recognition, and RNA-cleaving enzymatic activity. We reported that a hairpin ribozyme designed to cleave HIV-1 RNA in the 5' leader sequence suppressed virus expression in Hela cells co-transfected with proviral DNA from diverse HIV-1 strains. Moreover, the antiviral effect was primarily due to the catalytic rather than antisense property of the ribozyme. Human $CD4^+$ T cell lines (Jurkat and Molt 4/8) transduced by murine retroviral vectors carrying the ribozyme gene persistently expressed the ribozyme gene with no apparent deleterious effect on cell proliferation or long term viability. These cells were resistant to challenge from diverse strains of HIV-1, including an uncloned clinical isolate. Furthermore, we showed that the ribozymes inhibited both early and late steps in the replication cycle of retroviruses, presumably by cleaving incoming virion RNA as well as the transcribed genomic and subgenomic mRNAs. Our more recent efforts focused on transduction of primary lymphocytes and hematopoietic progenitor cells, as these would be the realistic targets for gene therapy for AIDS patients. Primary PBL selected in culture after transdcution with the ribozyme vector also completely resisted infection by HIV-1. High transduction efficiency was obtained with enriched CD34+ cells pre-stimulated with a variety of growth factors. Persistent expression of the ribozyme was also detected in the colonies established from these progenitor cells. Experiments are in progress to determine if ribozyme transduced progenitor cells will yield progeny cells that are resistant to HIV-1 infection.

Delivery Systems; Cancer Genetics

DZ 100 REPLICATION DEFECTIVE ADENOVIRUS ENABLES TRANSDUCTION BY RETROVIRAL VECTORS OF CELLS OUTSIDE OF THEIR HOST RANGE. R. Mark Adams, Mary Wang, David Steffen, Fred D. Ledley. Departments of Cell Biology and Pediatrics, Baylor College of Medicine, Houston, TX 77030 and GENEMEDICINE, INC, Houston, TX 77054.

Replication defective adenoviral particles enhance the efficiency of receptor-mediated gene transfer of DNA vectors into cells. We now show that replication defective adenovirus also enhances retroviral mediated gene transfer, increasing both the apparent titer of the retroviral vector, and, more importantly, enabling efficient transduction of cells outside of the retroviral host range. In initial experiments 5 x 10^5 (murine) NIH3T3 cells were infected with 5x10^5 cfu of a xenotropic vector (GP+X/N2) and the efficiency of transduction assessed by quantitative DNA analysis as # provirus/ genome equivalent. Under normal conditions the efficiency of infection of murine cells with this vector is $<10^{-6}$. With the addition of 10^8 particles of DL312 adenovirus to the incubation medium (100 particles/cell) there was a 2-3 order of magnitude increase in the efficiency of transduction with the xenotropic vector. In similar experiments, human cell lines HeLa and PLC/PRF were infected with ecotropic zen-ß-gal which carries the E. coli ß-galactosidase reporter gene and transduction assessed by X-gal staining. In the absence of retrovirus, no X-gal positive cells were observed. In infections performed in the presence of adenovirus, up to 10% of cells were transduced. The calculated titer of the ecotropic vector on human cells was only 10-100 fold lower than in control experiments performed on NIH3T3 cells. The addition of adenovirus increased the apparent titer of ecotropic vectors on NIH3T3 cells 2-4 fold. These experiments demonstrate infection of cells outside of the retroviral host range with the addition of replication defective adenovirus to the medium. This suggests that gene therapy might be performed with non-infectious particles formulated for efficient entry into target cells.

DZ 101 GENETIC MODIFICATION OF RODENT GUT EPITHELIUM BY GENE THERAPY USING LIPOSOMAL DELIVERY SYSTEMS, Richard Arenas, Steven J. Chmura, Glen Otto, Carol A. Westbrook, Department of Medicine, University of Chicago, Chicago, IL 60637

The expression of tumor-suppressor genes in the epithelial lining of the colon might be applied to the prevention of colorectal cancer in individuals with hereditary or environmental susceptibility to cancer. To explore this concept, methods were developed to obtain high level expression of exogenously-introduced genes in rat colonic epithelium. Liposomal vectors (DOTMA and others) were used to deliver the beta-galactosidase gene in plasmid form by rectal enema. Forty-eight hours after application, animals were sacrificed and the tissues stained with X-gal/IPTG. Expression was fairly uniform, with close to 100% of cells demonstrating the stain in the treated areas, with no evidence of cellular toxicity. Expression was continuous rather than patchy, with uniform distribution along the crypt axis. Gene expression persisted at 4 days, but dropped off at 6 days. These results indicate that twice-weekly rectal application of the gene will be sufficient to maintain a consistent level of exogenous gene expression for an extended period of time. Studies are underway to express tumor-suppressor genes for cancer prevention studies in animal models of colorectal cancer. We believe this method has considerable potential for human therapeutic intervention in the prevention and adjuvant treatment of colorectal cancers, as well as for the treatment of non-malignant disorders of the bowel.

DZ 102 SECOND GENERATION ADENOVIRUS VECTORS FOR CYSTIC FIBROSIS GENE THERAPY. D. Armentano, C. Sookdeo, G. White, V. Giuggio, D. Souza, L. Couture, L. Cardoza, K. Vincent, S. Wadsworth and A. Smith. Genzyme Corporation, One Mountain Rd., Framingham, MA 01701.

Our first generation adenovirus vectors for CF gene therapy are based on a parental vector deleted for only the E1 region. Because of the large size of the CFTR cDNA, 4.5kb, these vectors approach the upper size limitation for efficient viral packaging. Therefore, we explored the possibility of deleting additional regions from the parental vector which are not required for virus growth in culture.

The E4 region of adenovirus is known to function in viral DNA replication, late mRNA accumulation and host cell protein synthesis shut-off. Expression of E4 open reading frame 6 alone is sufficient to provide E4 functions required for normal DNA replication, late protein synthesis *and virus production in vitro*. We have constructed Ad2-E4/ORF6 which contains and expresses only ORF6 and is deleted for the non-essential ORFs of E4. This increases the cloning capacity of previously existing Ad2 based vectors by approximately 1.8kb.

The E3 region of adenovirus is believed to function in allowing the virus to escape immune surveillance *in vivo* and most of this region is dispensible for virus growth in culture. We have therefore constructed various Ad2 based vectors which are deleted for this region. It has been shown that viruses which express gp19 of the E3 region are less pathogenic *in vivo* than mutant viruses that do not. Because this may be a desirable safety feature for recombinant vectors, we also chose to construct a vector that is deleted for most of E3 but retains the ability to express gp19.

By combining E1, E3 and E4 deletions in an adenovirus vector, the cloning capacity of the vectors approaches 7.7 kb. The construction and characterization of viruses with deletions in E3 and E4 will be presented.

DZ 103 EVALUATION OF RECOMBINANT ADENOVIRUS VECTORS FOR GENE THERAPY OF LIPOPROTEIN DISORDERS. Patrick Benoît[1], Steve Hughes[2], Emmanuelle Vigne[3], Michel Perricaudet[3], Patrice P.Denèfle[1] and Edward Rubin[2]

[1]Rhône-Poulenc Rorer SA, Vitry (France), [2]Lawrence Berkeley Laboratory, Berkeley (USA) and [3]Institut Gustave Roussy, Villejuif (France).

HDL and its major associated apolipoprotein, apoAI, has been demonstrated in several studies to exert antiatherogenic properties regardless of the genetic and environmental etiology contributing to heightened atherosclerosis susceptibility. In order to evaluate the feasibility of an intervention at the genetic level on such a complex disease, we selected to develop new recombinant adenoviral vectors which were designed so as to achieve high levels of expression of human apolipoprotein AI (apoAI).

Different adenoviral vectors were tested *in vitro* on various cell lines to compare their relative strength. Two vectors which expressed apoAI under the control of either the CMV or the LTR-RSV promoters were prepared for animal studies.The animal model in which we performed the assay is the mouse line C57Bl/6, which develops pre-atherosclerotic lesions upon induction by an hypercholesterolemic diet. Since apoAI is extremely abundant in the plasma (1.3 g/l in humans), the protocol was also developed in apoAI knock-out mice (AI-KO) so as to measure the net increase in total HDL in animals with extremely low HDL.

After a single-dose intravenous injection of viral preparations (performed either in the tail or eye vein), which corresponds to approximately 10^{10} pfu, the plasma levels of human apoAI were found surprisingly high (0.2 to 15 mg/dl) and lasted for several weeks with a plateau of three weeks. The LTR-RSV adeno construct proved much more stable than the CMV construct in terms of levels and stability of the recombinant apoAI expression. A second injection performed at day 11 was also shown to further raise apoAI plasma levels.

HDL levels were significantly increased in the treated versus inbred control animals. In treated apoAI-KO mice, gradient gel electrophoresis analysis revealed a lipoprotein profile which was similar to that of human apoAI transgenic animals, with a characteristic bimodal density distribution of HDL. Following these very encouraging results, an atherosclerosis regression study on C57Bl/6 mice with pre-developed aortic lesions is currently underway.

DZ 104 USE OF A COMBINED POSITIVE / NEGATIVE SELECTABLE GENE FOR RETROVIRAL-MEDIATED GENE TRANSFER, Fabio Candotti, Craig A. Mullen and R. Michael Blaese, N.C.I., National Institutes of Health, Bethesda, MD - 20892.

Retroviral-mediated gene-transfer into mammalian cells is extensively used for gene therapy. Many retroviral vectors are constructed to contain an encoded drug resistance gene in order to allow the isolation of cells that have integrated the exogenous viral genome. Beside these positive selection systems, a variety of negative selectable markers have been developed, providing the ability to selectively eliminate cells engineered to produce various non-mammalian enzymes encoding drug sensitivity (*Herpes* thymidine kinase, *E. Coli* cytosine deaminase). We took advantage of a recently constructed fusion gene (TNFUS69) between the *Herpes simplex virus* thymidine kinase (HSV-*tk*) and bacterial neomycin phosphotransferase (*neoR*) to generate a retroviral vector carrying a positive and negative selective system within a single gene. The expression of the fused gene was compared with wild type *neoR* and HSV-*tk* in transduced mammalian cell lines. Equivalent efficiency of expression between the wild type and the "fused" *neoR* genes was seen *in vitro.* Cells carrying the HSV-*tk/neo* fusion gene were able to grow in HAT selection medium, demonstrating functional thymidine kinase enzymatic activity. However, cells expressing the "fused" HSV-*tk gene* showed a reduced (up to ten fold) sensitivity to Ganciclovir when compared with the wild type *tk*-gene. In order to determine whether the fusion gene would be sufficient to clinically eliminate growing tumors, a comparative HSV-*tk*/GCV "suicide" tumor model system in mice was established. Preliminary results and possible applications of the HSV-*tk/neo* fusion gene for gene transfer and clinical gene therapy will be discussed.

DZ 105 A SCALE-UP IMMUNOISOLATION DEVICE THAT RESEMBLES AN ORGANOID STRUCTURE FOR THE IMPLANTATION OF GENETICALLY ENGINEERED CELLS. V. Carr-Brendel, S. Neuenfeldt, R. Clarke, D. Hodgett, C. Vergoth, R.C. Johnson and J. Brauker. Gene Therapy Unit, Baxter Healthcare, Round Lake, IL 60073.

Immunoisolation is the encapsulation of therapeutic tissue in a membrane-bound device to prevent immune rejection of the implant. We developed a bilaminar membrane-based device that promotes vascular structures at the membrane-tissue interface and protects allogeneic tissue for a year (Sprague Dawley rat lung implanted into Lewis rat). The device was used to demonstrate long-term (2 months) expression of canine factor IX in athymic rats (J. Cell. Biochem. Supple.17E:224 (1993)). The immunoisolation device reported previously was a top-loaded device that was too small to support the number of cells required to cure hemophilia. In the process of increasing the size of the device, several difficulties were encountered: 1) the bilaminar membrane was separated by host cellular infiltration and parallelism between the membranes was not maintained *in vivo*, 2) cells implanted within this device configuration were often necrotic, and 3) good tissue morphology was not consistently obtained. To correct these difficulties, a ported device was constructed with a woven polyester mesh that was sonically sealed to the outer membrane of the device. The addition of the mesh helped maintain membrane parallelism, allowed for neovascularization at the membrane interface, and restricted the lumen of the device. The port allowed for loading cells after implantation. A model tissue, fetal lung, was loaded into this device after implantation into a Lewis rat fat-pad, and histologically analyzed. Allogeneic lung survival in the scale-up device scored consistently higher (survival=6) than the same tissue implanted in the top-loaded, smaller device (survival=4.6) (tissue survival defined as: 1=no living tissue, 2=scattered living cells, 3=less than 50% of tissue alive, 4=more than 50% of tissue alive, 5=living epithelial cells predominant, and 6=differentiated tissues). Single cell suspensions of fibroblasts or epithelial cells also grew well in the device. This device appears to fully integrate into the soft tissues of the host, allowing vascularization to occur at the membrane interface, thus creating an organoid structure. The advantages to this organoid device include: 1) cells can be added to the device after a vascular bed has developed, 2) maintenance of membrane parallelism and volume of the device, and 3) better survival of implanted tissues. This approach has advantages over other gene therapy delivery systems because: 1) it segregates the genetically engineered cells from the host, 2) the implanted cells are retrievable, and 3) allogeneic cells can be used, and if they escape they will be rejected.

DZ 106 *IN VIVO* GENE THERAPY OF BRAIN TUMOR IN MICE USING THE ADENOVIRUS VECTOR. Shu-Hsia Chen[1,2], H. David Shine[2,3], J. Clay Goodman[3,4], Robert G. Grossman[3] and Savio L.C. Woo[1,2]. [1]Howard Hughes Medical Institute, Departments of [2]Cell Biology, [3]Neurosurgery and [4]Pathology Baylor College of Medicine, Houston, TX 77030.

Direct introduction of a therapeutic gene into malignant cells *in vivo* can provide an effective treatment of localized and non-resectable tumors. In previous studies, Culver *et al.* reported regression of rat brain tumors by direct intra-tumor inoculation of cells producing a recombinant retroviral vector containing HSV-TK, followed by treatment with ganciclovir(GCV). The retroviral vector system however,is limited by low viral titer,and relatively low transduction efficiency *in vivo*. The recombinant adenoviral vector doesn't suffer from these limitations and was utilized as a vector for HSV-TK gene delivery to solid tumors *in vivo et situ*.

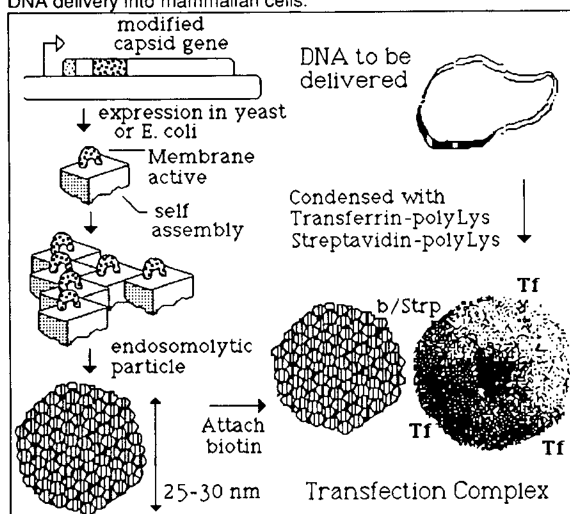
The C6 glioma brain tumor was selected as the initial target, since brain tumors are the most difficult to manage by pharmacological or surgical treatment. *In vitro*, 100% of C6 cells could be transduced by a recombinant adenoviral vector containing the ß-galactosidase gene. When transduce with the recombinant AD/RSV-TK vector, the TK gene is highly expressed in C6 cells, which are then sensitive to GCV treatment. To assess the efficiency of this approach *in vivo*, C6 cells were stereotaxically injected into the brains of nude mice. After 8 days, the resultant tumors were injected with AD/RSV-TK. The mice were then divided into two groups, one of which was treated with GCV for 6 days and the other with phosphate buffer (PBS). The resultant brain tumors were analyzed by microscopic pathological examation and computerized morphometric analysis. While tumors developed in all PBS-treated animal group, dramatic tumor regression was observed in the GCV treated animal group. AD/RSV-TK was also injected into the normal brains of nude mice, followed by treat with ganciclovir. No necrosis, demyelination, loss of neurons or inflammatory responses were observed. These results are the first demonstration that adenovirus can function as an efficient vector for the treatment of cancer by *in vivo* gene therapy.

DZ 107 TRANSIENT CYTOPLASMIC EXPRESSION OF LUCIFERASE AND β-GALACTOSIDASE GENES IN MAMMALIAN CELL LINES AND ANIMALS USING VECTORS CONTAINING BACTERIOPHAGE T7 PROMOTER AND RNA POLYMERASE GENE, Xiaozhuo Chen, Yunsheng Li, Keyong Xiong, Simona Aizicovici, Doros Platika, and Thomas Wagner. Progenitor Inc., and Edison Biotechnology Institute of Ohio University, Athens, OH 45701

Most gene expression vectors currently used in gene therapy study are viral in origin and require nuclear penetration or chromosomal integration for gene expression. However, in some targets of gene therapy, "correcting" gene expression ideally should be terminated and vector degraded when the disorder is corrected.

In order to find an alternative, transient cytoplasmic expression vectors have been constructed. These vectors contain a bacteriophage T7 promoter which has been linked to either a T7 RNA polymerase gene (T7 RNAP in T7-T7), or a reporter gene (T7-gene, gene = luciferase or β-galactosidase). T7-T7 construct also has been fused to T7-gene construct to make a single T7-T7/T7-gene plasmid. When introduced by lipofection following pre-binding of the T7 RNAP to the plasmid(s) *in vitro*, mouse L, human HepG2, CHO, and NIH3T3 cells were found transiently transfected by the T7 vectors. High levels of luciferase gene expression could be detected immediately after 3 hours' transfection. The expression was found to peak between 16 to 48 hours mainly depending upon initial cell plating densities, and last more than one week as determined by luciferase assays and β-gal stainings. Gene expression levels in L and HepG2 cells were increased approximately 10-fold when lipofectin was replaced by lipofectAMINE, and β-gal staining revealed that more than 10% of the cells were transfected when lipofectAMINE was used. When directly injected into various tissues of experimental mice, luciferase activities could be detected in the brain, liver, muscle, and tail connective tissues. These results suggest that the transient expression vectors based on T7 RNAP may be potential alternatives to animal viral vectors in certain targets in gene therapy.

DZ 108 DEVELOPMENT OF ENDOSOMOLYTIC VIRUS-LIKE PARTICLES FORGENE DELIVERY. Susanna Chiocca, Ernst Wagner and Matt Cotten, IMP Dr. Bohr-gasse 7 1030 Vienna
In the most efficient forms of receptor-mediated gene delivery, polylysine-condensed DNA is coupled to adenovirus particles with the virus providing a useful endosome disruption activity. We are working to replace the adenovirus with streamlined, nonviral endosomolytic elements. We reasoned that the presentation of endosomolytic peptides on proteins with the ability to self-assemble into ordered particles might provide a better reagent for augmenting gene delivery. Therefore, we have genetically modified simple virus capsid proteins to express membrane-active peptides on their surface. We find that the resulting particles are useful for DNA delivery into mammalian cells.



DZ 109 EXPRESSION OF SECRETED AND INTRACELLULAR PROTEINS FOLLOWING RECEPTOR-MEDIATED GENE TRANSFER TO HEPATOCYTES *IN VIVO*
Henry C. Chiou, June R. Merwin, Steven M. Levine, Kim M. Wimler, Michelle A. Salafia, and George L. Spitalny, TargeTech Inc., Meriden, CT 06450
Asialoorosomucoid-polylysine conjugates (ASOR-PL) bind DNA and selectively deliver it to hepatocytes *in vivo* via the asialoglycoprotein receptor (ASGPr). This method of gene transfer is easily achieved by intravenous injection. The ASOR-PL-DNA complex is rapidly taken up by the liver, with exclusive and uniform distribution to all hepatocytes. Internalization by non-parenchymal liver cells such as endothelia, smooth muscle cells, or Kupffer cells was observed to be negligible. We have employed ASOR-PL conjugates to deliver plasmids that express either hepatitis B virus surface antigen (HBsAg), driven by the SV40 early promoter, or firefly luciferase, driven by the CMV promoter. These two proteins serve as models, respectively, for potential therapeutic proteins such as Factor IX, which is secreted into the circulation, and cholesterol 7 α-hydroxylase, which remains intracellular. Though delivery of transgenes via ASGPr-mediated endocytosis is highly efficient, expression can be quite low since most of the DNA is destroyed within the endosome. Partial hepatectomies have been shown to enhance both the level and duration of expression. However, it is not practical for clinical use. We have found that treatment of mice with colchicine and chloroquine, which disrupt endosomal maturation and inhibit endosomal acidification, respectively, significantly enhanced transgene expression. Injection of 100-300 μg of DNA resulted in detectable circulating levels of HBsAg for up to 40 days, with protein production attaining 1.2 ng/ml in blood. Luciferase expression peaked between one and two weeks post-injection at 250 pg/gm of liver. We are examining the differences in expression of HBsAg and luciferase to determine if they are due to the nature of the genes or gene products, or due to differences in plasmid sequences and promoters.

DZ 110 CELLULAR GENES AND SEQUENCES DISRUPTED BY HUMAN PAPILLOMAVIRUS INTEGRATION IN CERVICAL CANCER, Kong-Bung Choo and Chuan-Mu Chen, Department of Medical Research, Veterans General Hospital, Taipei, Taiwan 11217, Republic of China

Human papillomavirus (HPV) infection has been associated with over 80% of cervical cancer cases. In HPV16-positive cervical cancer cases, the viral genome is found mostly in integrated form. The transforming E6 and E7 genes are invariably retained on integration. HPV integration appears to be random although sequences in the host chromosomal sequences may exert influence on the choice of integration sites. Integration has often resulted in deletion of some of the cellular sequences. Despite reports on a close association of HPV integration sites with the *myc* class oncogenes at a gross level, little is known about the cellular genes or sequences disrupted by HPV integration in cervical cancer cells. Since tumorigenesis is a multi-step process, some of the HPV-disrupted cellular genes may contribute to the genesis of cervical cancer.

As a first step towards addressing this issue, we have developed a PCR-based approach for rapid and large scale sequence analysis of the cellular loci disrupted by HPV16 integration in cervical cancer cells. Essentially, the techniques of multiplex PCR, nested PCR using only a single specific (HPV16) primer for amplification of the viral-cellular junctions, and direct cycle sequencing of the PCR products obtained were applied. To date, we have observed integration-induced disruption of the jun-B and MAP-2 genes in two separate cases. In a third case, although the cellular locus determined does not match any of the known sequences in the database, hybridization using a sequence from this locus as a probe has revealed rearrangement and amplification of this locus in at least one other case of cervical cancer. Details of these other cellular loci currently being analysed will be presented.

DZ 111 GENE TRANSFER INTO RETINAL TISSUE USING AN AMPLICON VECTOR SYSTEM, Maria E. Fotaki, Sonja Robaey-Mansour, Garth M. Bray, Albert J. Aguayo, Robert J. Dunn, Center for Research in Neuroscience, Montreal General Hospital, McGill Univ., Montreal, H3G 1A4
We have been investigating the utility of Herpes amplicon vectors for the introduction of recombinant expression vectors into retinal tissue. The amplicon vector system uses a plasmid that carries Herpes viral sequences which allow for the packaging of multiple copies of vector DNA into Herpes viral capsids in the presence of helper virus. We have developed an amplification protocol that results in improved ratios of amplicon to helper virus particles as indicated by PCR analysis. The resulting viral stocks are used to infect neuronal tissue *in vitro* and *in vivo*. We have used two series of amplicon vectors: i) pHermes-lacZ expressing the bacterial *lac z* gene under control of the human CMV immediate early promoter. ii) pHermes-L which retains the reporter gene under SV40 early promoter control and recombinant gene expressed from the CMV promoter. We are studying the neurons in the rat retina after gene transfer through intraocular injection of Hermes-lacZ. The long term goal is to effect a continuous supply of neurotrophins to the regenerating optic nerve.

DZ 113 TARGETED DNA DELIVERY INTO TUMOR CELLS UTILIZING THE FOLATE RECEPTOR.

Stephen Gottschalk[2], Richard J. Cristiano[2], Louis C. Smith[4], and Savio L. C. Woo[1,2,3]. (1) Howard Hughes Medical Institute, Departments of (2) Cell Biology, (3) Molecular Genetics, and (4) Medicine. Baylor College of Medicine, Houston, TX 77030.

We have combined a direct DNA delivery system with the membrane lysis ability of adenovirus to show that DNA can be delivered into tumor cells overexpressing the folate receptor. Folate (FOL) was activated with 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDC) in dimethylsulfoxide and was than coupled under hydrous conditions to Poly-L-Lysine (PLL). Various concentrations of the purified FOL/PLL conjugate were incubated with pCMV/ß-Gal DNA and the products were analyzed on agarose gels to determine the extent of FOL/PLL/DNA complex formation. A complex containing a PLL/DNA ratio of 200/1 was used to deliver the E. coli ß-galactosidase gene into KB-cells. Twenty-four hours after addition of the complex to the cells histological staining showed, that less than .1% of the cells could be positively stained with X-gal. The percentage of stained cells was greatly enhanced by the cointernalization of the FOL/PLL/DNA complex with a replication deficient adenovirus. The percentage of blue cells was proportional to the titer of adenoviral particles and 20 to 30% of the cells could be positively stained under these conditions. Beside KB cells other cell lines were tested and folate-mediated gene delivery was restricted to tumor cells that highly overexpress the folate receptor. Therefore this methodology shows great promise to selectively target genes into tumor cells overexpressing the folate receptor and has great potential for gene therapy of cancer.

DZ 112 IMMUNIZATION WITH INFLUENZA NUCLEOPROTEIN PLASMID DNA USING A PNEUMATIC GUN,

Stanislaw H. Gromkowski, H. Lee Vahlsing, Michelle Yankauckas, Michael Sawdey, and Marston Manthorpe, Vical Incorporated, 9373 Towne Centre Drive, San Diego, CA 92121

The Med-E-Jet pneumatic gun used for human vaccination can be used to propel intact, supercoiled plasmid DNA through skin and into skeletal muscles of mice. Pneumatic intramuscular injection of plasmids containing the firefly luciferase gene regulated by the human cytomegalovirus promoter resulted in the expression of several hundred picograms of luciferase enzyme activity in quadriceps muscles. Intramuscular injections of a plasmid containing the influenza A nuclear protein gene resulted in generation of potent and specific anti-nuclear protein humoral and cellular immune responses in eight out of eight BALB/c mice. This convenient and rapid injection method could be well-suited for genetic immunization of humans.

DZ 114 FILAMENTOUS PHAGE FOR CELL TARGETING AND GENE DELIVERY,

Stephen L. Hart, Andrew M. Knight[1], Richard Harbottle, Daniel F. Cutler[1], Robert Williamson and Charles Coutelle, Department of Biochemistry and Molecular Genetics, St. Mary's Hospital Medical School, London W2 1PG, UK and [1]MRC Laboratory of Molecular Cell Biology University College London, London, UK

Vectors for gene therapy have been developed which exploit the physiological process of receptor-mediated endocytosis. Such systems do not cause the deleterious side-effects which can be associated with viral vectors, and have the potential for both specificity and flexibility with regard to the targeting of different cell types, and the DNA constructs delivered. Peptides containing the amino acid sequence arginine-glycine-aspartic acid in a restricted conformation have a high affinity of binding to cell surface integrin molecules. Ligands which bind integrins on the mammalian cell surface with high affinity can mediate internalization. We show that particles of the bacteriophage fd which display a cyclic RGD-containing peptide in a proportion of their major coat protein subunits are internalized by cells in tissue culture. Phage displaying this peptide were used to coat wells and shown to bind cells efficiently from a suspension. Cellular internalization was demonstrated by immunofluorescent detection of phage in optical confocal sections of tissue cultured cells incubated with RGD-containing phage particles. This cell targeting and entry system can be exploited in the development of new vectors for gene therapy. In vitro gene delivery experiments using various marker genes will be reported.

DZ 115 STUDIES ON FIREFLY LUCIFERASE GENE EXPRESSION AFTER INTRAMUSCULAR INJECTION OF PLASMID DNA,

Jukka Hartikka, Francine Cornefert-Jensen, Jiin Felgner, Ann Y. Kuwahara-Rundell, Michal Margalith, Varavani J. Dwarki, Lee Vahlsing, Grace Hong and Marston Manthorpe, Vical Inc., 9373 Towne Centre Drive, San Diego, CA 92121

Direct injection of nonviral, covalently closed circular plasmid DNA into muscle results in the expression of the DNA in myofiber cells. Here we have compared the expression levels of different luciferase constructs after the injection into mouse rectus femoris muscle. We performed dose-response experiments using constructs containing various regulatory elements, including promoters from human cytomegalovirus immediate-early gene (CMV), Rous sarcoma virus, adenovirus, and crystallin B. Constructs containing enhancer elements from muscle creatine kinase, bovine growth hormone, and crystallin B were also tested. DNA was delivered as a single injection in 50 µl of sterile saline and the level of luciferase expression was determined 7 days postinjection. With all plasmid constructs, the highest expression was obtained with approximately 50 µg of DNA (the range of tested DNA concentrations was 1-500 µg per injection), indicating that some feature of muscle limits the expression when more than 50 µg of DNA is injected per site.

The luciferase construct containing the CMV promoter plus intron A ("nCMVintLUX") showed the highest expression yielding 7,500 pg luciferase per muscle with 50 µg of DNA injected. Time course analyses of nCMVintLUX DNA injections showed that maximal luciferase expression was achieved at 7-14 days post-injection. By day 60, the expression declined to about 5 - 10% of the maximum level and remained at this level for at least twelve months.

In order to correlate luciferase expression with the number of transfected muscle fibers, we performed a histochemical study using nCMVintLacZ as the reporter gene. As many as 200 myofibers out of 4000 present were stained positively with X-gal-based histochemistry at 7 days postinjection.

DZ 116 Mechanism of "Bystander Effect" Killing in the Herpes Simplex Thymidine Kinase Gene-Modified Tumor System.

Ishii H, Agbaria R, Hirano H, Ram Z, Oldfield E, Johns DG, Blaese, RM. NIH, Bethesda, MD 20892

"Bystander effect" killing of wild type (WT) tumor cells seen in the herpes-tk gene transfer system was reproduced *in vitro* using a ^{3}H-thymidine incorporation assay. Evaluation of the sensitivity of different tumor cell lines to the "bystander effect" demonstrated that some tumors were sensitive (9L, 38, A375) while other tumor cells were resistant (888, K562). A good correlation was found between *in vivo* bystander tumor elimination and the *in vitro* assay. *In vitro* mixtures of "bystander sensitive" tumor cell lines derived from the same or different species demonstrated cross transfer of the bystander effect, while mixtures of different resistant tumors remained resistant and mixtures of resistant with sensitive tumors were also resistant. Using ^{3}H-ganciclovir (GCV) and HPLC analysis, the formation of the toxic nucleotide analogue GCV-triphosphate (GCV-TP) in the herpes-tk transduced tumors was documented. Xenogeneic mixtures of "sensitive" cell lines were separated by immunomagnetic beads bound to species specific cell membrane antigens. After a period of coculture without GCV exposure, herpes-tk enzyme activity was found only in the gene-transduced cell partner indicating that the "bystander effect" is not dependent on direct transfer of the product of the inserted tk gene. In identical cell mixtures cocultured with ^{3}H-GCV, high levels of GCV-TP was found in both cell populations indicating its transfer from the gene-modified tumor cells to adjacent wild type (WT) cells. By striking contrast, tumor cell combinations "resistant" to bystander killing showed little or no GCV-TP transfer between transduced and WT tumor cells. The most likely mechanism of GCV-TP transfer between the tumor cells is via cellular gap junctions, with "resistant" tumors expressing lower levels of these channels for metabolic cooperation and small molecule exchange. The capacity of the intracellularly produced toxin, GCV-TP, to spread from cell to cell within a tumor mass effectively amplifies the apparent efficiency of gene transfer, thus permitting the use of gene transfer procedures which do not successfully transduce all of the tumor cells in a localized deposit of cancer.

DZ 117 DIRECT IN VIVO INTRODUCTION OF VARIOUS GENES INTO RAT KIDNEY BY HVJ-LIPOSOMES

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we developed efficient method for in vivo gene transfer using HVJ (Sendai virus), liposomes and nuclear protein. In this gene transfer system, DNA - nuclear protein complex in liposomes is directly introduced into the cytoplasm by the fusion activity of HVJ, and the DNA is delivered rapidly into the nucleus with nuclear protein. By this method, foreign genes can be introduced into the nucleus of all the cells but lymphocytes independent on cell cycle. So far, we have succeeded in introduction and expression of many genes in various organs of adult animals. Here, we present the results for direct in vivo gene transfer into rat kidney using HVJ-liposomes.

First, HVJ-liposomes containing SV40 large T antigen (SVT) DNA driven by chick-beta actin promotor was infused into rat renal artery by a cannula. On day 4 after the transfer, the expression of SVT was immunohistochemically detected exclusively in the glomerulus. Approximately 20-30 % of glomeruli of the kidney were immunopositive. The expression of SVT was continued for about 7 days in the kidney. However, no pathological change was observed in the kidney by the expression of SVT gene.

Next, we attempted to overexpress of TGF-beta1 or PDGF-B in rat glomerular cells by HVJ-liposome method. By the same approach, TGF-beta or PDGF gene was successfully introduced and expressed in rat glomerulus. In the TGF-beta 1 gene transfected rats, an extensive extracellular matrix (ECM) expansion with a moderate mesangial cell proliferation was observed. In contrast, the major alteration was a striking increase in cellularity with an ECM expansion in the PDGF-B gene - transferred rats. The conspicuous lobular formation of the glomerulus was also observed in rats transfected with PDGF-B gene. Pathological proteinuria was present in both TGF-beta and PDGF-B gene transferred rats, while it was not detected in CAT gene transferred rats. These pathological changes in rat kidney mimic glomerulosclerosis. This model rat may be useful to identify the responsible factor inducing glomerular lesions.

DZ 118 **TOWARDS A NEW GENE TRANSFER SYSTEM: SHOCK WAVE-MEDIATED DNA TRANSFER**

Ulrich Lauer[1,3], Zoë Squire[1,3], Elisabeth Bürgelt[1], Peter Hans Hofschneider[1], Michael Gregor[3], Stefan Gambihler[2] & Michael Delius[2].
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Lithotripter shock waves are pressure pulses of high amplitude and short duration used for the disintegration of urinary and biliary calculi *in vivo*. Recently, it was discovered that cells upon exposure to shock waves display a temporary increase in membrane permeability [1].

Starting from this observation, we investigated whether this phenomenon might support the transfer of plasmid DNA into eucaryotic cells. Plasmid pSV-MHBs expresses the secreted middle hepatitis B virus (HBV) surface protein (MHBs); plasmid pRSV-ß-gal gives rise to the cytoplasmic enzyme ß-galactosidase. HeLa cell suspensions mixed with reporter DNAs (2.5 ml; 10^7 cells/ml; 30µg DNA/ml) in polypropylene vials were exposed to 250 shock waves generated at 25 kV with an experimental Dornier XL1 lithotripter, seeded out subsequently and analyzed 36-48 hrs later for reporter protein expression.

Shock wave treated pSV-MHBs mixed HeLa cells accumulated increasing amounts of MHBs over time within cell culture supernatants as determined by ELISA-testing for HBsAg. Untreated control cells were found not to express/secrete MHBs. Shock wave treatment including reporter plasmid pRSV-ß-gal followed by histochemical staining led to the identification of individual transfected HeLa cells, whereas untreated control cells did not react at all. This points out that the shock wave-induced temporary increase in membrane permeability is necessary and sufficient for the transfer of DNA molecules. Other experiments suggest that cavitation, i.e. shock wave-triggered generation and movement of bubbles in a fluid, is responsible for this phenomenon.

In vivo, shock waves can be well focused within body regions of interest. Therefore, a combined *in vivo* gene therapy approach, i.e. application of extracorporally generated shock waves (1) to organs simultaneously perfused with defined DNA solutions or (2) following direct *in vivo* injection of DNA, might open up a new possibility to achieve a regionally enhanced DNA uptake *in vivo*.

[1] Gambihler, S. et al. (1992) Naturwissenschaften 79, 328-329.
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DZ 119 **NOVEL METHOD FOR DELIVERY OF GENE MEDICINES TO THE EPIDERMIS**

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A novel method is described for non-invasive and non-destructive delivery of DNA vectors to the epidermis which results in efficient the uptake and expression of the encoded gene product. Gene transfer, persistence, and expression was studied using vectors containing the CMV immediate early promoter and reporter genes including chloramphenicol acetyltransferase (CAT) and E. coli ß-galactosidase (ß-gal) after administration by hypospray to the dorsal skin of the rat. CAT activity was measured in dissected tissue samples harvested at various times after delivery. Reproducible activity was measured after delivery using different volumes, concentrations, and conditions for delivery. The cellular site of expression was identified within the epidermis by histochemical analysis of tissue samples after administration of vectors expressing ß-galactosidase. CAT activity was apparent for 3-4 days after injection and correlated with the elimination of DNA from tissue samples. This method represents a non-invasive approach for gene delivery to the skin which does not produce histological damage to tissues over the site of delivery, does not result in integration of DNA, and does not leave residual (non-biodegradable) material in the targeted cell. The ability to employ clinically validated methods for delivery of gene therapy, the safety of this method for administration of vectors, and the predictable clearance of the gene and gene product from tissues after delivery significantly expands the potential utility of gene medicines in clinical practice for the treatment of common diseases.

DZ 120 LONG TERM PERSISTENCE OF PLASMID DNA AFTER INTRAMUSCULAR INJECTION IN MICE,

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Muscle cells are capable of exogenous gene expression *in vivo* upon direct injection of plasmid DNA. Studies with reporter genes show that enzymatic expression from the plasmid peaks 7 to 14 days postinjection and declines to a stable, non-zero level by 30 days. Expression then continues for at least a year in many mice. Here, we address the question of how long the DNA persists after injection into the quadriceps muscle.

Persistence of the injected plasmid DNA was measured by utilizing a PCR assay. A time course study was undertaken using plasmids carrying the luciferase gene under the control of the CMV promoter. We detect reporter gene sequences in muscle at 1 day, 1 week, 1, 2, 4, 6, 9 and 12 months postinjection. Plasmids encoding the influenza virus nucleoprotein gene driven by either the RSV or CMV promoter were detectable for as long as 15.5 months postinjection. Virtually every injected muscle assayed by this PCR method has shown stable persistence of plasmid DNA with as little as 3 µg of DNA being injected. The plasmids in each of these experiments persist almost exclusively in the injected muscle, but can occasionally be detected in other organs within the mouse.

We have investigated the amounts of plasmid DNA remaining in muscle by Southern blots and quantitative PCR. The vast majority of injected DNA is degraded within 2 hours. One day after injection, we find 1/25,000 of the injected DNA remains as full length plasmid. These molecules are presumably internalized in the muscle cells. Within the first week, the amount of DNA continues to decline, though not as quickly, to ~1/2,500,000 or ~0.00004% of the original amount injected.

Our studies indicate that most of the injected DNA is rapidly degraded but that a small fraction appears to persist for many months, perhaps for the lifetime of the animal. We conclude that the injection of plasmid DNA can result in permanent transfection of the muscle tissue.

DZ 121 TRANSIENT EXPRESSION OF HERPES SIMPLEX VIRUS THYMIDINE KINASE (HSV-TK) GENE BY T7 VECTORS IN TIB-90 TUMOR CELLS,

Yunsheng Li, Xiaozhuo Chen, Yuefeng Xie, Keyong Xiong, Simona Aizicovici, Doros Platika, and Thomas Wagner. Edison Biotechnology Institute of Ohio University, and Progenitor Inc., Athens, OH 45701

HSV-tk gene expression coupled with ganciclovir treatment has been used to treat brain and liver cancers with promising results. In those studies, producer cell lines which stably express HSV-tk were used. This approach is able to provide stable and high levels of HSV-tk expression but the system is complicated by the use of retroviral vectors and cells.

We have developed a transient cytoplasmic gene expression system based on bacteriophage T7 RNA polymerase (RNAP). This vector, when introduced with pre-bound T7 RNAP, has been shown to transiently express reporter genes in several mammalian cell lines and in mice. In this study, an HSV-tk gene was inserted into the T7 vector, and transiently expressed in both mouse L and TIB-90 tumor cells. TK assays have revealed that HSV-tk expression by the T7 vector has been ~ 3 times higher than that provided by a traditional vector with a metallothionein promoter 24 hrs post transfection, and about same levels at 48 hrs. β-gal staining study following transfection by pT7βgal plasmid demonstrated that more than 10% of TIB-90 cells could be transfected. Preliminary result on TIB-90 tumor-bearing SCID mice suggested that pT7-tk DNA injection/ganciclovir treatment was inhibitory to the tumor growth. A large scaled study is under way to determine the effectiveness of this approach. Because of its simplicity and its rapid and high level of expression, this transient cytoplasmic gene expression system may become an alternative or complementary method to treat cancers.

DZ 122 RAPID PROTECTION AGAINST HIV-1 REPLICATION MEDIATED BY HIGH EFFICIENCY NON-RETROVIRAL DELIVERY OF GENES INTERFERING WITH HIV-1 TAT AND GAG

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Efficient transduction of inhibitory genes is a critical requirement in the development of a gene therapy strategy against human immunodeficiency virus type 1 (HIV-1). Commonly used systems based on retrovirus mediated gene delivery are characterized by low efficiency gene transfer into the target cell. Genes were transduced in the absence of cell selection into 60-90% of human CD4+ cells by using a novel technique that allows high efficiency gene transfer mediated by adenoviruses coupled with DNA-polylysine complexes. Protection of these cells against HIV-1 acute infection was evaluated by transducing them with three different inhibitory genes which interfere with HIV-1 replication at separate levels (polymeric-TAR decoy, dominant-negative mutant of the gag gene and antisense sequences of the gag gene) and subsequent challenging with HIV-1. The polymeric TAR decoy inhibited HIV-1 replication over 95%. Both the dominant-negative mutant and the antisense sequence of the gag gene were less potent inhibitors than the polymeric-TAR decoy. Combinations of either polymeric-TAR with dominant-negative mutant or antisense of the gag gene synergistically enhanced the inhibitory effects of the single genes. These data suggest that the combination of a highly efficient transduction technique with effective HIV-1 inhibitory genes confers rapid protection against HIV-1 acute infection in vitro, thus suggesting potential application in vivo.

DZ 123 EFFECTS OF VIRAL SEQUENCES UPON HUMAN GLOBIN GENE EXPRESSION IN TRANSGENIC MICE,

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The human β-globin Locus Control Region (LCR) is essential for high-level expression of human ε-, γ- and β-globin genes. Developmentally stable, DNase I hypersensitive sites (HS) mark sequences within this region which are important for LCR activity. A 1.1kb KpnI-XbaI fragment containing 5' HS 2 enhances human β-globin gene expression 80-fold in transgenic mice and also confers relative position independent expression. Constructs containing this 5' HS 2 fragment upstream of the human β-globin gene were used to examine the effects of viral sequences upon globin gene expression. The 5' HS 2 site and β-globin gene were cloned into the polylinker of the Moloney murine leukemia virus vector LXSN. The SV40 promoter and neomycin resistance gene were removed, leaving 1.7kb of viral sequence flanking the HS 2/β-globin gene. This construct was microinjected into fertilized oocytes and RNA from d16 fetal livers was analyzed. Expression of the human β-globin gene was undetectable in these samples. Constructs containing 5' HS 2 upstream of the human α-globin gene were also dramatically repressed when flanked by the same 1.7kb of retroviral sequence. Smaller fragments containing particular regions of the retrovirus were then cloned next to the HS 2/β-globin gene construct. Two of these constructs have been analyzed in transgenic mice. A 260 bp NheI-XbaI fragment containing the retroviral enhancer was found to have no deleterious effect upon expression. Conversely, a 250 bp KpnI-SpeI fragment containing parts of R, U5, and ψ greatly reduced β-globin expression. This work indicates that the presence of flanking retroviral sequence is sufficient to dramatically reduce expression from the human α- and β-globin promoters. These sequences are presumably responsible for the inhibition of gene expression observed in primary culture cells and animals infected with retroviral vectors. Additional work is being conducted to identify the specific sequences responsible for repression of genes inserted into retroviral vectors.

DZ 124 INTACT RETROVIRAL TRANSDUCTION OF AN INTRON-CONTAINING HUMAN PURINE NUCLEOSIDE PHOSPHORYLASE GENE AFTER IDENTIFICATION AND MODIFICATION OF SEQUENCES PREVENTING INTACT TRANSDUCTION, R. Scott McIvor, Donald E. Habel and Jon J. Jonsson, Institute of Human Genetics and Dept. of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN 55455.

Absence of purine nucleoside phosphorylase (PNP) in humans results in T-cell immunodeficiency, a candidate condition for gene therapy because the symptoms of the disease are largely restricted to the lympho-hematopoietic system. We previously determined in transfection experiments that optimum PNP expression is observed when introns are included in the PNP expression construct. However, insertion of an abbreviated PNP gene into a retroviral vector in the reverse orientation so as to allow transduction of an intronic sequence resulted in deletions apparently associated with inadvertant splicing signals and errors in reverse transcription of the PNP reverse complement (rcPNP) sequence. Here we report several modifications of this vector construct to allow intact transduction; (i) In-frame modification of an inadvertant splice donor in the rc of the PNP coding sequence; (ii) Modification of a splice donor located in intron 1 of the rcPNP sequence; and (iii) Deletion of a splice acceptor and several potential RT misalignment sites in the rc of the PNP 5' untranslated region. This construct, LN-PMG11, was transfected into GP + E86 ecotropic packaging cells, collecting supernatants for infection into PA317 amphotropic packaging cells. Of 22 G418-resistant PA317 clones isolated, 5 clones (23%) contained high-levels of human PNP identified by isozyme analysis on isoelectric focusing gels. However, when virus was collected from two of the PNP+ PA317 clones and used to transduce NIH 3T3 cells, 90% of the G418-resistant clones thus isolated contained intact PNP minigenes resulting in high-level human PNP expression. PCR analysis of clones expressing human PNP confirmed the presence of intact PNP minigenes. We conclude that offending sequences, particularly splicing signals, in reverse orientation retroviral vectors can be modified by *in vitro* mutagenesis to provide a system of intact transduction.

DZ 125 PURIFICATION AND CHARACTERIZATION OF THE UNILAMELLA FUSOGENIC LIPOSOME AS A NEW TOOL FOR GENE THERAPY, Mahito Nakanishi[1], Ken-ichi Ashihara[1,2], Takao Senda[3] and Tadanori Mayumi[2]. Institute for Molecular and Cellular Biology[1], Faculty of Pharmaceutical Sciences[2], Medical School[3], Osaka University, Suita, Osaka, 565, Japan.

We previously reported fusogenic liposome-mediated gene transfer *in vitro* and *in vivo*. Recently we have succeeded in preparing and purifying unilamella fusogenic liposomes (FLs) and have examined their properties in detail. Unilamella liposomes were prepared by the reverse phase evaporation method. The liposomes were mixed with UV-inactivated Sendai virus. The mixture was then incubated for 2 h at 37 °C and purified by sucrose gradient centrifugation. We examined the presence of FLs using the delivery of the A fragment of diphtheria toxin as a marker. We found that most FLs were recovered between 12 and 30% sucrose. No free liposome or Sendai virus was recovered in these fraction. Nearly 100% of the particles in these fraction could fuse with various types of cell membranes and deliver their contents directly into the cytoplasm. The average diameter of the fusogenic particles examined by dynamic light scattering was 379 nm, whereas that of unilamella liposomes and Sendai virus was 304 nm and 332 nm, respectively. Electron microscopic examination revealed that the fusogenic particles have a unilamella membrane with spikes similar to Sendai virus envelope on the surface and an empty inner space. All of these data supported our conclusion that a unilamella fusogenic liposome was generated by the fusion of an unilamella liposome and a Sendai virus particle. The FLs could be concentrated by centrifugation (20,000 rpm, 20 min) without loss of activity and could be stored in liquid nitrogen indefinitely. The FLs could deliver their contents to a wide variety of cells with sialic acid on the cell surface. A concentrated FL suspension usually contains $2x10^{11}$ particles/ml and can transform $2x10^{7}$ human cells stably in combination with an Epstein-Barr virus vector. These data demonstrate that the unilamella fusogenic liposome is a useful tool for gene therapy.

Ref. 1. Nakanishi, M. et al. *Exp.Cell Res.* **159**, 399-409, 1985.
2. Kato, K. et al. *J.Biol.Chem.* **266**, 3361-3364, 1991.

DZ 126 RECOMBINANT AAV VECTORS: VIRAL AND LIPOSOME MEDIATED TRANSFECTION OF RECOMBINANT AAV GENOME LEADS TO HIGH EFFICIENCY GENE TRANSFER INTO PRIMARY CELLS. Kevin W. Page, Ramila Philip, Elisa Brunette, Maureen McNally, Thomas B. Okarma and Jane Lebkowski, Applied Immune Sciences, Inc., Santa Clara, CA 95054

Adeno-associated virus (AAV), a single stranded DNA parvovirus, is endogenous to humans yet has never been associated with any disease. AAV has a unique replication strategy which makes it ideal as a vector to introduce foreign DNA into eukaryotic cells. We have engineered recombinant adeno associated virus (rAAV) vectors which contain various combinations of promoter/enhancer sequences and one or two genes including the marker genes ß-galactosidase and chloramphenicol acetyl transferase as well as the human interleukin-2 gene. These recombinant AAV have been shown to express their gene products efficiently in several established cell lines including those of hematopoietic origin. Our recent work involves using rAAV to infect and rAAV plasmid DNA: liposome complexes to transfect primary T-lymphocytes and a variety of fresh and cultured tumor cells. Both rAAV transduction and AAV plasmid: cationic liposome mediated transfection induced significant levels of expression of the indicator gene products (CAT, ß-gal) as well as a therapeutic gene (IL-2). Although direct comparison between AAV infection and lipofection of AAV plasmid is difficult, both systems appear efficient methods to introduce transgenes into primary cell types. Data will be presented comparing expression efficiencies using biological assays and southern blot analysis. The relative merits of using viral or non-viral transduction systems will be discussed. Potential clinical applications include tumor vaccine studies and the modulation of T lymphocytes and hematopoietic stem cells for AIDS and cancer therapies.

DZ 127 COMPLETE NUCLEOTIDE SEQUENCE OF 4070A, AN AMPHOTROPIC MURINE RETROVIRUS. [1]Michael N. Pensiero, [1]Jonathan Hirsch, [1]Michelle G. Jacobson, [2]W. French Anderson, [1]Paul Tolstoshev, and [1]Bruce C. Trapnell, [1]Department of Virology, Genetic Therapy Inc, Gaithersburg, MD 20878; and [2]University of Southern California, Norris Cancer Center, CA 90033.

Amphotropic retroviruses, which are able to infect human tissues, are important in generation of recombinant retroviral vector packaging cell lines for use in human gene therapy. In this context, the envelope gene (env) of the amphotropic retrovirus 4070A has been cloned and used in such cell lines (e.g. PA317). Despite this critical role and the potential for use as a backbone in future retroviral vectors, the complete nucleotide sequence of the 4070A retrovirus has not been determined. To accomplish this, a 4070A clone was isolated from persistently infected Mink lung fibroblasts by co-cultivation with naive NIH3T3 TK- cells. A 48 hr Hirt supernatant was used to isolate DNA for the non-integrated provirus which was then cloned into a λ library. Plaques were identified using as a probe sequences from pPAM-3, a plasmid known to contain a partial sequence of 4070A. Individual clones were isolated and transferred into a plasmid (Bluescript KS-) and one clone (4070A-11) was sequenced completely. This clone (8298 bp) was shown to be circularly permuted at the LTR and contained one open reading frame (ORF) for gag and pol genes, and a second ORF for env. Comparison of nucleotide sequences of 4070A ORFs with corresponding regions of Moloney murine Leukemia virus showed homology in the gag and pol regions (89.5% and 92.5%), respectively and considerably less in the env region (55.7%). To test whether these sequences encoded functional proteins an infectious 4070A was constructed; this was made by reconstruction of the 3' LTR using 5' LTR sequences. The resultant plasmid was transfected into NIH3T3 TK^- cells and supernatant used to transduce naive NIH3T3 cells. Infectivity was proven by demonstration of env expression in 4070A transduced, but not control cell populations by Western and FACS analysis. Further proof was obtained by the ability of 4070A-11 to rescue a standard env^- β-galactosidase-expressing retroviral vector. This clone will be important in construction of future generation retroviral vectors as well as 'split-packaging' cell lines where the gag-pol and env gene are separated within the genome of the cell.

DZ 128 GENE TRANSFER INTO HEMATOPOIETIC PROGENITOR CELLS USING *ACCELL®* PARTICLE BOMBARDMENT, Ping Qiu*, Ze-Qing Ye, Joseph Burkholder*, Nasrollah T. Shahidi and Ning-Sun Yang*, Department of Mammalian Genetics, Agracetus, Inc.*, Middleton, WI and Hematology/Oncology Division, Department of Pediatrics, University of Wisconsin, Madison, WI 53792

To transfer genes into hemopoietic stem cells, most investigators rely on retroviral vectors which require prior induction of the stem cells into S phase. We have recently used an alternative technique for gene transfer which obviates the need for retroviral vectors or cell cycle coordination. In this study, CD 34+ cells, isolated from human cord blood, were subjected to particle bombardment using a particle-mediated gene delivery device (*Accell®*). In this technique, microscopic gold beads coated with plasmid DNA are bombarded into target cells by a shock wave generated by a high voltage electric-discharge mechanism. The CD 34+ cells were isolated from fresh cord blood using soybean agglutinin and anti-CD 34+ antibody coated flasks. The highly purified CD 34+ cells were bombarded with two transgenes: firefly Luciferase (Luc) gene and GM-CSF gene, both driven by a cytomegalovirus (CMV) promoter. Particle bombardment was performed either immediately after separation of CD 34+ cells or after 4- and 10-day expansion *in vitro* in the presence of SCF, IL-3 and IL-6. Both genes were expressed, not only in growth factor stimulated CD 34+ cells, but also in freshly prepared CD 34+ cells. Cells bombarded after 4-day culture exhibited a 4- and 10-fold increase in expression of Luc and GM-CSF respectively. After 10 days of incubation there was a 17-fold increase in Luc expression. As determined by FACS, 91% of cells in 4-day culture were CD 34+. Whereas 78% of cells in 10-day cultures were already differentiated into CD 33+ cells. To optimize the transgene expression in hematopoietic stem cells the relative strength of various viral and cellular promoters and special vector designs for gene expression were also examined and yielded reproducible results. The present study suggests that *Accell®* particle bombardment is an effective technique for gene transfer into human hemopoietic stem cells.

DZ 129 FUNCTIONAL INTERACTION OF WILD - TYPE AND MUTANT P53 TRANSFECTED INTO HUMAN TUMOR CELL LINES CARRYING ACTIVATED RAS GENES, Reinhold Schäfer, Irmgard Schwarte-Waldhoff, Hermann Oberhuber and Sanjai Sharma, Division of Cancer Research, Department of Pathology, University of Zürich, CH-8091 Zürich, Switzerland

The mutational inactivation of the *P53* tumor suppressor gene and activation of *RAS* genes constitute very frequent genetic alterations in human cancer. Both lesions may occur in the same tumor. We asked the question if overexpression of the suppressor gene would inhibit the proliferation of human cancer cells harboring activated *RAS* genes. The anti-proliferative activity of *P53* was analyzed in the HT1080 fibrosarcoma cell line carrying an activated *NRAS* gene, the SW480 colon carcinoma line (activated *KRAS*) and the EJ bladder carcinoma line (activated *HRAS*). The levels of p53 protein and incorporation of bromodeoxyuridine (BrdU) were determined simultaneously in transiently transfected cells by immuno-flow cytometry. HT1080, SW480 and EJ cells were equally sensitive toward wild-type *P53*-mediated inhibition of DNA synthesis, independent of the state of the endogenous p53 protein. Overexpression of *P53* mutated at amino acid codon 143 resulted in increased proliferation of SW480 cells that have two mutated endogenous alleles. To mimic the genetic constitution of evolving tumor cells that have sustained a mutation in one *P53* allele, we transiently coexpressed both wild-type and mutant *P53* controlled by cytomegalovirus promoters in HT1080 cells. BrdU uptake was reduced to a similar level as in cells into which only wild-type *P53* had been introduced. The wild-type gene is a dominant growth-suppressor over mutant genes modified at amino acid codons 143, 175 or 273 in all cell lines analyzed. The presence in coexpression experiments of both the wild-type and mutant conformations of the p53 protein was demonstrated by immunoprecipitations with antibodies Pab 122, 420 and 1620.

DZ 130 A RAT MODEL FOR STUDYING DNA UPTAKE AND EXPRESSION BY THYROID CELLS AFTER DIRECT INJECTION. Michael L. Sikes, Bert W. O'Malley, Jr., Fred D. Ledley. Departments of Cell Biology, Pediatrics, and Otorhinolaryngology, Baylor College of Medicine, Houston, TX and GENEMEDICINE, INC, Houston, TX 77054.

Direct injection of recombinant DNA vectors into rabbit thyroid leads to uptake and expression of the gene product by thyroid follicular cells. Previous studies have demonstrated that the kinetics of DNA uptake and elimination from the thyroid, as well as the persistence of gene expression, differs significantly from that observed after injection of DNA into muscle or receptor-mediated targeting of DNA to the liver. This observation suggested that the mechanisms of uptake, compartmentalization, or degradation of the administered DNA is different in these model systems. We now report that rat thyroid, like rabbit thyroid, expresses recombinant genes following direct interstitial injection of plasmid vectors. Individual rat thyroid lobes were visualized after surgical exposure and injected with 10-20 ul of PBS containing 2 ug/ul DNA. The plasmid used for these experiments contains the CMV immediate early promoter driving a chloramphenicol acetyltransferase (CAT) reporter gene. Twenty four hours after injection, thyroids were harvested, protein extracts were prepared, and CAT assays were performed using 50 ug of total protein. The level of CAT activity was similar to that observed previously in rabbit thyroid. These observations demonstrate that the expression of DNA after intrathyroid injection is not species specific, and establishes a controlled and reproducible in vivo model in which to study the mechanism and characteristics of DNA uptake, trafficking, and expression. This model is being used to study the effect of various expression constructs, different formulations, and various pharmacological manipulations of the target cell which alter uptake, compartmentalization, metabolism, or proliferation by the cell.

DZ 131 RECOMBINANT ADENOVIRUS VECTOR MEDIATED *IN UTERO* FETAL GENE THERAPY. Eric M. Toloza, Alexander R. Miller, Weihong Guo, Kristina Rhoades, William McBride, Kelly Hunt, Beatrice L. Schuck, Sousan Karimi, Thomas Drake, Robert Moen, Bruce Trapnell, Eric W. Fonkalsrud, and James S. Economou, Divisions of Surgical Oncology, Pediatric Surgery, Radiation Oncology, and Pathology, UCLA School of Medicine, Los Angeles, CA 90024, and Genetic Therapy, Inc., Gaithersburg, MD 20878.

A recombinant adenovirus vector bearing the *E. coli* beta-galactosidase (lacZ) gene with nuclear localization sequences was used to transduce fetal rabbits *in utero*. Adenovirus vectors are packaged at high titers (10^9-10^{11}pfu/ml), have the capacity to infect throughout the cell cycle, and result in greater transduction efficiencies. We introduced 10^8pfu of purified recombinant adenovirus vector into fetal rabbits on day 26 of the 31-day gestation period. The rabbit pups were delivered by Caesarian section on day 31 of gestation and reared by foster dams. The routes of viral delivery found to be most successful for gene transfer were direct injection into the fetal liver, and administration into the fetal peritoneal cavity or into the amniotic sac. Consistently high expression of the lacZ gene product in newborn rabbits was demonstrated by histochemical staining and by polymerase chain reaction. Intrahepatic injection resulted in gene transfer solely into the liver, while injection into amniotic and peritoneal fluid resulted in transduction of the upper gastrointestinal tracts and livers, respectively, of these animals. Beta-galactosidase expression persisted for at least ten days postpartum in the livers of intraperitoneally-injected rabbits. Long term studies are ongoing. Adenovirus-based vectors thus are useful tools in the development of efficient *in vivo* fetal gene therapy.

DZ 132 *IN VIVO* REDUCTION OF RAT ANGIOTENSINOGEN BY HVJ-LIPOSOMES CONTAINING ANTISENSE OLIGONUCLEOTIDE

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Renin angiotensin system (RAS) plays a very important role in the regulation of water electrolyte balance and blood pressure. Many reports indicate the existence of local RAS, but functions of these are still unknown. Circulating angiotensinogen (ANG) is mostly produced in liver. In this study we tried to block the production of AGN in liver using antisense oligonucleotides by HVJ-liposomes to investigate the function of local RAS. First antisense and sense oligomers were synthesized complementary to the transcription start site of rat AGN. Prior to *in vivo* study, we comfirmed the inhibitory effect of the antisense oligomer using rat primary hepatocytes. Then HVJ-liposomes containing antisense and sense oligomers (4μM) were injected into liver via portal vein. On day 2 after the transfer, rats were decapitated, and plasma and liver were removed. Northern blot analysis showed the significant reduction of mRNA of AGN in liver by the antisense oligomer. Moreover AGN concentrations both in plasma and in liver were also decreased. The sense oligomer had no effect on mRNA of AGN and AGN concentrations. We already reported the model rat produced by *in vivo* transfer of HVJ-liposomes with human renin gene and that expressions of introduced genes by HVJ-liposomes were enhanced by repeated injections. Thus these rats produced by an efficient and safe gene transfer method, HVJ-liposomes, could be good tools for investigating RAS and for the therapy of hypertension.

AGN concentrations

	Antisense	Sense	Control
n	8	8	8
Plasma(ng AI/ml)	49.8±6.7*	83.7±4.2	87.4±2.3
Tissue(ng AI/g tissue)	6.2±0.9*	7.4±0.7	7.7±0.4

*p<0.01

DZ 133 PHARMACOKINETICS OF SINGLE AND REPEATED *IN VIVO*, ADENOVIRUS-MEDIATED GENE DELIVERY TO AIRWAY EPITHELIUM,

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Adenoviral vectors (Av) represent potentially important *in vivo* delivery vehicles for gene therapy for diseases where *in vivo* administration is essential. While preliminary investigations have been encouraging, the feasibility of Av for human gene therapy remains to be demonstrated. Due to the non-integrated, epi-chromosomal localization of the transduced Av genome, an important concern is the pharmacology of single and repetitive dosing. To evaluate this, a replication-deficient, recombinant adenoviral vector, AvlLucl, expressing a cytoplasmic luciferase reporter was constructed. Reporter expression was sequentially, and quantitatively evaluated in lung tissue homogenates (n = 20) after pulmonary administration of AvlLucl ($2x10^8$ plaque forming units) to cotton rats. In this model, >90% of the pulmonary dose is delivered to the medium and small airway epithelium. Reporter gene expression was maximal (32,410 ± 10482 light units (lu)) three days after administration, and declined with first order kinetics and a terminal elimination half life ($T_{1/2\beta}$) = 3.5 days reaching baseline by 21 days. Efficacy of repeat administration was evaluated exactly as above in rats (n = 10) 120 days after pulmonary administration of AvlCf2, an identical vector expressing normal human CFTR instead of luciferase. Importantly, repeated Av transduction was successful as indicated by luciferase expression significantly above control (6047 ± 1292 lu vs 167 ± 13 lu; p<0.01), but was significantly lower (p<0.001) than when given as the first vector dose. Efficacy of repeat administration showed a strong inverse correlation (correlation coefficient = 0.92) with the serum level of neutralizing anti-human adenovirus antibodies, but such antibodies were unrelated to the time course of the decline in expression following the first vector dose. These observations suggest that 1) factors other than humoral immunity are responsible for the decline in transduced gene expression after a single pulmonary Av administration; and 2) humoral immunity may play a significant role in the efficacy of repetitive Av dosing. This will likely have important implications in the design of dosing regimes for human clinical trials.

DZ 134 TRANSFER OF THE HUMAN GLUCOCEREBROSIDASE GENE INTO CANINE AND HUMAN REPOPULATING CELLS,

Christof v. Kalle, Hans-Peter Kiem, Jordi Barquinero, Boris Darovsky, Mikhail Duboikin, Shelly Heimfeld, John Barringer, A. Dusty Miller, Rainer Storb, Friedrich G. Schuening, Fred Hutchinson Cancer Research Center, Seattle, WA, 98104, CellPro, Bothell, WA, 98021 and University of Pittsburgh, Pittsburgh, PA, 15261

Transferring the human glucocrebrosidase (GC) gene into hematopoietic repopulating cells of Gaucher disease patients could be curative. To investigate whether GC transduction of repopulating cells can be achieved, six dogs received autologous marrow and peripheral blood mononuclear cells transduced either by 24h cocultivation on MFG/GC vector-producing packaging cells followed by 4-day incubation in a vector-containing long-term marrow culture (LTMC) system (n=4) or by 5 day incubation in a vector-containing LTMC only (n=2). Dogs were treated before intravenous infusion of transduced cells with either an otherwise lethal dose of 920cGy total body irradiation (TBI) (n=2), a sublethal dose of cyclophosphamide (n=2), local irradiation of both humeri (n=1) or no conditioning (n=1). Only the four dogs conditioned with either TBI or cyclophosphamide showed persistence of the human GC cDNA in peripheral blood granulocytes and lymphocytes for so far up to 30 weeks after transplant, suggesting that prior myeloablative treatment is necessary for engraftment of transduced hematopoietic repopulating cells. GC gene expression in vivo has not been detected above the normal background, probably due the low percentage of transduced cells. Two human marrow and three peripheral mononuclear cell samples were transduced in a 5 day vector-containing LTMC system with and without prior enrichment for $CD34^+$-cells. GC enzyme activity of transduced cells was increased up to 4.9fold above normal control cells. Enrichment for $CD34^+$-cells increased GC activity up to 2.7fold above unselected transduced cells. Between 15 and 40% of CFU-GM colonies and 15% of LTMC-initiating cells had vector GC cDNA integration by PCR. We conclude that canine and human hematopoietic repopulating cells can be transduced with a GC gene containing retrovirus vector and that prior myeloablative treatment appears to be necessary for engraftment of transduced hematopoietic repopulating cells.

DZ 135 PARTICLE BOMBARDMENT-MEDIATED RNA DELIVERY AS AN APPROACH FOR GENE THERAPY,

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Current gene transfer and gene therapy studies are focusing mainly on DNA delivery technologies; progress in using RNA delivery for transgene expression has been very limited. For gene therapy, RNA-mediated gene transfer may be clinically more desirable than with DNA, since RNA molecules do not integrate into chromosomes and will not cause insertional mutagenesis in transgenic cells. High-level transient expression of functional genes may have a number of applications to gene therapy, including genetic immunization. Efficient delivery of RNA transcripts to *in vivo* or *in vitro* cell systems may also provide new means for basic research on regulation of post-transcriptional, translational and ribozyme activities. In this study, we report that *Accell®*, a particle bombardment-mediated gene transfer technology, can offer a simple, efficient and reliable means for RNA delivery into various mammalian somatic tissues, under both *in vivo* and *in vitro* conditions. Transgene expression of RNA transcripts of three reporter genes, firefly luciferase (luc), human growth hormone (hGH) and human alpha-1 antitrypsin (hAAT), were detected in mouse epidermal or rat liver tissues bombarded *in vivo* and in cell cultures (CHO, B16 and KG-1) bombarded *in vitro*. Time course of transgene expression via RNA delivery was established. *In situ* bombardment of mouse epidermal skin tissue with hAAT-mRNA followed by boost-bombardments elicited high level *in vivo* immune response in antibody production against the transgenic hAAT antigens. Each boost-bombardment resulted in an increasingly higher antibody response, with a titer of 1:12800 from the third one. In addition to humoral immune response, cellular immunity against transgenic antigens are also expected from this RNA gene therapy approach.

DZ 136 LACTOFERRIN-MEDIATED GENE TRANSFER: USE OF A NONANTIGENIC, DNA-BINDING PROTEIN FOR RECEPTOR TARGETED GENE THERAPY.

Jason Yovandich, Jozsef Stankovics, Fred D. Ledley, Departments of Cell Biology and Pediatrics, Baylor College of Medicine, Houston TX 77030 and GENEMEDICINE, INC, Houston, TX 77054.

The coupling of DNA to receptor-binding ligands represents an effective method for delivering DNA vectors into cells in vitro and in vivo. Previous approaches have used ligand/polylysine/DNA complexes to target hepatocytes using asialoglycoprotein/polylysine complexes or other cells using transferrin/polylysine complexes. Covalent modification of peptide ligands with polylysine, however, renders these proteins antigenic, and the development of antibodies may prohibit repetitive dosing regiments in clinical practice. We now describe a novel method for gene transfer using lactoferrin. Lactoferrin is a DNA binding glycoprotein in its unmodified form and is a ligand for specific receptors. Complexes formed between lactoferrin and DNA were characterized in band-shift gel assays. Retention of the complex was proportionally related to the ratio of lactoferrin:DNA (1:1 - 100:1). Complexes were stable over a wide range of salt concentration (0-500mM), pH (4-10), and temperature. The size of the complex was critically dependent upon the iron content of lactoferrin and the incubation reaction. Lactoferrin/DNA (pCMV-CAT) complexes transfected several cell types representing hepatocytes (hepG2), skeletal muscle (sol8), and pulmonary epithelium (LA4). Further studies in hepG2 cells showed that both lactoferrin-DNA complexes and asialolactoferrin/ DNA complexes produced CAT activity, and activity was increased by coincubation with attenuated adenovirus. There was extreme variability in the efficiency of gene transfer of different lactoferrin-DNA preparations. These studies demonstrate that lactoferrin may be used effectively as a carrier for gene transfer. The utility of this system is dependent upon constructing consistent and colloidally stable lactoferrin/DNA complexes.

Gene Therapy: Somatic Tissue I; Homologous Recombination/Mouse Models

DZ 200 TOWARD GENE THERAPY FOR GAUCHER DISEASE: TRANSFER AND EXPRESSION OF THE GLUCOCEREBROSIDASE GENE IN CD34+ CELLS,

John Barranger, Alfred Bahnson, Maya Nimgaonkar, Sallie Boggs and Edward Ball, Department of Human Genetics and Division of Hematology/Bone Marrow Transplantation, University of Pittsburgh, PA 15261

Storage of glucosylceramide occurs in tissue macrophages as a result of the inherited deficiency of glucocerebrosidase (GC) in patients with Gaucher disease. In this most common of the lysosomal storage disorders and the most prevalent Jewish genetic disease, augmentation of macrophage enzymatic activity either by its direct replacement (ERT) or allogeneic transplantation of donor marrow (BMT) is therapeutic. Gene therapy should be an efficacious treatment provided 1) GC gene transfer is efficient, 2) it results in satisfactory levels of enzymatic activity and 3) sufficient numbers of genetically reconstituted bone marrow stem cells engraft in the autologously transplanted patient. We have demonstrated efficient GC transfer and sustained expression in animals. Human CD34+ enriched cells from normal bone marrow, Gaucher bone marrow, peripheral blood, and cord blood are good targets for the retroviral vector carrying the GC gene. These cells express up to five times the normal GC activity. These results permit investigation of competitive engraftment of genetically corrected CD34+ cells in patients with Gaucher disease.

DZ 201 ANALYSIS OF LEUKOCYTE INTEGRIN PROMOTERS IN RETROVIRAL VECTORS: APPLICATION TOWARDS HUMAN GENE THERAPY,

Thomas R. Bauer, Jr., William A. Osborne, William W. Kwok, and Dennis D. Hickstein, Medical Research Service, Seattle Veterans Affairs Medical Center, Seattle, WA 98108 and Department of Medicine, University of Washington School of Medicine, Seattle, WA 98195

Human gene therapy for diseases such as leukocyte adherence deficiency, ADA deficiency and Gaucher's disease may be facilitated by the identification of promoter / enhancer sequences which direct high levels of expression of the replacement gene in specific populations of leukocytes.

We utilized the leukocyte integrin promoter sequences in retroviral vectors to direct the expression of a reporter gene, adenosine deaminase or ADA, in human leukocyte cell lines.

The leukocyte integrins consist of a common β or CD18 subunit which is non-covalently associated with one of three different α subunits designated CD11a (LFA-1), CD11b (Mac-1), and CD11c (p150). The CD11a subunit is expressed preferentially on lymphocytes and macrophages, whereas the CD11b subunit is expressed on neutrophils.

The promoters for CD11a, CD11b, and the CD18 subunits were cloned upstream of the ADA gene in retroviral vectors and transduced into the human leukocyte cell lines K562 and HL-60 and in EBV-transformed human B-cell lines. The activity of the leukocyte promoters in these cell lines were compared to the Moloney Murine Leukemia Virus 5' LTR promoter and the cytomegalovirus promoter by using RNA isolated from these transduced cell lines on Northern blots and by examining ADA levels in cell extracts.

This study should facilitate the identification of promoter sequences expressing reporter genes at high levels in leukocytes and allow comparison of the specificity of the integrin promoters. In summary, these studies tested the efficacy of endogenous integrin promoters in retroviral constructs, using a marker gene, ADA. Retroviral constructs utilizing the leukocyte integrin promoters may be important for directing high levels of the replacement gene in human leukocytes.

DZ 202 THE MAINTENANCE AND PROLIFERATION OF CD34$^+$ CELLS IN SCID-hu MICE. Amy E. Berson, Diane Rood, Karen Chen, Maureen A. McNally, Karla Knobel, Thomas B. Okarma, and Jane S. Lebkowski, Applied Immune Sciences, Inc., Santa Clara, CA 95054

Severe combined immunodeficient (SCID) mice have proven to be a valuable animal model for the study of human hematopoiesis due to the ability of the SCID mouse to support xenogeneic grafts. We have used this model to compare hematopoiesis from different human CD34$^+$ cell sources. For this analysis, SCID-hu mice were made by placing HLA typed human fetal thymus and liver under the kidney capsule of CB.17 *scid* mice. Once the human tissue engrafted, the graft was then seeded with donor HLA mismatched human cells. Ten weeks later, the grafts were analyzed by flow cytometry for the maintenance and differentiation of the seeded cells. In these studies, we have shown that G-CSF mobilized peripheral blood and normal bone marrow can repopulate and differentiate in the transplanted fetal thymus. CD34$^+$ cells isolated from adult bone marrow have also proliferated in the thymus/liver xenografts. As few as 25,000 bone marrow CD34$^+$ can survive, proliferate 50-500 fold, and differentiate into mature CD4$^+$ and CD8$^+$ cells. Currently, we are using this system to compare the hematopoietic capacity of CD34$^+$ cells from mobilized peripheral blood and of normal bone marrow CD34$^+$ cells. We are also investigating the proliferative potential of primitive CD34$^+$ subpopulations in these xenografts. Further studies will look at the gene expression of transduced CD34$^+$ cells.

DZ 203 REGULATION OF *STEEL* FACTOR EXPRESSION IN HEMATOPOIETIC MICROENVIRONMENTAL CELLS: POTENTIAL TARGETS FOR GENE THERAPY, Jeffrey S. Buzby, Eva M. Knoppel, Carmella van de Ven, and Mitchell S. Cairo, Children's Hospital of Orange County, Orange, CA 92668.

The multipotent cytokine known as *Steel* factor (SLF), stem cell factor, Kit ligand, or mast cell growth factor is a product of mesenchymal cells comprising hematopoietic microenvironments. SLF acts synergistically with other cytokines to enhance proliferation and colony formation by bone marrow progenitor cells and may be useful in bone marrow transplantation for the expansion of hematopoietic progenitors (van de Ven, et al, *Exp Hematol* 21:1134, 1993). However, phase I clinical trials with subcutaneous, recombinant hSLF have encountered substantial toxicity (McGuire, et al, *J Clin Oncol* 12:135 & 142, 1993). The recent successful utilization of vascular endothelial cells (EC) and fibroblasts (Fb) as targets for somatic gene transfer and expression suggested their potential to produce SLF in bone marrow via gene transfer, since they are critical components of bone marrow microenvironments. To determine the normal pattern of SLF expression, we measured its soluble, membrane-bound, and mRNA levels in human umbilical vein EC (HUVEC), adult aortic EC (HAEC), and dermal Fb. Cultures were stimulated with 30 ng/mL PMA, 10^{-5} *M* A23187, 5 U/mL hIL-1, or 100 U/mL hTNF-α for 1–72 hrs prior to harvest. RNA was analyzed by Northern blot hybridization with a ^{32}P-labeled 900 bp *Bam*HI-*Hin*dIII probe for hSLF from pBs:hMGF (Immunex) and a 670 bp *Rsa*I probe from pHFβA-1 for β-actin, a constitutive indicator of total RNA loading. Autoradiographic signal strength of hybridized transcripts was estimated densitometrically. Soluble SLF in EC and Fb culture supernatants and cell-associated SLF in EC cultures extracted with RIPA buffer were measured by ELISA (Amgen-Boulder). SLF mRNA expression was increased 4-fold by IL-1 (2 h) or TNF-α (4 h) and 50-fold by PMA (4 h) or A23187 (4 h) from a low basal level in HUVEC and HAEC. HUVEC expressed twice as much SLF mRNA as HAEC before and after stimulation. In contrast, basal expression of SLF mRNA in Fb was nearly as great as the maximal level induced by PMA or A23187 in EC, and it was unaffected by these four agonists. Equivalent levels of SLF mRNA were observed in Fb from neonatal (NFb) and adult (AFb) sources. Production of EC-associated SLF was also induced 2-fold above basal level by PMA (8 h), with HUVEC producing 2-fold more than HAEC (16.4 ± 2.4 vs. 7.3 ± 1.0 ng/mg protein). Soluble SLF was induced 6-fold above basal levels in HUVEC (0.25 ± 0.01 ng/mL) and HAEC (0.22 ± 0.01 ng/mL) after 24 h PMA, while Fb produced a non-inducible, 3-fold lower level than that induced in EC (0.07 ± 0.01 and 0.08 ± 0.01 ng/mL in NFb and AFb, respectively). Thus, the ability of these cells to produce secreted and membrane-bound SLF indicates their potential as targets for engineering enhanced hSLF expression to generate an activated *ex vivo* and/or *in vivo* hematopoietic microenvironment.

DZ 204 The Development and Use of Adenovirus Vectors for Cystic Fibrosis Gene Therapy. Larry Couture, Judith St. George, Donna Armentano, Jennifer Arcand, Lisa Cardoza, Alex Floyd, Vicki Giuggio, Michael Lukason, Lynne Keyes, John Martin, Pamela Peterson, Sara Pennington, Joseph Zabner*, Michael Welsh*, Bruce Pratt, Sam Wadsworth and Alan Smith. Genzyme Corporation, 1 Mountain Rd. Framingham MA, 01701 and The Howard Hughes Medical Institute, University of Iowa College of Medicine, Iowa City, Iowa, 52242.

We have developed adenovirus vectors for gene therapy of cystic fibrosis. These adenovirus vectors have been extensively tested in animals and tissue culture models with a dual focus on safety and efficiency of gene transfer. Towards addressing the safety of E1 deleted adenovirus vectors we have assayed for early and late events in the viral life cycle in tissue culture models. In general, we have not detected Virus production, viral DNA synthesis or early and late gene expression at low or high MOI's with the exception of low levels of E2A in HeLa cells at all MOI's.

To address the efficiency of gene transfer, we have employed an adenovirus vector containing the LacZ' gene driven by the CMV early promoter. Cotton rats, hamsters, and primates were administered virus by transtracheal instillation. Expression of ß-galactosidase is readily detected in cotton rats up to eight weeks post instillation, albeit at a significantly reduced level compared to early time points. Similar experiments in hamsters showed continued high level expression to at least 17d. Studies in primates using the LacZ reporter gene or CFTR have detected viral DNA to 96 days post instillation and reporter gene messenger RNA for at least two weeks.

The immune response to single and multiple doses of adenoviral vectors was examined in rodents and primates. Initial exposure resulted in the development of moderate antibody titers to Adenovirus. In all cases antibody titers returned to near pre-immune levels within several weeks. Repeat administrations produced similar responses. Prior adenovirus vector exposure did not significantly inhibit gene transfer as judged by expression of the ß-gal reporter gene. We conclude from these studies that adenovirus represents a viable vector system for gene transfer to lung epithelium. We are continuing our development of the adenovirus system to further insure the safety and efficacy of the vector.

DZ 205 MYOBLAST TRANSFER OF HUMAN ERYTHROPOIETIN GENE YIELDS LONG-TERM HEMATOCRIT INCREASE, Yasuo Hamamori[1], Babru Samal[2], and Larry Kedes[1], Institute for Genetic Medicine[1], University of Southern California School of Medicine, Los Angeles, CA, and Amgen Inc.[2], Thousand Oaks, CA.

We have investigated the ability of myoblast-mediated gene transfer to elicit sufficient recombinant protein production for a physiologically significant and sustained systemic response. We used the production of recombinant human erythropoietin (EPO), a proven therapy for anemia related to diminished red blood cell production, as a gene therapy model. C2 murine myoblast cells were transfected with a neomycin resistance plasmid containing the EPO gene under the control of a cytomegalovirus promoter. G418 resistant cells were selected and screened for EPO secretion by radio immunoassay. EPO secretion levels paralleled EPO function as measured by bioassay on UT-7/EPO cells, an EPO-responsive human leukemic cell line. 4 x 10^7 cells from the single most productive of twelve EPO-secreting clones (each >15 units/10^6 cells/ml/day) was used for injection into hind limb skeletal muscle of syngeneic C3H mice and nude mice. 10^6 cells in 10 μl were injected at each of 40 sites. Micro-hematocrits (Hct) showed dramatic increases after several days (Table). The Hct peaked 2 w after injection in C3H mice but began to decline at 3 w and by 1 Mo. was at a level lower than the initial level. RT-PCR of injected C3H mouse muscle demonstrated persistence of human EPO mRNA at 4 weeks. The Hct rise was sustained in nude mice for at least for 2 Mo. and human EPO was detectable in their serum. C2 myoblast control injections did not change Hct or generate EPO mRNA.

Mean Hematocrits in C3H Mice (A, n=7) *and Nude Mice* (B, n=5)

pre-injection	3 d	1 w	2 w	3 w	4 w	5w	6w	7w
(A) 42.9	45.1	53.5	58.6	50.2	41.8	36.5	33.1	34.4
(B) 43.0	55.8	60.0	67.0	59.1	67.4	57.3	64.0	66.3

To monitor the fate of injected cells, another C2 cell line which stably expresses β-galactosidase (β-gal) gene was established by infecting cells with a ψ2 BAG retrovirus. At 3 Mo. β-gal positive myotubes, but not myoblasts, were detected in the entire area of injected muscle in C3H mice, indicating differentiation and fusion with endogenous myotubes of the injected myoblasts and long-term expression of an integrated exogenous gene.

DZ 206 RIBOZYME AND ANTISENSE DNA REGULATION OF bcr/abl EXPRESSION IN CML PATIENTS, PP Kearney, LA Wright, S Milliken, JC Biggs and SB Wilson, Department of Haematology, St Vincents Hospital, Sydney, Australia.

The etiological role of *bcr/abl* expression in chronic myeloid leukaemia (CML) is well established and such a novel mRNA is a logical target for potentially therapeutic agents such as ribozymes and antisense oligomers.

The exposure of chronic phase patient cells to antisense oligomers specific to the junction region had no effect on clonogenicity, whereas acute phase patient cells were acutely sensitive to such molecules, with up to 90% of colony forming units inhibited. PCR analysis of those colonies which were able to grow in the presence of oligo, indicated these colonies did not express *bcr/abl* mRNA. We are currently examining the effect of these molecules on CML patient xenografts on SCID mice.

In a second strand to our research we have designed a ribozyme to the *bcr/abl* mRNA. In vitro studies have demonstrated this ribozyme was able to cleave upto 90% of a synthetic *bcr/abl* substrate at physiological temperature and ion concentrations. Unfortunately a synthetic *bcr* substrate was also cleaved by this ribozyme reducing its utility. To counter this, a series of 4 further ribozymes were designed with altered arm sequences and these were examined for their ability to cleave both *bcr* and *bcr/abl* substrates. Two of these ribozymes were found to be specific for *bcr/abl.*

DZ 207 CONVERSION OF MYOCARDIAL INFARCT TO SKELETAL MUSCLE BY RETROVIRAL GENE TRANSFER, **Larry Kedes[1], Howard Prentice[1], Vittorio Sartorelli[1], Robert A. Kloner[2]**, [1]Institute for Genetic Medicine, [1,2]University of Southern California School of Medicine and [2]Heart Institute, Hospital of the Good Samaritan, Los Angeles, CA 90033

A non-contracting scar from myocardial infarction can adversely effect ventricular topology and hemodynamic function. We are attempting to modify myocardial scar structure in vivo by gene transfer. We examined (1) whether ischemic myocardium can take up and express injected DNA; (2) whether cardiac fibroblasts convert to skeletal muscle cells by cellular transduction with a retroviral vector harboring the MyoD cDNA; (3) whether transduction with MyoD leads to cellular conversion of fibroblasts to muscle cells in the ischemic canine ventricle.

(1) Luciferase gene under the control of the Rous sarcoma virus promoter was injected directly into the anterior left ventricular wall after 15 minutes or 60 minutes of proximal coronary occlusion or sham operation.. At one week, high expression of luciferase was observed in both the ischemic/reperfused and non-ischemic hearts. Thus not only are foreign genes taken up by direct injection into ischemic/reperfused myocardium but they are transcribed and the protein synthetic machinery of the injured cells produce recombinant, enzymatically active, polypeptides.

(2) LXSN retroviral-transduced neonatal rat cardiac fibroblasts conserve the typical rounded fibroblast morphology. In contrast, the same cells transduced with the MyoD cDNA containing construct, LMDSN, were elongated and frequently contained several nuclei. These morphological changes were consistent with a conversion to the myogenic phenotype because only the LMDSN transduced cells immunostained with antibody recognizing skeletal muscle (non-cardiac) myosin heavy chain (α-MHC).

(3) Myocardial infarcts were created in dogs by injection of helical coils through a percutaneously-introduced intracoronary catheter and verified by angiography. After 1 week the infarction was visualized and the scars of 5 dogs were injected with 300 μl of LNPOZ ± LMDSN at three sites within a marked area of 1 cm^2. During the following week, the scar tissue of 3 out of 4 dogs injected with LNPOZ and LMDSN and one dog injected with LNPOZ alone stained positively for β-galactosidase. Frozen sections revealed that multiple clusters of cells stained with anti α-MHC antibody. These cells co-stained with the muscle marker α-actinin. Whether true skeletal muscle myotubes will form within the scar and contribute to ventricular function remeains to be determined.

DZ 208 EXPRESSION OF DOG FACTOR IX PROTEIN AFTER INTRAMUSCULAR DNA INJECTIONS IN MICE, Ann Y. Kuwahara-Rundell[1], Michal Margalith[1], Gary Rhodes[1], Michelle Yankauckas[1], and VJ Dwarki[2],; [1]Vical Inc., San Diego CA 92121 and [2]Somatix Corp., Alameda CA 94501

Intramuscular (im) injections of plasmid DNA encoding the canine factor IX gene produced measurable serum protein levels in Balb/c mice. The plasmids were retroviral vectors containing the human cytomegalovirus enhancer/promoter with or without mouse muscle creatine kinase enhancer (mck) element [Dai et al. Proc Natl Acad Sci USA. 89(22):10892-5, Nov 15 1992].

Canine factor IX protein could be detected by a standard ELISA in serum 24 hours after injection, peaking at 5-9 days post injection. A multiple injection regimen of 1 injection/day for 5 days increased factor IX expression over a single injection. However, the results were not additive. At 50 ug DNA dose, multiple injections gave peak levels of 1500-2400 pg/ml protein 7 days post injection 1. A single injection of 50 ug DNA gave 600-1100 pg/ml protein on day 7. By day 40, the factor IX levels, regardless of injection regimen, were undetectable .

When the mice were boosted with plasmid DNA on day 69 or day 75, factor IX levels in 8 out of 14 mice remained at zero. By 2 weeks post boost, all animals showed no detectable protein. We believe an immune response to canine factor IX contributed to this "turn off" effect. Antibodies to factor IX first appeared 26 days post injection, peaking at 60 days. These antibodies were neutralizing in an in-vitro canine factor IX ELISA assay.

Nude mice, injected with plasmid, showed constant serum factor IX levels from 19 to 40 days post injection. A decline was first seen at day 68. The immune response occured later in nude mice than in normal Balb/c mice. Antibodies were first detected at day 40 as compared to day 26 for Balb/c.

Thus, intramuscular injection of the FIX gene produced peak steady state serum levels of 1-3 ng/ml of factor IX a week after injection. Serum concentrations declined at later times and were correlated with the appearance of neutralizing antibodies. We conclude im injection of canine factor IX plasmid is a useful model system for DNA based delivery of therapeutic proteins.

DZ 209 DEVELOPMENT OF AN ANIMAL MODEL FOR β-THALASSEMIA AND SICKLE CELL ANEMIA BY TRANSPLANTATION INTO SCID MICE, A. Larochelle[1], T. Lapidot[1], J. Vormoor[1], G. Stamatoyannopoulos[2], N.F. Olivieri[1], J.E. Dick[1], [1]Department of Genetics, Hospital for Sick Children, Toronto, Canada, M5G 1X8, [2]Department of Medical Genetics, University of Washington.

Progress in understanding the regulation and organization of the human hematopoietic system has been hampered, until recently, by the lack of a suitable *in vivo* reconstitution assay for human stem cells. Recently described methods of engraftment of human hematopoietic cells into immune-deficient mice provide the basis for such an assay and constitute a powerful approach to create animal models for diseases such as leukemias, infectious diseases, autoimmunity and hemopathies. We report the establishment of a SCID model for β-thalassemia and sickle cell anemia to test the feasibility of human gene therapy. Ficolled bone marrow cells from 8 thalassemic and 3 sickle patients were delivered into sublethally irradiated SCID animals by IV injection. Mice have been treated with human recombinant growth factors (MGF, PIXY 321 and EPO) for 30 to 60 days. As assessed by morphology and Southern analysis, high levels of engraftment were attained with 7 of 8 (83%) thalassemic individuals and 3 of 3 (100%) sickle children. The bone marrow from 18 of 27 (66%) transplanted thalassemic mice was reconstituted with ≥10% human hematopoietic cells; 5 of 8 (63%) sickle animals showed significant (≥10%) levels of engraftment. Myeloid, erythroid and multipotent human progenitors developed in the mouse bone marrow. The presence of human thalassemic erythroid progenitors was independently confirmed by detecting human hemoglobin F (HbF) in BFU-E lysates after electrophoretic separation on cellulose acetate plates, similar to the red cell progenitors from the original bone marrow donor. Gene expression studies indicated that the BFU-Es were positive for the γ-globin chains as shown by RNase protection assay and RT-PCR. A low number of mature human erythrocytes were also released in the peripheral blood of the transplanted SCID mice as revealed by agglutination with antibodies directed to the human blood group antigens. The establishment of these unique animal models for β-thalassemia and sickle cell anemia will enable the development and testing of high efficiency gene transfer procedures for long term functional correction in mature red blood cells, as a prelude to human gene therapy trials.

DZ 210 RETROVIRAL VECTOR-MEDIATED GENE TRANSFER INTO HUMAN PRIMARY MYOGENIC CELLS LEADS TO EXPRESSION INTO MUSCLE FIBERS IN VIVO.

F. Mavilio[1], G. Ferrari[1] G. Salvatori[2], C. Rossi[1], R. Giavazzi[3], G. Cossu[2].
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Gene transfer into muscle tissue has a number of potentially relevant applications in medicine, including somatic cell gene therapy of muscular dystrophies and systemic delivery of recombinant proteins for correction of genetic or acquired disorders. We have developed an in vivo model for testing the *expression of foreign* genes into human adult muscle fibers after retroviral vector-mediated gene transfer into primary myogenic cells. Satellite cells isolated from adult muscle biopsies were infected by co-culture with a high-titer, MoMLV-derived retroviral vector expressing the β-galactosidase gene under LTR control. Gene transfer efficiency averaged 50% as revealed by β-gal staining. The reporter gene was stably integrated, faithfully inherited, and expressed at significant levels in myogenic cells for at least 10 generations under clonal growth conditions, and throughout the culture life span upon differentiation into myotubes. Comparable gene transfer efficiency was obtained in myogenic cells from muscle biopsies of patients affected by a number of genetic or acquired myopathies, including Duchenne muscular dystrophy. Transduced normal human satellite cells were injected into regenerating muscle of immunodeficient mice, where they formed new muscle fibers in which expression of the reporter gene was detectable for at least 2 months after injection. These results show that retroviral vectors can be used to transfer foreign genes with high efficiency into normal or abnormal primary human myogenic cells, leading to stable expression into mature muscle. Satellite cells engineered in this way might represent an effective tool for gene therapy of muscular dystrophies as well as for systemic delivery of recombinant gene products for correction of inherited and acquired disorders. The human-mouse model described here will allow direct in vivo testing of the function of genetically modified muscle fibers, and therefore pre-clinical evaluation of a number of gene therapy strategies based on muscle tissue.

DZ 211 LIVER DIRECTED GENE THERAPY: OPTIMIZING EXPRESSION OF SERUM PROTEIN GENES AFTER *IN VIVO* RETROVIRAL TRANSDUCTION, K.P. Ponder, D.G. Hafenrichter, S.D. Rettinger, S.C. Kennedy, and M.W. Flye, Departments of Surgery, Internal Medicine, and Biochemistry, Washington University School of Medicine, St. Louis, MO, 63110

Liver directed gene therapy could dramatically alter the treatment of many inherited hematologic and metabolic diseases. We have developed a rapid, reliable low mortality method of *in vivo* hepatocyte transduction in which we deliver an intraportal bolus of RV during a 3 minute period of hepatic in-flow occlusion. Using an amphotropic Mo-MLV based RV containing the human α_1-antitrypsin (hAAT) reporter gene, under the control of the promoter for the large subunit of RNA polymerase II (Pol II), we have found that up to 15% of hepatocytes integrate the provirus *in vivo*, and stable hAAT serum protein levels were sustained for at least 12 months (mean serum hAAT level = 148 ng/ml). Serum hAAT levels detected by ELISA were consistent with hAAT mRNA levels, proviral DNA incorporation, and were proportional to the number of RV particles injected. Without 70% hepatectomy, rats receiving intraportal RV failed to express serum hAAT. Despite high transduction efficiencies, expression for the RV *in vivo* has been disappointingly low. A logical way to increase expression in the hepatocytes is to use strong, liver-specific promoters. We evaluated hAAT expression under the control of each of 4 liver specific promoters; murine Albumin (mALBp), hAAT promoter (hAATp), Phosphoenolpyruvate Carboxykinase (PEPCKp), and Liver Fatty Acid Binding Protein (LFABPp). Although the *in vitro* titers were significantly lower for the mALBp and hAATp containing RV's compared to the Pol II containing RV, the mean serum hAAT levels were 94 ng/mL and 43 ng/mL respectively, similar to the Pol II containing RV. After normalization for the approximate number of RV particles injected, the mALBp and hAATp driven hAAT gene expression was 74 and 29 fold higher than the Pol II driven RV. The *in vivo* hAAT expression under the control of the PEPCKp and LFABPp was negligible. We are currently quantitating the amount of proviral DNA in the liver to confirm the transduction frequency of the liver specific promoters. We conclude that liver specific promoters do indeed lead to higher levels of gene expression than the cellular Pol II promoter. We are currently obtaining high titer RV's and developing promoter element RV constructions to further maximize our *in vivo* gene expression.

DZ 212 EXPRESSION OF HUMAN CLASS II MHC GENES IN MURINE NEUROBLASTOMA CELLS ABROGATES TUMORIGENICITY, INDUCES IMMUNITY TO UNTRANSDUCED NEUROBLASTOMA, AND FACILITATES THE REJECTION OF SMALL ESTABLISHED TUMORS

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Recombinant retroviruses were used to infect and direct the expression of class II MHC molecules in a highly malignant murine neuroblastoma cell line, Neuro-2a. The expression of xenogeneic human MHC Dw14 genes in Neuro-2a cells completely abrogated tumorigenicity in immunocompetent syngeneic A/J mice; however, Neuro-2a Dw14$^+$ cells retained tumorigenicity in immune-deficient nude mice. Notably, mice which rejected Neuro-2a Dw14$^+$ cells were rendered immune to subsequent tumor challenge with unmodified Neuro-2a (36 of 39). Tumor immunity developed quickly in that simultaneous administration of 10^6 Dw14$^+$ and 10^6 unmodified Neuro-2a prevented tumor development and resulted in long-term survival (26 of 30), while inoculation of unmodified Neuro-2a alone caused rapid tumor development and death (40 of 46). Additionally, small established Neuro-2a tumors undergo regression and are ultimately eliminated when mice are immunized with Neuro-2a Dw14$^+$ cells (22 of 24). Studies designed to address the mechanism of tumor rejection revealed that splenocytes obtained from mice immunized with Neuro-2a Dw14$^+$, but not from saline injected control mice, conferred tumor immunity to naive recipient mice (9 of 10). Furthermore, splenocytes isolated from immune mice, but not saline injected control mice, were capable of lysing both Dw14$^+$ and unmodified Neuro-2a in cytotoxicity assays <u>in vitro</u>. These data indicate that tumor rejection is a cell-mediated phenomenon. These experiments show that retroviral-mediated transduction of human class II MHC genes into a malignant murine neuroblastoma is an effective means of altering its tumorigenicity and that immunization with class II-modified tumor cells may have the potential to cure small residual tumors. Finally, preliminary experiments utilizing the transduction of syngeneic (I-A^k) class II MHC genes into Neuro-2a cells indicate that syngeneic class II molecules are not as effective at promoting tumor rejection and immunity as are xenogeneic class II molecules.

DZ 213 DEVELOPING A MICROENVIRONMENT FOR THE DIFFERENTIATION OF PRIMITIVE CD34+ HEMATOPOIETIC CELLS IN HU-BM-SCID MICE

David W. Ritchey#, Eric R. Hall#, Sumesh Kaushal#, Vincent LaRussa+, Steven Kessler^, Suzanne Gartner#, Daniel C. St. Louis#, Douglas Mayers^, L. Para Perera §, Zhipeng Yu#, Jerome H. Kim+, Karl Sitz+, XU J.#, Donald S. Burke+ and Joseph D. Mosca#. #Jackson Foundation, + WRAIR, Rockville, MD; §NIH, ^NMRI, Bethesda, MD

In this study, we sought to determine *in vivo* models which will ultimately be used to produce and maintain target cells for retroviral transfer of anti-HIV genetic material into primitive CD34+ bone marrow derived progenitor cells. Our objectives were to establish a microenvironment that would provide a substratum for primitive human CD34+ hematopoietic cells to adhere and at the same time be nurtured by the production of human cytokines within the SCID mouse model. For this purpose we injected 5 x 10^7 Lof11-10 cells (a SV40 T-antigen immortalized human stromal cell line, whose condition media will support CD34+ differentiation *in vitro*) IP into recipient SCID mice. All mice were gamma irradiated with either 200 or 400 rads, with the idea that irradiation would provide space within the mice for the cells to repopulate. In our initial experiments, mice were irradiated with 400rads. Mice (n=2) injected with media without Lof cells died at 7 days, whereas mice (n=5) receiving Lof cells lived to 50 days post-irradiation, suggesting that Lof cells reconstituted the mice and transiently protected the mice from lethal irradiation. Subsequent experiments at 200rads demonstrated similar methycellulose colony numbers (30 to 50) in non-irradiated mice and irradiated mice receiving Lof cells, compared to few to no colonies (0 to 3) in irradiated mice not receiving Lof cells. Gross morphologic differences were seen in the spleens of irradiated mice in the presence and absence of Lof cells. Spleen and bone marrow cells are presently being analyzed for the presence of Lof cells by DNA PCR and HLA tissue typing, as well as the serum for human cytokines by ELISA. Once characterized, the Lof reconstituted mice will be tested for their ability to support human CD34+ bone marrow derived progenitor cells *in vivo*, compared to non- reconstituted SCID mice.

DZ 214 CHRONIC THROMBOCYTOPENIA IN TRANSGENIC MICE EXPRESSING MEGAKARYOCYTE TARGETED SV40 T ANTIGEN

Murray O. Robinson, Wen Zhou, Martha Hokom, Dimitry Danelinko, Pamela Hunt and Robert Bosselman. Amgen, Inc., Thousand Oaks, California 91320.

Thrombopoiesis is a highly regulated process functioning to maintain platelet homeostasis. To perturb this process *in vivo*, we generated transgenic mice expressing the tsA58 mutant of the SV40 T antigen via the megakaryocyte-specific Platelet Factor 4 promoter. Ten of the seventeen transgenic lines established exhibited chronic thrombocytopenia evident from birth, each line displaying a distinct and heritable platelet level. The platelet deficiency appeared to correlate with transgene expression, as homozygotes exhibited a more severe platelet reduction than hemizygotes within any given line. Megakaryocytes from the thrombocytopenic lines exhibited morphological abnormalities, similar to those observed in experimentally induced models of thrombocytopenia. In affected mice, the megakaryocyte numbers were inversely correlated with the platelet level, and at least one affected line showed an increase in mean megakaryocyte ploidy.

The role of Retinoblastoma protein (Rb) in the observed phenotype was investigated by mating the transgenic mice to mice heterozygous for the Rb knockout. Transgenic mice with an inactivated copy of the Rb gene had even lower platelet counts than those with both copies of the Rb gene.

In older mice (>7months) megakaryocytic neoplasia, characterized by striking splenomegaly and neoplastic infiltration into many tissues, was often observed in lines with both normal and reduced platelet counts. Infrequently, other tumors of unknown cell type have been seen, suggesting the possibility of transgene expression in other tissues.

These findings indicate that T antigen is interfering with terminal differentiation of the megakaryocyte lineage, and that T antigen target proteins such as the Retinoblastoma (Rb) gene product may be important for normal differentiation of megakaryocytes. Investigations into the role of Rb and p53 in megakaryocyte maturation and attempts to establish megakaryocyte cell lines are currently underway.

DZ 215 EFFECTS OF AUTOCRINE AND PARACRINE SECRETION OF CYTOKINES ON INDUCTION OF ANTITUMOR IMMUNITY,

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Cytokine gene transfer into tumor cells has been shown to lead to primary rejection of transfected tumor cells in syngeneic hosts as well as to induction of a systemic cellular antitumor immune response in several experimental systems. While it would be preferable for practical reasons to use a standardized cellular source of cytokine secretion, e.g. by cytokine gene transfected fibroblasts, and to avoid *in vitro* culture and transfection of primary tumor cells, efficacy in the paracrine situation is still controversial. We have previously shown that the simultaneous secretion of IL-2 and IFN-γ by retrovirally transduced tumor cells can lead to increased effects on the *in vivo* induction of antitumor immunity as compared to the secretion of only one cytokine. We have now investigated the effects of autocrine and paracrine secretion of cytokines on tumor rejection and specific immunity in more detail. Murine CMS-5 fibrosarcoma cells and autologous or allogeneic murine fibroblasts were transfected by retroviral infection, electroporation or lipofection with IL-2 or IFN-γ cDNA. BALB/c mice were injected with different mixtures of irradiated and non-irradiated IL-2, IFN-γ or IL-2/IFN-γ double transduced CMS-5 cells, as well as with mixtures of parental CMS-5 cells and IL-2 secreting autologous or allogeneic fibroblasts. Preliminary data suggest that paracrine effects of cytokines can be demonstrated in this setting, however, higher cytokine concentrations seem to be necessary when compared to autocrine secretion.

DZ 216 INHIBITION OF CHRONIC MYELOID LEUKEMIA CELL GROWTH BY CIRCULAR ANTISENSE OLIGONUCLEOTIDES,

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Gene therapy efforts to date have focused on the correction of a deficient activity through the transfer of a normal gene. An analogous approach is the antagonism of a deleterious activity through the transfer of an antisense sequence. Leukemias due to chromosomal translocations represent attractive candidates for antisense attack. The antiproliferative activity of conventional linear oligodeoxyribonucleotides is transient because their phosphodiester internucleotide linkages are subject to cleavage by exonucleases in serum and within cells. We have investigated circular oligodeoxyribonucleotides as antisense agents, using as a model chronic myeloid leukemia cells. K562 is a chronic myeloid leukemia cell line in which *bcr* exon 3 is joined to *abl* exon 2. A circular 34-mer targeting a 12-nucleotide polypurine sequence in *bcr* mRNA located 385 nucleotides 5' to the *bcr-abl* junction inhibited K562 cell growth by day 6 with an $I.C._{50}$ of 7 μM. A circle with the same nucleotide composition but with a random sequence had no effect; therefore this effect was sequence-specific. This circular antisense oligonucleotide was more active than a linear 12mer targeting the same 12-nucleotide site; the latter had no effect at 13 μM. The other common mutation found in chronic myeloid leukemia joins *bcr* exon 2 to *abl* exon 2 and is found in the BV173 cell line. A circular 34-mer targeting the purine-rich 12-nucleotide sequence at this *bcr-abl* breakpoint inhibited BV173 cell growth; the $I.C._{50}$ was 9 μM. A concentration of 32 μM arrested growth completely. Thus circular oligodeoxyribonucleotides (1) inhibit proliferation of CML cells, (2) have sequence-specific activity, and (3) are more active than linear oligonucleotides containing only the base-pairing region, presumably because of resistance to exonucleases.

DZ 217 SPECIFIC CELLULAR AND HUMORAL IMMUNITY IS INDUCED BY INTRAVENOUS INJECTION OF PLASMID DNA CONTAINING INFLUENZA A NUCLEOPROTEIN GENE,

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Intramuscular (i.m.) injections of the influenza A virus nucleoprotein (NP) naked plasmid DNA into mice induces NP-specific cytolytic T cells (CTL) and antibodies (Ab) (Science 259, 1745-1749). Since a DNA solution injected into muscle is expected to mix with blood, we investigated whether a direct intravenous injection of naked NP plasmid could induce NP-specific immune responses.

Four mice each received a single tail vein injection of 200µg of NP plasmid in 100 µl of saline. Control groups of four animals were each injected with either 200 ug of NP plasmid i.m. or with 100 ul of saline i.v. Mice were assayed for anti-NP Ab in a standard ELISA three weeks post injection and sacrificed one week later. Anti-NP mixed lymphocyte cultures were prepared and assayed for cytolytic activity. Animals that received NP plasmid via the i.v. or i.m. route, but not the saline injected mice, produced anti-NP Ab. Similarly, three out of four i.v. injected mice and all i.m. injected mice displayed high levels of the NP-specific CTL activity. Ab titers and levels of cytolytic activity were similar for both routes of injection. Thus, NP-specific Ab and CTL responses can be generated in mice by a single i.v. injection of naked NP plasmid DNA.

Human Diseases I; Gene Therapy: Somatic Tissue II

DZ 300 ADOPTIVE TRANSFER OF HUMAN LONG TERM MARROW CULTURE CELLS IN IMMUNE DEFICIENT MICE: A MODEL SYSTEM FOR GENE TRANSFER INTO HAEMOPOIETIC PROGENITORS, Julia Ackland-Snow, Ian D. Dubé, and Suzanne Kamel-Reid, Department of Molecular and Cellular Pathology, University of Toronto, and the University of Toront Hospitals' Cancer Cytogenetics and Molecular Oncology Program, 100 College St., Toronto, Canada, M5G 1L5.
We developed a novel method for retroviral transduction of haemopoietic progenitor cells in long term marrow cultures (LTMC). In a canine model system, we demonstrated the presence of LTMC-derived transduced haemopoietic progenitor cells *in vivo* for up to two years post-infusion into non-marrow ablated autologous recipients. Our data supported the concept that LTMC provide an *in vitro* environment in which haemopoietic progenitors proliferate, differentiate and possibly self-renew. Our results further demonstrated that upon infusion into non-marrow ablated recipients LTMC-derived canine progenitor cells continued to proliferate, differentiate, and contribute to haemopoiesis *in vivo*. We now wish to determine if human LTMC-derived haemopoietic progenitor cells can be similarly stimulated *in vitro* and contribute to long term haemopoiesis *in vivo*. LTMC were established from seven normal human bone marrow samples and maintained by weekly demi-depopulation and media replenishment. After 21 days cultured cells were injected into homozygous *scid/scid* and *scid/scid;beige/beige* immune-deficient mice. Mice were sacrificed at 4, 12 and 20 weeks post-injection and tissues analysed by Southern for the presence of human DNA. Analysis of data indicate that human bone marrow cells maintained in LTMC for three weeks can proliferate in *Scid* mice in the absence of exogenously added growth factors. The primary tissues exhibiting engraftment were the lung, spleen, bone marrow, and lymph nodes of recipient mice. Similar proliferation was not observed when uncultured marrow cells were infused into murine recipients. Levels of human cell engraftment observed in these preliminary studies range from 0.1-1.0%. We are currently manipulating LTMC conditions to increase the engraftment potential of cultured haemopoietic progenitor cells. Assessment of the long term reconstitutive ability of these human LTMC-derived cells and their potential to contribute to multiple haemopoietic lineages is in progress using immunohistochemistry, Southern ananysis and flow cytometry. Data from these experiments will help to determine if human LTMC cells are good targets for gene transfer and may further support the hypothesis that LTMC favour the activation of quiescent human haemopoietic progenitor cell populations.

DZ 301 RETROVIRAL TRANSDUCTION AND PERSISTENCE OF THE HUMAN ALKYLTRANSFERASE GENE INTO HEMATOPOIETIC CELLS, James A. Allay, Luba L. Dumenco and Stanton L. Gerson, Depts of Medicine and Biology and the Cancer Center, Case Western Reserve University, Cleveland, OH 44106 and the Dept of Pathology and Laboratory of Medicine, Brown University, Providence, RI 02912.
Myelosuppression is the dose-limiting toxicity for nitrosoureas and related chemotherapy. We have shown that low levels of the O^{6}alkylguanine DNA alkyltransferase repair enzyme in myeloid cells is responsible for this observed sensitivity. This enzyme repairs the cytotoxic and mutagenic O^{6}alkylguanine adduct in a suicide reaction. To improve hematopoietic cell resistance to alkylating agents, we used gene transfer of the alkyltransferase gene. A retroviral vector, pM5neoMGMT, was constructed that contains the human alkyltransferase gene, MGMT, cloned into the BamHI restriction site of the myeloproliferative sarcoma virus (MPSV) based vector, pM5neo. MPSV has been shown to be active in hematopoietic progeny. 5-FU pretreated mouse bone marrow was transduced with vM5neoMGMT and transplanted into lethally irradiated recipients. Efficient transduction into hematopoietic progenitors was detected by PCR in 28/32 CFU-S from 11 recipient mice. The provirus was persistently detected in bone marrow, spleen, thymus and blood of animals sacrificed between 12 and 28 weeks post transplant. CFU-GM from the bone marrow also contained provirus. The presence of provirus in 14/18 CFU-S from secondary bone marrow transplant recipients indicated that early hematopoietic precursors had been transduced. The bone marrow, spleen and thymus from secondary transplants also showed presence of integrated provirus between 8 and 30 weeks post transplant. Expression of the transduced MGMT gene could be detected in bone marrow and spleen by reverse transcription PCR (RT-PCR). Our data indicate that retroviral transduction of the human MGMT gene into hematopoietic cells using an MPSV vector leads to long term persistence and expression of MGMT. Increased alkyltransferase activity in hematopoietic cells may reduce myelosuppression caused by nitrosourea chemotherapy and lead to clinical trials using MGMT to protect the bone marrow.

DZ 302 MUTATION ANALYSIS IN HAEMOPHILIA A, Bidichandani S.I., Lanyon W.G., Shiach C., Lowe G.D.O., Connor J.M. Department of Medical Genetics, University of Glasgow, Glasgow G3 8SJ, U.K.
We have used a combination of PCR-based mutation detection methods to detect disease producing mutations in the factor VIII gene of 15 unrelated haemophilia A patients. At least one sequence variant was localized in every patient, on the basis of a distinct positive result with a mutation screening method. We have so far identified the causative mutation in 9 of the 15 patients in our series, along with a silent mutation in one of the patients. The mutations detected included 7 point mutations (R-5STOP, V162M, G701D, R1869I, F1880F & 2 identical A→G at position +3 in intron 6), 2 deletions (5 bp in exon 4 and the entire exon 16) and 1 mRNA abnormality involving intron 22 (Naylor et al. 1993 Hum. Mol. Genet. 12, 11-17). Results of the genotype-phenotype correlations are presented. The nonsense mutation at position 15 in the FVIII leader peptide (-5) and the out of frame 5bp deletion will clearly result in a markedly truncated FVIII protein and have both resulted in clinically severe haemophilia A. The in-frame deletion of exon 16 has also resulted in a severe phenotype. This is probably due to the resulting exclusion of the highly conserved Cys1832 residue, thought to be important for disulphide bonding and accurate protein folding. The intron 6 splice donor mutation which was seen in two of our patients was shown to result in the skipping of exons 5 and 6 by RT-PCR of the FVIII ectopic transcripts from peripheral lymphocytes. This splicing of exons 4 and 7 results in an in-frame shortening of the FVIII mRNA and the predicted shortening of the conserved "A1" domain of the FVIII protein has caused disease of moderate severity. The missense mutations were analysed using the ProSearch 2.0 sequence analysis program. The V162M mutation was seen in a mildly affected patient and was shown to involve a caesin kinase II phosphorylation site. The G701D mutation which resulted in a severe phenotype involves the consensus sequence pattern of multicopper oxidases, highly homologous to ceruloplasmin with which the FVIII protein shares its characteristic domain structure.

DZ 303 DEVELOPMENT AND ANALYSIS OF RETROVIRAL VECTORS EXPRESSING HUMAN FACTOR VIII AND FACTOR IX AS A POTENTIAL GENE THERAPY FOR HEMOPHILIA. Marinee Chuah, Thierry VandenDriessche, Kurt Newman, Craig Mullen*, and Richard Morgan. National Heart Lung and Blood Institute, and *National Cancer Institute, National Institutes of Health, Bethesda, MD.

In consideration of a gene therapy approach for the treatment of hemophilia it will be important to optimize gene transfer and expression. Of the gene transfer systems currently in clinical trials, only retroviral vector-based gene transfer results in stable chromosomal integration and thus offers the potential for long-term gene expression. We constructed several retroviral vectors designed to produce the two major clotting factors associated with hemophilia, factor VIII and factor IX. It has been suggested that the factor VIII mRNA is unstable and, when inserted into a retroviral vector, makes the vector mRNA similarly unstable. To circumvent this problem, two approaches are being pursued. First, we will construct retroviral vectors in which the factor VIII gene is in the opposite transcriptional orientation to the vector LTR (this construction requires the mutation of a polyadenylation signal found in the opposite strand of factor VIII). This orientation may lessen the negative effect of factor VIII sequences on the stability of vector RNA. Second, the domain responsible for mRNA instability has been identified and site-directed mutagenesis will be employed in an attempt to change the structure of the resultant RNA (without changing the protein coding potential). In an attempt to optimize factor IX gene expression in various cell types (including lymphocytes and bone marrow stem cells), we have analyzed the ability of various murine leukemia virus enhancer/promoter elements to promote gene expression in specific cell lines. These studies revealed that the enhancer/promoter elements from the SL3-3 and MPSV viruses may be more efficient promoters in lymphocytes than the standard Moloney murine leukemia virus promoter. Based on these results we are currently constructing new factor IX vectors using lymphocyte-specific promoter elements. In addition to the emphasis on lymphocyte/bone marrow delivery and expression of factor IX, we have begun to investigate alternative gene delivery systems, including selectively permeable membrane devices and direct in vivo gene transfer.

DZ 304 IMMUNIZATION WITH CYTOKINE-SECRETING ALLOGENEIC MOUSE FIBROBLASTS EXPRESSING MELANOMA ASSOCIATED ANTIGENS PROLONGS THE SURVIVAL OF MICE WITH MELANOMA, Edward P. Cohen and Tae Sung Kim. Department of Microbiology, University of Illinois at Chicago, Chicago, IL 60612

Immunization of C57BL/6 mice (H-2^b) with a mouse fibroblast cell-line of C3H origin (H-2^k) genetically modified for IL-2-secretion and the expression of melanoma associated antigens (MAA) resulted in a specific, generalized anti melanoma response that was capable of prolonging the survival of mice with established melanoma. Both Lyt-2.2+ (CD8+) cells and NK/LAK cells with anti melanoma cytotoxicity were responsible. The anti melanoma immunity was insufficient to fully eradicate all the neoplastic cells, however, and tumor growth eventually recurred. Cells from the recurrent neoplasms formed melanin were histologically indistinguishable from melanoma cells in C57BL/6 mice injected with B16 cells alone. Cells from the recurrent melanomas were resistant to additional rounds of immunotherapy, as indicated both by cytotoxicity assays performed *in vitro* and by the lesser periods of survival of mice with established recurrent melanomas treated with the IL-2-secreting cell construct. The resistant melanoma cells were MHC Class I-deficient. The potential for combination immunotherapy was indicated by the survival of mice with melanoma immunized with allogeneic fibroblasts modified to secrete both IL-2 and interferon-gamma. It was significantly longer than that of mice immunized with cells modified for secretion of either cytokine alone.

DZ 305 GENE THERAPY FOR PHENYLKETONURIA (PKU): PHENOTYPIC CORRECTION IN A MOUSE MODEL BY ADENOVIRUS-MEDIATED HEPATIC GENE TRANSFER .

((B. Fang[1], R. C. Eisensmith[2], X. H. C. Li[1], A. Shedlovsky[3], W. Dove[3] and S. C. L. Woo[1,2,4].)) [1]Howard Hughes Medical Institute, [2]Department of Cell Biology and [4]Institute of Molecular Genetics, Baylor College of Medicine, Houston, Texas and [3]Department of Oncology, University of Wisconsin Medical School, Madison, Wisconsin.

Classical phenylketonuria (PKU) predisposes affected individuals to severe mental retardation and is caused by a deficiency of hepatic phenylalanine hydroxylase (PAH). The initiation of a low-phenylalanine (PHE) diet early in life can reduce or prevent the mental retardation characteristic of this disease. However, the success of this therapy can be limited by poor compliance, especially in older individuals. We have therefore examined the potential application of somatic gene therapy for the phenotypic correction of PKU. A recombinant adenoviral vector containing the human PAH cDNA (Adv/RSV-hPAH) was constructed and administered to PAH-deficient mice (strain Pah^{enu2}). These animals exhibit a biochemical phenotype similar to human PKU patients, with extremely high serum PHE levels and hepatic PAH activities less than 1% of that in wild-type animals. Both hepatic PAH activity and serum PHE levels in these animals were completely normalized five days after treatment, but remained unchanged following infusions of a similar number of particles of a control adenovirus. Although this therapeutic effect did not persist, these studies conclusively demonstrated that only 10%-20% of normal enzymatic activity in the mouse liver is sufficient to restore normal plasma phenyalanine levles. These results suggest that PKU and other metabolic disorders secondary to hepatic deficiencies can be corrected in the future by gene therapy with long-lasting vectors.

DZ 306 DEVELOPMENT OF A COMPETITIVE RT-PCR ASSAY FOR THE QUANTITATIVE DETECTION OF gp91 GENE EXPRESSION IN PATIENTS WITH CHRONIC GRANULOMATOUS DISEASE

Mauro Giacca[1], Lorena Zentilin[1,2], Gabriele Grassi[1], Sabrina Tafuro[1], Alessandro Ventura[2], and Arturo Falaschi[1]; [1]International Centre for Genetic Engineering and Biotechnology,Trieste (Italy); [2]Children Hospital *Burlo Garofolo*, Trieste (Italy)

We are interested in the development of a gene therapy protocol for the X-linked form of chronic granulomatous disease (CGD), an inherited disorder resulting from the inability of phagocytic cells to produce superoxide anion (O_2^-) due to the absence or to the non functionality of the gp91 protein, a component of cytochrome b^{245}.

We have studied the genetic defect in four Italian patients with the disease, and established lymphoblastoid cell lines by EBV infection, which were used for Southern and Northern hybridization analysis. No gross abnormalities could be detected in the DNA of the four patients; the amount of gp91 mRNA was present in normal levels in three patients, and was undetectable in the fourth one.

We have developed a competitive RT-PCR assay to precisely quantify the amount of gp91 mRNA. This assay is based on the addition to the RNA sample of a competitor RNA fragment having exactly the same sequence as the target transcript, except for the addition of 20 nt in the middle. By this method, all variables which affect reverse transcription and amplification of the target RNA have the same effect also on the competitor. The absolute amount of RNA molecules in the sample is determined by the ratio between the two amplification products. The competitor RNA was obtained by a recombinant PCR technique followed by in vitro transcription [Diviacco et al., 1992, Gene 122, 313-320] [Menzo et al., 1992, J. Clin. Microbiol. 30, 1752-1757].

This strategy has allowed:

- to detect the presence of low levels of transcription (about 100 times less than the normal ones) even in the patient which scored negative in Northern blotting;
- to directly measure the amount of gp91 mRNA in the peripheral blood granulocytes of the patients. This approach indicated that the physiological level of transcription is about 10^4 times less than the one of the ß-actin mRNA; the amounts of transcripts detected in the granulocytes precisely reflect the ones found in immortalized B-cell lines;
- to screen for the efficiency of gp91 mRNA expression in the packaging cell line used for the production of an amphotropic retrovirus; this retrovirus was constructed by the insertion of the coding region of the mRNA in the pBabeHyg vector;
- to monitor the expression of the gp91 mRNA in retrovirus-infected B-lymphoblastoid cell lines from the patients.

DZ 307 TUMOR SUPPRESSOR GENE THERAPY OF CANCER: ADENOVIRAL MEDIATED GENE TRANSFER OF THE p53 cDNA INTO HUMAN TUMOR CELL LINES, Richard J. Gregory, Daniel C. Maneval, Suganto Sutjipto, Shu Fen Wen, Matthew P. Harris, Karen Nared-Hood, Patricia Menzel, Whei Mei Huang, Mei-Ting Vaillancourt, Wendy Hancock, Mia Moulton, Margarita Nodelman and Kenneth N. Wills, Canji Inc, 3030 Science Park Rd, San Diego, CA, 92121.

Mutation or loss of the p53 tumor suppressor gene is the most frequently detected genetic alteration associated with human malignancies. Introduction of wild-type p53 into p53 altered human tumor cells suppresses their tumorigenic properties and in some cases induces apoptosis. In principle p53 gene therapy would seem to be a viable means of treating many cancers, and we have therefore constructed a series of adenovirus vectors which direct expression of the human wild-type p53. These vectors are deleted for the adenoviral E1a *and E1b genes* required for viral replication and have substituted in their place expression cassettes in which p53 expression is driven from the adenovirus major late promoter or the CMV promoter. Infection of $p53^{null}$ or $p53^{mut}$ tumor cell lines with these viruses or appropriate controls indicates that they can express p53, suppress DNA replication, inhibit cell growth and induce apoptosis in certain cell lines. Other human tumor cells appear to be refractory to these effects. Interestingly, preliminary evidence suggests that those cells which do not respond to the p53 adenoviruses may not be efficiently infected by Ad5 based vectors. These results suggest that adenovirus mediated p53 gene transfer may be an effective treatment for certain cancers, a hypothesis we are currently testing in animal models.

DZ 308 A TUMOUR-SPECIFIC PRODRUG ACTIVATION STRATEGY FOR BREAST AND PANCREATIC CANCER,

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Most current treatments available for metastatic malignant disease of the breast and pancreas are ineffective. One of the most promising of the selective genetic strategies against cancer is VDEPT (virally directed enzyme prodrug therapy). This uses a viral vector to carry a prodrug activating enzyme gene into both tumour and normal cells. By linking the enzyme gene downstream of tumour-specific transcription units, tumour-specific prodrug activation is achieved. Overexpression of the oncogene, c-*erb*B-2, occurs in many breast, pancreatic and gastric tumours (often due to transcriptional upregulation of a single copy c-*erb*B-2). We have determined that sequences within the first 500bp of the 5' flanking region of this oncogene drive specific transcription within these tumour cell types. We are developing a genetic therapy strategy using VDEPT against cancers of the breast and pancreas. We have constructed a chimeric minigene consisting of the c-*erb*B-2 promoter linked to cytosine deaminase (converts the prodrug 5-fluorocytosine into the cytotoxic 5-fluorouracil, a drug commonly used in the cancer clinic). We have made viral particles containing the chimeric gene and have used these to transduce a panel of c-*erb*B-2 positive and negative pancreatic and breast cell lines. Significant cell death was observed in 5-FC treated *erb*B-2 positive transduced cell lines. When we assayed for cytosine deaminase activity, we found that it was low in c-*erb*B-2 negative transduced cell lines but was increased at least 10 fold in *erb*B-2 positive transduced cells. This tumour cell-specific cytotoxic system is currently being developed for use in a clinical protocol that could be used against tumours that overexpress c-*erb*B-2.

DZ 309 Local, long-term delivery of recombinant secretory form of TGF-b1 to the myocardium by using somatic gene transfer,

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Intracardiac grafting of genetically modified skeletal myoblasts could provide a useful approach for the local, long-term delivery of recombinant molecules to the heart. We have shown previously that C2C12 myoblasts form stable, differentiated grafts when introduced into the ventricular myocardium (J.Clin.Invest. September 1993, in press). In this study, C2C12 myoblasts were stably transfected with a recombinant gene comprised of the metallothionein promoter and a cDNA encoding the secretory form of porcine TGF-ß1. Transgene expression in the resulting cell lines was induced by the addition of $ZnSO_4$ to a final concentration of 100 uM in the culture media. TGF-ß1 expression was then monitored by Northern blot analyses, as well as by the ability of conditioned medium to induce mitosis in CCL-64 cells. The cell line exhibiting the highest levels of transgene expression was used to produce intra-cardiac grafts in the left ventricular myocardium of syngeneic adult C3Heb/FeJ mice. Graft-bearing animals were maintained on water containing 50 mM ZnSO4 for a period of 2 weeks. TGF-ß1 expression was readily detected by immunohistologic analyses in the grafted animals; no signal was evident in control grafts from non-transfected C2C12 cells. The myocardium bordering TGF-ß1 expressing grafts was highly vascularized, consistent with the angiogenic properties of the peptide. We conclude that intraventricular grafting of genetically modified skeletal myoblasts is a novel method for local delivery of recombinant protein to the myocardium without the potential complications associated with systemic delivery.

DZ 310 GENE THERAPY FOR NEONATES WITH ADA-DEFICIENT SCID BY RETROVIRAL-MEDIATED TRANSFER OF THE HUMAN ADA cDNA INTO UMBILICAL CORD CD34+ CELLS.

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Umbilical cord blood has been shown to contain sufficient hematopoietic stem cells for allogeneic BMT and to contain progenitor cells susceptible to retroviral-mediated gene transduction. Three neonates were identified *in utero* as having ADA deficiency, because of prior siblings with that form of SCID. Umbilical cord blood was collected at birth and processed to isolate CD34+ cells, using the Ceprate SC Concentrator. The CD34+ cells were cultured for three days in medium containing IL-3, IL-6 (Sandoz) and SCF (Amgen) with daily additions of LASN retroviral vector-containing supernatants. The cells were then washed and infused I.V. into each child. The transduced cells contained 12-20% G418-resistant CFU-GM. The cell infusions were tolerated without adverse effects. All three infants have also been treated with twice weekly injections of PEG-ADA, which has resulted in correction of metabolic abnormalities in red blood cells. Each child has shown marked increases in T lymphocyte numbers with development of normal PHA responses. The infants were discharged to home between 6-8 weeks after birth. PCR evaluations of peripheral blood leukocyte DNA from the first patient have been negative over the first ten weeks after infusion. The second patient, who received a log more CD34+ cells than the first, has shown approximately 1/1000 vector positive cells at 10 and 17 days after transplant, none detectable for the next six weeks, and again 1/1000 DNA-positive cells at day 72. These and other studies in progress will determine the efficacy of the gene transfer protocol to transduce umbilical cord blood stem cells and to express the ADA cDNA in mature lymphoid progeny cells.

DZ 311 IN VIVO COMBINATION GENE THERAPY WITH THE HERPES SIMPLEX THYMIDINE KINASE (HS-tk) AND INTERLEUKIN-2 (IL-2) GENES FOR THE TREATMENT OF OVARIAN CANCER.

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Relapsed ovarian cancer patients with disease confined to the peritoneal cavity is a common and difficult clinical problem. Retroviral gene therapy offers a novel approach in the treatment of this localized disease. The direct injection of retroviral vector producer cells (VPC) into growing tumors results in the efficient transduction of tumor cells in vivo. This method of delivery has been shown to have the selective ability to transduce the tumor in preference to normal surrounding tissues which are not actively dividing. We have explored gene therapy with the HS-tk and IL-2 genes to combine direct tumor cell killing with enhancement of the immune response. We tested this hypothesis in an human xenograft animal model of ovarian cancer localized in the peritoneal cavity. OVCAR-3 cells, a human ovarian cancer cell line, were introduced into the peritoneal cavity of athymic nude mice and subsequently treated with HS-tk VPC, IL-2 VPC or both 7-14 days later. One week later the animals recieved ganciclovir IP and followed for tumor growth. The animals in the HS-tk VPC group showed tumors that were significantly smaller than controls treated with LacZ VPC alone. The IL-2 VPC group showed no significant effect. Those animals treated with IL-2 and HS-tk VPC had no measurable disease in 3 of 4 animals. Animals showed no evidence of toxicity from the gene therapy. Animals that received as therapy OVCAR-3 cells transduced with either the IL-2 gene or HS-tk gene showed no efficacy alone or in combination. These findings suggest that the injection of HS-tk and IL-2 VPC may be more effective than either alone in the treatment of ovarian cancer.

DZ 312 MECHANISMS LEADING TO IL-2 MEDIATED TUMOR REJECTION IN MURINE MELANOMA

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Murine melanoma M3 cells were transfected with cytokine gene constructs condensed with transferrin-polylysine and linked to endosome-disruptive adenovirus particles. Transfected cells produce high levels of cytokines (100,000 units IL-2 / 10^6 cells/24 hrs) in vitro for at least several days after transfection. Subcutaneous injection of 1×10^5 untransfected M3 cells into syngeneic DBA/2 mice resulted in tumor development in all animals, whereas 1×10^5 M3 cells transfected with IL-2 have lost their tumorigenicity. Vaccination of DBA/2 mice with irradiated, IL-2 transfected M3 cells results in a systemic protection in all animals against a subsequent challenge with tumorigenic doses of unmodified M3 cells.
In an attempt to explore the cellular mechanisms responsible for IL-2 induced tumor rejection in the model described, we used immunohistochemical methods to identify contributing cells, such as natural killer cells, macrophages and T-cells at the time of immunization as well as after challenge with carcinogenic M3 cells. Immunohistological characterization of the challenge site showed a substancial increase in both CD4 and CD8 positive T-cells in the animals immunized with IL-2 compared to the control group. In addition we found at least a four-fold increase in the number of infiltrating macrophages in IL-2 expressing tumor cells compared to mock-DNA transfected cells. The importance of T cell mediated tumor rejection was further explored in nude mice. In this model, native IL-2 expressing tumor cells were efficiently rejected. However, IL-2 immunized mice were not protected against a subsequent challenge with native tumor cells indicating the role of CD4 and CD8 positive T-cells in the generation of a long-lasting anti-tumor response. In addition, results from cell transfer experiments point to T-cell involvement in IL-2 mediated tumor rejection.

DZ 313 NOVEL STRATEGIES TO ENHANCE THE EFFICACY OF ANTISENSE OLIGONUCLEOTIDES FOR IN VIVO GENE THERAPY, Ryuichi Morishita, Gary H. Gibbons, Yasufumi Kaneda*, Wendy Lee, Lunan Zhang, Toshio Ogihara*, Victor J. Dzau. Falk Cardiovascular Research Center, Stanford University, CA, *Osaka University Medical School, Osaka, Japan.
To enhance the utility of antisense (AS) oligodeoxynucleotide (ODN) technology for in vivo gene therapy, we have developed a transfection system using a liposome-ODN complex coated with an inactivated Sendai virus (HVJ). We postulated that the therapeutic efficacy of AS ODN in vivo may be limited by: 1) ODN degradation in lysosomes and 2) a limited capacity to promote RNase H-dependent mRNA degradation. To test this postulate we targeted angiotensin converting enzyme (ACE), an enzyme that generates angiotensin II and is associated with vascular lesion formation in vivo. To minimize ODN localization in lysosomes, we co-transfected AS ACE ODN with the nuclear protein high mobility group-1 (HMG-1). Co-transfection with HMG-1 significantly potentiated the inhibitory effect of AS ACE ODN on vascular smooth muscle cell ACE activity in vitro. To examine the role of an RNase-H dependent mechanism, we inhibited endogenous RNase H by administration of excess substrate (co-transfection with poly A(DNA)-polyU (3uM). Co-transfection of excess RNA-DNA hybrids attenuated the inhibitory effect of AS ACE ODN on ACE activity whereas DNA-DNA hybrids did not. Based on this evidence that endogenous RNase-H may be a important rate-limiting step, we assessed the effect of increasing intracellular RNase H activity by incorporating RNase H into the HVJ complex. Cells transfected with AS ACE ODN plus exogenous RNase H (6 units in liposome) exhibited a further decrease in ACE activity compared to treatment with AS ACE ODN alone. This potentiating effect of exogenous RNase H was abolished by co-transfection of excess RNA-DNA hybrids (15uM). The combination of both HMG-1 and RNase H within the HVJ complex was additive in further enhancing the efficacy of AS ACE ODN. We tested the hypothesis that inhibition of ACE activity after vascular injury would inhibit lesion formation in the rat carotid model. A single intraluminal transfection of the AS ACE ODN (10 uM) with HMG-1 and RNase H significantly inhibited vascular lesion formation whereas transfection of the AS ACE ODN alone had no effect. Conclusion: The effectiveness of an AS ODN strategy for in vivo gene therapy is significantly enhanced by an HVJ-liposome delivery system that incorporates both HMG-1 and RNase H.

DZ 314 IMPLANTS OF GENETICALLY MODIFIED SKIN FIBROBLASTS FOR LYSOSOMAL ENZYME DELIVERY: STUDIES IN NEWBORN MUTANT MICE AND IN NORMAL DOGS, Philippe Moullier[1], Delphine Bohl[1], Jorge Cardoso[2], Jean-Michel Heard[1] and Olivier Danos[1], (1) Laboratoire Rétrovirus et Transfert Génétique, Institut Pasteur, 75015 Paris, France. (2) Laboratoire de Recherche Chirurgicale, Faculté de Médecine Cochin-Port Royal, 75014 Paris, France
Genetic deficiencies in lysosomal hydrolases result in severe storage diseases with various extent of hepato-splenomegaly, skeletal abnormalities and CNS involvement. Treatments based on enzyme replacement have been proposed. In mice lacking ß-glucuronidase, which develop a disease homologous to human mucopolysaccharidosis type VII (MPS VII, Sly syndrome), we have used syngenic implants of genetically-modified skin fibroblasts for the continuous in vivo delivery of the enzyme. In the liver and spleen of adult MPSVII mice intraperitoneally implanted with collagen lattice secreting human ß-glucuronidase, we have observed a rapid and complete disappearance of lysosomal storage lesions, stable for at least 155 days (1). Although the overall condition of treated animals was improved, storage lesions were still present in the brain, kidney and joints. Moreover, skeletal abnormalities were not eliminated. We have now implanted modified fibroblasts in newborn MPS VII animals, in hope that providing enzyme to these sites early in life will prevent the development of lesions. Newborn animals were injected i.p. with 1-2 x 10^6 enzyme secreting fibroblasts within the first day of life and intraperitoneal lattices containing 5×10^6 cells were implanted at day 5. An analysis of the animals, performed after weaning, will be presented.
We are also investigating the potential of intraperitoneal neo-organs in a large animal. For this, we are currently testing the inocuity, stability and long term behaviour of these implants in dogs. Collagen lattices containing 10^9 autologous fibroblasts secreting human ß-glucuronidase were implanted in the omentum of three normal dogs. After 45 days vascularized neo organs were observed during a laparotomy and human ß-glucuronidase activity was detected in liver biopsies. As in mice, the activity was mostly localised to Kuppfer cells A long-term follow up of the animals will be presented.
(1). Moullier et al. (1993) Nature Genet. 4:154.

DZ 315 VEGF GENE TRANSFER INTO VASCULAR SMOOTH MUSCLE AND ENDOTHELIAL CELLS WITH A REPLICATION-DEFICIENT RECOMBINANT ADENOVIRUS VECTOR, Judith Mühlhauser, Marsha J. Merrill, Hiroyuki Maeda, Mima Bacic, Ronald G. Crystal and Maurizio C. Capogrossi, Pulmonary Branch, NHLBI and Surgical Neurology Branch, NINDS, NIH, Bethesda, MD 20892
Angiogenic factors may be useful in the treatment of ischemic disorders. However, efficient and localized delivery of such factors in vivo is technically difficult. Gene transfer with replication-deficient adenovirus vectors may overcome this problem by providing efficient transduction of selected tissues with the cDNA for an angiogenic factor. Among the growth factors which can cause angiogenesis, Vascular Endothelial Growth Factor (VEGF) is of interest because it is a secreted protein that acts selectively on endothelial cells in a paracrine manner. We constructed the replication-deficient adenovirus vector AdCMV.VEGF by homologous recombination of the plasmid pJM17, containing the Ad5 genome, and the plasmid pS5.VEGF. The latter plasmid contains the CMV promoter and the cDNA coding for the mature human $VEGF_{165}$ and its signal sequence. Cultured human umbelical vein endothelial cells (HUVEC) and rat aorta vascular smooth muscle cells (VSMC) were infected either with AdCMV.VEGF or with the control vector Ad.RSVβgal (5 and 20 pfu). VEGF mRNA expression and secretion into the supernatant were assessed by Northern and Western analyses respectively at 1, 3 and 7 days after infection. In HUVEC and VSMC both mRNA expression and protein secretion were already maximal at 1 day and did not decrease by the 7th day. Both gene expression and protein secretion were more marked at the higher pfu. Neither VEGF mRNA nor secreted protein was found in either HUVEC or VSMC infected with Ad.RSVβgal. The effect of the adenovirus vectors on HUVEC growth was assessed in 1% serum 3 days after infection. HUVEC number was twofold higher in AdCMV.VEGF than in Ad.RSVβgal infected (20 pfu) and control uninfected cells. Thus, AdCMV.VEGF causes gene expression and protein secretion in infected HUVEC and VSMC. Furthermore, AdCMV.VEGF enhances endothelial cell survival and growth in low serum conditions. AdCMV.VEGF may provide a novel approach for efficient local delivery of VEGF in the treatment of ischemic disorders.

DZ 316 LONG TERM IN VIVO EXPRESSION OF RETROVIRALLY TRANSFERRED ADA GENES IN LYMPHOCYTE CLONES FROM AN ADA-SCID PATIENT TREATED WITH GENE THERAPY, Craig A. Mullen, Karen Snitzer and R. Michael Blaese, Metabolism Branch, National Cancer Institute, NIH, Bethesda, MD 20892

In the past three years patients with severe combined immunodeficiency (SCID) due to adenosine deaminase (ADA) deficiency have been treated with autologous T cells derived from peripheral blood transduced with a retroviral vector containing an ADA gene (LASN). One patient received her final treatment with gene corrected cells nearly one year ago and continues to have readily detectable vector sequences and increased ADA levels in her peripheral blood. To assess the long term expression of the transferred ADA gene in individual lymphocytes, 9 T cell clones were derived from a peripheral blood sample 10 months after her last infusion. ADA vector was detected by PCR in 8 clones. ADA enzyme activity was measured in each clone. Activity in the clones containing vector ranged from 22% to 94% of normal. Less than 10% normal activity was found in the clone lacking the vector, compatible with uncorrected moderate ADA deficiency. Analysis of vector copy number and immunophenotyping of the clones is underway. It is likely that the cloning procedure imposed selection pressure for cells expressing the ADA gene; ADA negative cells do not grow well in culture. However, the recovery of lymphocytes continuing to express the transferred ADA gene at therapeutic levels at least 10 months in vivo after gene transfer provides encouraging evidence for the utility of retrovirally mediated gene transfer in the treatment of genetic disease.

DZ 318 GENE TRANSFER INTO PERIPHERAL BLOOD LYMPHOCYTES FOR IN VIVO IMMUNOMODULATION OF DONOR ANTI-TUMOR IMMUNITY IN A PATIENT AFFECTED BY EBV-INDUCED LYMPHOMA.

C. Traversari, G.Ferrari, C.Bonini, N.Nobili, S. Verzelletti, L.Vago, A.Faravelli, A.Vanzulli, P.Servida, S.Rossini, F.Mavilio, and C.Bordignon, Istituto Scientifico H. S. Raffaele, Milan; L. Sacco Hospital, Milan, Italy.

We devised a simple protocol based on vector-mediated transfer and expression in transduced cells of a modified (non-functional) cell surface marker not expressed on human lymphocytes, followed by positive immunoselection of the transduced cells. This results in virtually 100% gene-modified PBL. An additional advantage of this strategy is related to the possibility of PBL infection during antigen stimulation of effector cells. Since retroviral vectors infect almost exclusively dividing cells, positive selection of transduced cells allows a significant enrichment of antigen-specific lymphocytes. Based upon earlier observations made at Memorial Sloan-Kettering on the therapeutic use of donor lymphocytes (R.J.O'Reilly, personal communication), our first clinical application of this strategy to in vivo anti-tumor adoptive immunotherapy of neoplastic disorders was in a patient who developed an EBV-induced lymphoproliferative disease, after receiving a T-cell depleted BMT from an HLA-identical donor for a hematological malignancy. This complication, caused by the severe immunosuppression, is usually fatal in BMT recipients. For this purpose, donor lymphocytes were transduced in vitro by a retroviral vector for transfer and expression of two genes: 1- a modified (non functional) form of the low affinity receptor for the nerve growth factor gene (ΔLNGFR), for in vitro selection of transduced cells and for in vivo follow-up of the infused donor lymphocytes; 2- the thymidine-kinase gene that confers to the transduced PBL in vivo sensitivity to the drug gancyclovir, for in vivo modulation of donor-anti tumor response, and for in vivo specific elimination of cells potentially responsible for GvHD. After approval of the ethical committee, 2 x 10^6/kg CD3+ donor PBL, transduced with this vector, were infused i.v. into a patient who presented with high fever, generalized lymphonode involvement, and marrow failure due to the extensive infiltration of an EBV-induced lymphoma. In the two weeks following the administration of vector-transduced donor cells, all clinical symptoms associated with EBV-induced B-cell proliferation regressed, with full hematopoietic recovery. During this time, marked donor cells increased progressively in the patient peripheral blood up to 13.4% of total mononuclear cells, as detected by FACS analysis of LNGFR-expressing cells. Circulating transduced donor lymphocytes were predominantly CD3+/CD8+. Four weeks after infusion of gene-modified lymphocytes, the patient progressively developed signs of acute GvHD, with increasing liver function enzymes, and a positive skin biopsy. The i.v. administration of two doses of 10 mg/kg of the drug gancyclovir resulted in reduction of marked lymphocytes to 3.1%, with disappearance of clinical signs of skin GvHD and reduction of over 50% of altered liver function enzymes. This strategy may provide a crucial tool against the GvH potential of alloreactive T-cells contaminating tumor-specific T-cells used in adoptive immunotherapy.

DZ 317 RETROVIRAL VECTORS FOR THE IN VIVO DELIVERY OF α-L-IDURONIDASE AND ARYL SULFATASE B IN HUMAN MUCOPOLYSACCHARIDOSES TYPE I AND VI., Anna Salvetti, Philippe Moullier, Olivier Danos and Jean Michel Heard, Laboratoire Rétrovirus et Transfert Génétique, Institut Pasteur, 75015, Paris, France.

The Mucopolysaccharidoses (MPS) are inherited metabolic disorders resulting from deficiencies in lysosomal enzymes. These enzymes can be secreted and captured by distant cells, providing a rationale for enzyme replacement therapy. Our approach is to obtain the secretion of enzyme from an autologous implant of genetically modified fibroblasts. This was tested in β-glucuronidase deficient mice which develop a disease homologous to human MPS type VII. The human β-glucuronidase cDNA was introduced with a retroviral vector into mutant mice skin fibroblasts and the animals were implanted intraperitoneally with these modified cells embedded into collagen lattices. Five months later, the animals expressed β-glucuronidase from vascularised neo-organs and the lysosomal storage lesions had disappeared in their liver and spleen. This protocol could be applied to the treatment of other, more common, MPS and particularly to MPS type I (Hurler syndrome) and type VI (Maroteaux-Lamy syndrome) which are due to α-L-iduronidase (IDUA) and aryl sulfatase B (ASB) deficiencies respectively. The human cDNAs encoding these two enzymes were isolated from a λgt11 liver cDNA library and inserted into a retroviral vector under the control of the phosphoglycerate kinase 1 promoter. Ψ-CRIP derived clones producing high titers of recombinant retroviruses were isolated and used to infect MPS I or MPS VI fibroblasts. The IDUA activity measured in the extracts of MPS I infected fibroblasts was 150-fold over non infected MPS I cells and 7-fold over normal human fibroblasts (HuF). Similarly, a 320-fold and a 9.5-fold increase of ASB activity were detected in the extracts of MPS VI infected cells over MPS VI and HuF cells respectively. This overexpression was associated with a secretion of the enzyme in the culture media of infected cells. The secreted IDUA was efficiently internalized by non infected MPS I fibroblasts. Antibodies against human IDUA were obtained from a Biozzi mouse implantated with autologous fibroblasts infected with the retroviral vector. The presence of high titer antibodies in the serum of this mouse was detected by Elisa, one month following implantation. These antibodies will be used to reveal the presence of human IDUA in the tissues of animals carrying neo-organs from which the enzyme is secreted.

Human Diseases II; Gene Therapy: Somatic Tissue III

DZ 400 TREATMENT OF HEMOPHILIA B BY SOMATIC CELL GENE THERAPY USING KERATINOCYTES AS A GENE DELIVERY SYSTEM. M. Yvonne Alexander, Sanjay I. Bidichandani, Caspar J.M. Robinson and Rosemary J. Akhurst. Duncan Guthrie Institute of Medical Genetics, University of Glasgow, Glasgow, G3 8SJ, U.K.
Factor IX is a clotting factor which is deficient in patients suffering from haemophilia B. This is an X-linked bleeding disorder currently treated with Factor IX concentrate infusion but due to the biological complications involved with this treatment it is considered to be a candidate disorder for a gene therapy approach. Factor IX has recently been shown to be expresssed albeit at low levels, in skin cells and secreted into the circulation (1). Using the reporter gene ß-galactosidase linked to strong keratin promoters, or to different general viral promoters, we are currently investigating optimal conditions for delivering and expressing exogenous genes in skin *in vivo* through the use of a gene gun and various lipofectin reagents. We have initially demonstrated the ability to deliver tungsten particles into mouse skin, with little if any, tissue damage. If successful, the method may then be used to investigate the possibility of expressing therapeutic proteins in skin cells for secretion into the circulation. This approach is unique in allowing transfer of foreign genes into skin cells *in vivo* thereby removing the need for two surgical procedures as is required for *ex vivo* gene therapy. Various constructs have been designed linking a Factor IX cDNA to different keratin promoters together with their regulatory sequences and the efficiency of these constructs is being tested *in vivo* using transgenic technology. Future work will assess the levels of therapeutic protein that can be synthesised and secreted by the transformed keratinocytes and will determine which delivery method fulfils our expectations and merits further research with therapeutic genes for gene therapy in the patient.
REFERENCES
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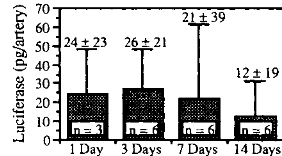
DZ 401 AN ALANINE TO THREONINE SUBSTITUTION IN THE INTESTINAL FATTY ACID BINDING PROTEIN IS ASSOCIATED WITH INSULIN SENSITIVITY AND OBESITY IN MEN, Leslie Baier, James Sacchettini*, Clifton Bogardus, Michal Prochazka, Clinical Diabetes and Nutrition Section, NIH Phoenix, AZ. 85016 and * Albert Einstein Medical College, Bronx, NY. 10461
The Pima Indians of Arizona have the highest reported prevalence of non-insulin-dependent diabetes mellitus (NIDDM) of any population. We have previously reported linkage between insulin action in non-diabetic Pimas at maximally stimulating insulin concentrations (MaxM), as well as fasting insulin concentration, with the FABP2 locus on 4q26. The coding sequences of the most frequent FABP2 alleles (1 and 3) were analyzed by single-stranded conformational polymorphism (SSCP). One nucleotide substitution (G to A) was identified that results in an alanine to threonine substitution at amino acid 54. The frequency of an alanine-encoding allele was 70% and the frequency of a threonine encoding allele was 30%. The FABP2 locus encodes the human intestinal fatty acid binding protein (I-FABP). Preliminary analysis suggests that Pima men encoding I-FABP-Thr are more obese and more insulin sensitive compared to men encoding I-FABP-Ala. The crystal structure of I-FABP reveals that the alanine to threonine substitution resides near the solvent accessible opening of the protein where fatty acids bind. Microcalorimetry studies with recombinant I-FABP-Ala and I-FABP-Thr demonstrate that the two proteins have distinct binding kinetics for unsaturated fatty acids.

DZ 402 PERSISTENCE OF LUCIFERASE GENE EXPRESSION FOLLOWING ADENOVIRUS-MEDIATED *IN VIVO* DIRECT GENE TRANSFER INTO PORCINE CORONARY ARTERIES, Brent A. French, Nadir M. Ali, Wojciech Mazur, George P. Rodgers, Daryl G. Schulz, Robert Roberts, Albert E. Raizner, Department of Medicine, Section of Cardiology, Molecular Cardiology Unit, Baylor College of Medicine, Houston, TX 77030

Restenosis following coronary angioplasty may be amenable to gene therapy provided that therapeutic levels of recombinant gene expression can be obtained in afflicted vessels over an appropriate period of time. Evidence from animal models and human autopsies indicates that the majority of the smooth muscle cell migration and proliferation responsible for restenosis occur during the first weeks following angioplasty. Thus to be effective, an antiproliferative gene therapy would have to provide for at least two weeks of therapeutic gene expression. We have previously used recombinant adenoviral vectors to obtain high levels of reporter gene expression in porcine coronary arteries 3 days after gene transfer; however, little is known about the time course of gene expression *in vivo* following adenoviral infection. The current study was undertaken to determine the duration of recombinant gene expression provided by a replication-deficient adenoviral vector containing the firefly luciferase expression cassette (Ad5/RSV/GL2) in porcine coronary arteries. Under fluoroscopy, 4 ml of virus (4×10^9 pfu) was infused into each porcine coronary artery at a pressure of 8 atms using perforated balloon catheters. Reporter gene activity was determined at 1, 3, 7, and 14 days following gene transfer using a luminometric assay for luciferase. Results are shown in the graph where values represent mean levels of luciferase recovered per artery in picograms (pg) ± standard deviation and n equals the number of coronary arteries assayed. Reporter gene expression was found to persist at significant levels from 1 to 14 days following gene transfer. We conclude that the duration of gene expression provided by adenoviral vectors appears to be suitable for antiproliferative gene therapies directed against restenosis.



DZ 403 THERAPEUTIC RESPONSE PRODUCED BY DIRECT LIVER INJECTION OF DNA CONSTRUCTS CONTAINING THE ERYTHROPOIETIN GENE, M. Anne Hickman[1], Karin Lehmann-Bruinsma[2], Mansour Bassiri[2], Tracey R. Sih[1,2], Don M. Carlson[1] and Jerry S. Powell[2], Section of Molecular and Cellular Biology[1] and Department of Med:Hematology and Oncology[2], University of California, Davis, CA 95616
Erythropoietin (Ep) is the glycoprotein hormone that stimulates production of red blood cells. Ep synthesis occurs in response to hypoxia, primarily in the kidney but also in the liver. Based on our recent studies of direct injection of DNA into liver, we investigated whether cat and rat liver could express therapeutic amounts of Ep from plasmids containing Ep coding sequences with heterologous promoters. Male Sprague-Dawley rats (250-400 g) and adult domestic short-hair cats were anesthetized and livers surgically exposed. Either vehicle alone, or varying concentrations of plasmid DNA (1-20 mg) were injected in multiple fractions into hepatic parenchyma. Dexamethasone, 1mg/kg initially, and continuing at varying dosages, was administered to most animals. Plasmids contained human Ep cDNA, Lac Z cDNA or luciferase cDNA and CMV, albumin or retroviral LTR promoter/enhancer sequences. Blood was obtained periodically for assays of Ep concentration and liver function and for indices of RBC production including hematocrit and reticulocytes. With rats, 16 of 19 animals injected with Ep DNA demonstrated increased Ep concentrations with maximum levels occurring between 2 and 18 days and persistent elevation of Ep for at least 23 days. In each animal with elevated Ep, the hematocrit and reticulocyte index rose. The increase in hematocrit correlated with both the amount and the duration of Ep production. Plasmids containing the CMV promoter-Ep cDNA produced higher initial (48 hours) serum Ep concentrations than albumin promoter-Ep plasmids (21.5 mU/ml ± 2.9 SEM vs 6.6 mU/ml ± 2.9 SEM, respectively). However, the albumin promotor functioned more persistently *in vivo* than the CMV-promotor and did not require continued administration of high doses of dexamethasone for expression. Serum Ep concentrations at day 23 were 40% of maximal values with the albumin promotor as compared to 12% with the CMV promoter. ß-Galactosidase staining demonstrated that Lac Z plasmid was expressed by hepatocytes near the injection sites. Similar results were obtained with cats. Analysis of DNA from feline liver homogenates showed that plasmids persisted as episomes. We conclude: 1) that direct intrahepatic injection of Ep DNA leads to therapeutic serum concentrations of Ep; 2) that the albumin promoter-enhancer results in more persistent Ep production than the CMV promoter-enhancer; 3) that the CMV promoter *in vivo* is less effective in the absence of dexamethasone. These findings support the efficacy of direct injection of DNA as a non-viral based strategy for gene augmentation therapy.

DZ 404 A GENE FOR FAMILIAL JUVENILE NEPHRONOPHTHISIS (NPH) MAPS TO CHROMOSOME 2q11.1-q21.1.

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Familial juvenile nephronophthisis (NPH), an autosomal recessive cystic disease of the kidney, leads to end stage renal failure in childhood. Since the pathology of NPH is obscure, we used linkage analysis to map a gene for NPH with the future goal of elucidating the disease mechanism. In 9 NPH families with at least 2 affected children exhibiting the typical history, physical signs and renal biopsy finding of NPH, linkage analysis was performed using highly polymorphic microsatellite markers. A region on chromosome 2p was studied for which Antignac et al. have described linkage with a gene locus for NPH (Nature Genet., 3:342-5, 1993).

Linkage with an NPH locus was confirmed by 3-point analysis using markers AFM262xb5 and AFM220ze3 at loci D2S176 and D2S160, respectively, with a maximum lod score of Z_{max} = 5.7 (θ_{max} localized 3.6cM(M) q-terminal of D2S160). The 95% support interval was 27.5cM(M). A family with 5 affected children yielded a maximum lod score of Z_{max} = 2.7 (θ=0) for the marker AFM220ze3. By analysis of recombination data in this family, flanking markers were defined, thereby mapping the NPH region to a 15cM(M) interval between AFM 172xc3 and AFM016yc5 at loci D2S135 and D2S110. Furthermore, the candidate region for NPH was assigned to 2q11.1 - q21.1 by demonstration of linkage with markers of defined cytogenetic localization.

We conclude that linkage for a disease locus for NPH on chromosome 2 was confirmed and specified cytogenetically. The definition of flanking markers will help further progress towards physical mapping and isolation of a gene for NPH.

DZ 405 ADENOVIRAL VECTOR-MEDIATED EXPRESSION OF THERAPEUTIC LEVELS OF HUMAN FACTOR IX IN MICE, Michael Kaleko, Theodore Smith, Michele Mehaffey, Dawn Kayda, June Saunders, Soonpin Yei, Bruce Trapnell, and Alan McClelland, Genetic Therapy, Inc., Gaithersburg, Md. 20878

To evaluate adenoviral vector-mediated expression of human factor IX in vivo, we constructed the vector Av1H9B, which encodes human factor IX cDNA downstream of the RSV promoter and adenovirus tripartite leader. The vector is devoid of most of the adenovirus E1 and E3 regions. Three routes of vector administration were evaluated to determine which would provide the highest level of gene transfer to mouse liver. 1 x 10^9 pfu of Av1H9B were infused into the tail vein, the portal vein, or directly into the liver parenchyma. Southern analysis of liver DNA demonstrated that all three routes of vector administration yielded extremely efficient gene transfer. Plasma levels of human factor IX, determined by ELISA, were in the range of 3-30 ng/ml during the first week after vector delivery. However, after tenfold higher doses of Av1H9B were injected into either the tail vein or the liver parenchyma, plasma levels of human factor IX in the range of 200-500 ng/ml were observed during the first two weeks after vector delivery, and levels slowly declined to baseline by nine weeks. An immunochromogenic assay indicated that the factor IX produced in vivo was completely biologically active. Southern analysis of liver DNA from mice which received a tail vein injection of vector demonstrated an average of 55 vector copies per cell during the first four weeks. By seven weeks, an average of three copies per cell were present. The tissue distribution of vector was determined in two mice by Southern blot analysis. Transduction levels were highest in the liver and tenfold lower in the lungs. Vector was demonstrated in each of the organs tested with the exception of the brain. A second injection of Av1H9B in mice ten weeks after an initial injection did not yield expression of human factor IX in plasma. An anti-adenoviral antibody assay indicated that mice which received a single injection of Av1H9B developed antibodies which could inactivate adenovirus in vitro, and which presumably inactivated adenovirus after the second administration. Antibody titers were significantly boosted after the second vector delivery.

DZ 406 IN VIVO CORRECTION OF LDL RECEPTOR DEFICIENCY IN THE WATANABE HERITABLE HYPERLIPIDEMIC RABBIT WITH RECOMBINANT ADENOVIRUSES, Karen F. Kozarsky and James M. Wilson, Institute for Human Gene Therapy and Departments of Molecular and Cellular Engineering and Medicine, University of Pennsylvania, and the Wistar Institute, Philadelphia, PA 19104

An animal model of LDL receptor deficiency in humans, the Watanabe heritable hyperlipidemic (WHHL) rabbit, was used to develop an *in vivo* approach to gene therapy based on recombinant adenoviruses. An LDL receptor expressing adenovirus was constructed and infused into the portal circulation of WHHL rabbits. Analysis of tissues harvested three days after virus infusion demonstrated human LDL receptor protein in the majority of hepatocytes that exceeded the levels found in human liver by 20-fold; transgene expression was stable for 7-10 days and diminished to undetectable levels within 3 weeks. This loss of expression was also observed in NZW rabbits infused with an adenovirus encoding a marker gene (*lacZ*). Infusion of LDL receptor expressing virus led to substantial reductions in serum cholesterol that returned to baseline within 3 weeks; this acute reduction in serum cholesterol was associated with accumulations of lipid in hepatocytes. The development of neutralizing antibodies to the recombinant adenovirus markedly diminished the effectiveness of a second dose. These studies illustrate the advantages of recombinant adenoviruses for the treatment of liver metabolic disease and define issues that need to be overcome before the promise of this technology can be fully realized.

DZ 407 GENERATION OF THERAPEUTIC T CELLS FROM LYMPH NODES DRAINING A TUMOR GENETICALLY ENGINEERED TO SECRETE INTERLEUKIN 4. John C. Krauss, Scott E. Strome, Alfred E. Chang, and Suyu Shu, Departments of Internal Medicine, Otolaryngology, and Surgery, University of Michigan, Ann Arbor, MI 48109

The adoptive transfer of immune T cells has been demonstrated to mediate regression of established tumors in animals with encouraging results in human clinical trials. A prerequisite of clinical adoptive immunotherapy has been the isolation of immune cells from patients. In animals, lymph nodes draining a progressive immunogenic tumor contain tumor sensitized but functionally deficient pre-effector cells, which can be activated in vitro by stimulation with α-CD3 and IL-2 to differentiate into functional effector cells. However, the pre-effector cell response is not evident during the growth of poorly immunogenic tumors such as the BL-6/B16 melanoma. In this study, a clone of BL-6/B16 (A9), was transfected with the cDNA encoding for murine IL-4, in an attempt to enhance the immunogenicity and/or the pre-effector cell generation in the draining lymph node. The IL-4 secreting clone (A94-3; 38U IL-4/mL/2x10^5 cells/24 hours) grew significantly slower than controls after sc. inoculation (42 $\pm$ 4 mm^2 vs. 115 $\pm$ 5 mm^2 at 2 wk.). The ability to establish pulmonary metastases after intravenous inoculation was also impaired as compared to controls (51 $\pm$ 6 vs. 183 $\pm$ 15 nodules at 2 wk.). The pre-effector cell activity was examined in lymph nodes draining the IL-4 secreting tumors for 10 days. After α–CD3/IL-2 activation, lymphocytes were adoptively transferred into animals bearing 3 day established parental pulmonary metastases. The transfer of cells derived from sensitization with A94-3 tumors was capable of reducing the average pulmonary metastases from 401$\pm$59 to 12 $\pm$ 6. This result appeared to be comparable to the efficacy of generation of immune effector cells with parental tumor admixed with C. Parvum. Thus genetic modification of tumor cells to secrete IL-4 can stimulate an increase in pre-effector cell function in the tumor draining lymph node, suggestive of an increase in T cell mediated immune function against the parental tumor.

DZ 408 **Liposome Mediated Transfection of Human GM-CSF Gene into Human Melanoma Cell Lines**

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Retrovirally produced murine GM-CSF in lethally irradiated B16 melanoma cells has been reported to greatly amplify the ability of the murine immune system to reject live B16 melanoma cells in murine hosts(Glenn Dranoff and Richard Mulligan, PNAS,90,pp3539-3343, April 1993). We are investigating the effeciency of cationic liposome mediated transfection , rather than retroviral infection, of human GM-CSF into a number of human Melanoma cell lines established at the Arizona Cancer Center. We have cloned the human GM-CSF gene into the MFG retroviral expression vector. We have introduced this construct into melanoma cells using the polycationic lipofectant, lipofectAMINE (BRL). Human GM-CSF protein is detected in the serum free media 24 hours post transfection. We are establishing a retroviral producer line by transfecting the human GM-CSF:MFG construct into Ψ crip cells. We intend to compare the level and duration of expression of human GM-CSF in human melanoma cell lines after either infection by human GM-CSF retroviruses or by lipofectAMINE transfection. A more rapid and direct means of producing cytokine expressing melanoma vaccines may be possible through the use of lipofectAMINE.

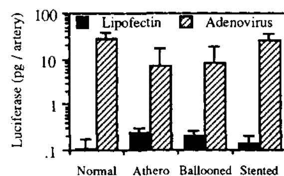
DZ 409 *IN VIVO* GENE THERAPY FOR HYPERCHOLESTEROLEMIA IN THE WATANABE RABBIT MODEL, J. Li[2], R. C. Eisensmith[2], B. Fang[2], X. H. L. Chen[1], I. Nasonkin[2], M. Mimms[3], C. Montgomery[4] and S. C. L. Woo[1,2]. [1]Howard Hughes Medical Institute, [2]Departments of Cell Biology, [3]Medicine and [4]Comparative Medicine, Baylor College of Medicine, Houston, Texas, 77030.

Hypercholesterolemia has been implicated in a number of human diseases, including coronary heart disease, atherosclerosis and stroke. It is estimated that approximately 16 million Americans might benefit significantly from procedures that lower serum cholesterol levels. We are assessing the potential of *in vivo* gene therapy to prevent or treat these cardiovascular disorders through the delivery of exogenous genes known to regulate lipid metabolism. These studies are being performed in the Watanabe heritable hyperlipidemia (WHHL) rabbit animal model.

A full-length cDNA for the rabbit LDL receptor was created by ligating overlapping oligonucleotides to the truncated 5' end of a cDNA clone kindly provided by Goldstein *et al.* The full-length clone was then introduced into a recombinant adenoviral vector, where its expression was controlled by the RSV promoter (ADV/RSV-rbLDLR). The functionality of this construct was determined by transduction of hepatocytes isolated from WHHL rabbits. Levels of binding, uptake and degradation of radioiodinated-LDL in the transduced hepatocytes were significantly increased relative to the background levels present in non-transduced WHHL rabbit hepatocytes. A second recombinant adenoviral vector containing the ß-galactosidase gene was used to establish optimal conditions for the *in vivo* transduction of hepatocytes in these animals. Portal vein infusion of 2-5 x 10^{11} recombinant adenoviral particles per kg body weight resulted in the transduction of 50%-80% of hepatocytes, with little or no apparent cytopathological effects. A similar infusion of the ADV/RSV-rbLDLR vector into WHHL rabbits resulted in a dramatic decrease in serum cholesterol levels. Although this effect persististed for only 1-2 weeks, this study indicates that hypercholesterolemia and similar metabolic disorders can be corrected by hepatic gene therapy when long-lasting vectors are developed.

DZ 410 REPLICATION - DEFICIENT ADENOVIRAL VECTORS MEDIATE EFFICIENT DIRECT *IN VIVO* GENE TRANSFER INTO NORMAL AND INJURED PORCINE CORONARY ARTERIES, Wojciech Mazur, Nadir M. Ali, J. Patrick Finnigan, Daryl G. Schulz, George P. Rodgers, Robert Roberts, Albert E. Raizner, Brent A. French, Department of Medicine, Section of Cardiology, Molecular Cardiology Unit, Baylor College of Medicine, Houston, TX 77030

The problem of restenosis following coronary angioplasty may be amenable to gene therapy provided that recombinant genes can be efficiently delivered to atherosclerotic and restenotic vessels. We therefore constructed an adenoviral vector carrying the luciferase reporter gene and compared the efficiency of adenovirus- vs Lipofectin-mediated direct gene transfer in four types of porcine coronary vessels: i) normal coronaries, ii) atherosclerotic coronaries, iii) atherosclerotic coronaries injured by oversized angioplasty balloons, and iv) atherosclerotic coronaries injured by intracoronary stents. For each transfected artery, 50 µg of reporter vector DNA (pRSVL) was complexed with 150 µg of Lipofectin (BRL) and diluted to 4 ml with Opti-MEM I (BRL). Viral infections were performed with 4 ml infusions ($4x10^9$ pfu) of a recombinant adenovirus harboring an analogous reporter cassette (Ad5/RSV/GL2). Under fluoroscopy, these solutions were infused at 8 atmospheres into the coronary arteries of intubated Hanford miniature swine via perforated balloon catheters. Reporter gene activity was determined 3 days later using a luminometric assay for luciferase. Each column in the graph at right indicates the mean level of luciferase recovered from 4 to 6 coronary arteries and error bars indicate the standard deviation. Adenovirus-mediated gene transfer was equally effective in normal, atherosclerotic, and restenotic arteries. When the data are combined, the luciferase levels obtained from adenovirus averaged 100 times higher than those from Lipofectin. We conclude that adenovirus-mediated gene transfer is an efficient process suitable for delivering gene therapy in both the balloon- and stent-injured porcine models of coronary restenosis.



DZ 411 **DEVELOPMENT OF RETROVIRAL VECTORS THAT OPTIMIZE THE INHIBITION OF HIV-1 AT MULTIPLE POINTS OF THE VIRAL LIFE CYCLE.** Richard A. Morgan, Jack Ragheb, Marinee Chuah, Thierry VandenDriessche, and Peter Bressler[#], Molecular Hematology Branch, National Heart, Lung, and Blood Institute; and [#]Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institute of Health, Bethesda.

In HIV-1 infection, it may be possible to apply the technology of gene therapy to deliver anti-viral agents directly to infected cells and potentially benefit the infected individual. Retroviral vectors were constructed that inhibit the HIV-1 virus at various points in the viral life cycle. The goal of this work is to develop the components of the ideal anti-HIV vector. This ideal vector would potentially attack HIV at three points in the viral life cycle. First, the vector should be able to inhibit the establishment of infection either by preventing HIV binding to cells or by stopping HIV proviral integration. Second, the vector should be capable of preventing viral protein production. This could be done early by inhibiting tat function, in the middle of the life cycle by preventing the rev-mediated production of structural RNAs, or at the end of the life cycle by the inhibition of virion assembly. As a third and final anti-viral approach, it may be possible to have the infected cell release a defective HIV particle and thus prevent a new round of replication. We have produced retroviral vectors that individually attack one or more of these points. These vectors include: sCD4 and CD4IgG binding decoy vectors, anti-sense tat and TAR vectors, antisense tat/rev and rev transdominant vectors, new HIV gag gene transdominant vectors, a CD4-KDEL ER retention signal vector, and a series of HIV inducible vectors that synthesize diphtheria toxin, cytosine deaminase, or interferon. These vectors were assayed in T-cell lines and primary culture PBL and shown to inhibit both laboratory strains of HIV and clinical isolates. The combination of different protein coding domains into one vector was facilitated by the use of internal ribosome binding sites. These multi-gene anti-HIV vectors can also be combined with anti-sense approaches to inhibit HIV. The evaluation of the single gene and multi-gene vectors as anti-HIV-1 agents will be presented.

DZ 412 MESOTHELIAL CELL GENE THERAPY, Janice A. Nagy, Ty R. Shockley *, and Robert W. Jackman, Departments of Pathology, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215 and * Renal Division Research, Baxter Healthcare Corporation, McGaw Park, IL 60085
Mesothelial cells line the coelomic cavities (peritoneal, pleural and pericardial), cover a large surface area, possess secretory function, and, by virtue of their anatomical location, offer the possibility of easy removal and subsequent reimplantation. We hypothesized that genetically modified autologous peritoneal mesothelial cells might be used as vehicles for *ex vivo* gene therapy and established an experimental model system in the rat to test our hypothesis. Briefly: 1) rat primary mesothelial cells were isolated from the parietal peritoneal wall and propagated in cell culture; 2) rat primary peritoneal mesothelial cells were stably transduced (using BAG virus carrying the E. coli *lacZ* gene) or transfected (using the strontium phospate co-precipitation method and the plasmid pSVTKgH) *in vitro* either to mark the cells with a reporter protein (β-galactosidase, β-gal) or to deliver a secreted product (human growth hormone, hgh); 3) such transduced or transfected rat primary mesothelial cells were implanted on the denuded peritoneal surface of syngeneic recipients; 4) these implanted mesothelial cells continued to express the reporter gene product β-gal *in vivo* for at least one month and measurable levels of hgh were detected in the peritoneal fluid and the blood of these animals for ~ one week. We then extended our studies to gene therapy using human mesothelial cells. Primary human mesothelial cells were isolated from discarded surgical specimens (omentum), propagated in cell culture and stably transfected *in vitro* (using the strontium phospate co-precipitation method) with the plasmid pSVTKgH (encoding hgh). These transfected human peritoneal mesothelial cells (marked with DiO, a lipophilic dye used for long-term tracing of transplanted cells) were implanted on the denuded peritoneal surface of athymic nude rat recipients. Fluorescent patches of transfected human mesothelial cells were observed covering the denuded areas and hgh was detected in the blood of these animals. Our results indicate that delivery of gene products released by transfected peritoneal mesothelial cells is not confined to the peritoneum, since the peritoneal cavity, by virtue of its draining lymphatics, is in direct communication with the blood. Therefore, we conclude that mesothelial cell gene therapy (both *ex vivo* and *in vivo*) may be useful in the treatment/prevention of acquired or inherited diseases localized to the peritoneal cavity (e.g., ovarian carcinoma, peritonitis, adhesions) as well as those requiring delivery of recombinant gene products directly into the systemic circulation.

DZ 413 PRE-CLINICAL STUDIES OF LYMPHOCYTE GENE THERAPY FOR HUNTER SYNDROME, Dao Pan, Stephen E. Braun, Jon J. Jonsson, Elena L. Aronovich, R. Scott McIvor, Chester B. Whitley, Gene Therapy Program, University of Minnesota Medical School, Minneapolis, MN 55455
Hunter syndrome is a lethal inborn error of lysosomal metabolism resulting from deficient iduronate-2-sulfatase (IDS) enzyme activity and the consequent systemic accumulation of glycosaminoglycan (GAG). Utilizing peripheral blood lymphocytes (PBL) from patients with Hunter syndrome, we evaluated IDS expression, metabolic correction, and cross-correction in vitro. Four retroviral constructs were studied: L2SN and LNC2 (constructed from LXSN) with the IDS cDNA transcriptionally regulated by the LTR or CMV promoter; L2 with no selectable marker; and LB2 utilizing the ß-actin promoter. Following 3.5 days of T-lymphocyte stimulation (OKT3, IL2), PBL were transduced on 4 consecutive days with PA317 supernatant in the presence of protamine sulfate. After culture for 3 - 6 days without G418 selection, PBL were: 1) assayed for IDS specific activity; 2) tested for $^{35}SO_4$-GAG accumulation vs. time; and 3) co-cultured for 2 days to assess $^{35}SO_4$-GAG accumulation in neighboring fibroblasts from patients with Hunter syndrome (reported as % of $^{35}SO_4$-GAG in fibroblasts cultured alone).

	PBL	PBL-LXSN	PBL-L2SN	PBL-LNC2	PBL-pLB2	PBL-pL2
[1]IDS	31	32	598	287	668	400
[3][^{35}S]	90-98%	87-92%	71-84%	75-77%	81%	---

Owing to overexpression of IDS in the portion of transduced PBL, IDS activity was increased above PBL-LXSN controls or leukocyte (WBC) levels of patients (<40 U/mg/h), and comparable to WBC levels of normal individuals (μ=806; SD 251). Such transduced PBL failed to show continued accumulation of $^{35}SO_4$-GAG indicating that recombinant IDS enzyme corrected the metabolic defect. Additionally, fibroblasts from patients co-cultured with transduced PBL had reduced accumulation of $^{35}SO_4$-GAG suggesting that recombinant IDS is taken up by neighboring non-transduced cells. These results illustrate the potential for treatment of mild, non-neuropathic Hunter syndrome by ex vivo lymphocyte gene therapy. Support by Daniel Molinaro Foundation

DZ 414 DIRECT DELIVERY OF CYTOKINE GENES INTO TUMORS FOR CANCER IMMUNOTHERAPY, Suezanne E. Parker and Shirin Khatibi, Vical Inc. San Diego CA 92121
Conventional gene therapy for the treatment of cancer typically involves removal of the tumor, in vitro gene transfer, selection of the genetically modified cells and reintroduction of the cells back into the same patient. A simpler approach is to introduce recombinant DNA directly into tumors. This approach has the advantage that it does not require a virus for the delivery of genetic material and virtually any gene containing the appropriate regulatory sequences can be delivered.
We tested this strategy by injecting plasmid DNA expression vectors containing lymphokine genes directly into subcutaneous B16 melanoma tumors in mice. The direct intratumor injection of 50ug of plasmid DNA encoding the IL-2 gene, 3 times/week, for three weeks, resulted in a smaller tumor mass and prolonged survival as compared to animals injected with blank plasmid DNA (tumor volume at three weeks of 526 vs 1423 mm^3 respectively). Co-injection of a mixture of 50ug of plasmid DNA encoding the IL-2 gene with 50ug of plasmid DNA *encoding* the Infγ gene as described above resulted in enhanced anti-tumor activity (tumor volume of 226mm^3). Injection of genes in the presence of cationic lipids resulted in a more effective anti-tumor effect than the DNA injections alone. For example, injection of Il-2 and Inf-γ DNA combined with cationic lipid resulted in a tumor volume of 63 mm^3 as compared to 2226 mm^3 for untreated animals and 1570 mm^3 in animals treated with the lipid alone.
Direct intratumor injection of plasmid DNA containing cytokine genes whether alone, or in combination with cationic lipids, significantly slowed tumor growth in the B16 melanoma model. Cationic lipids enhance the anti-tumor effect but are not required. A combination of IL-2 and Inf-γ DNA was more effective than Il-2 alone. These data suggest that local expression of a cytokine gene may be useful in tumor therapy.

DZ 415 BLADDER CANCER VACCINE ESTABLISHED BY TRANSFECTION OF IL-2 OR GM-CSF GENE INTO BLADDER CANCER CELLS, Shiro Saito, Bernd Gansbacher, Felicia Rosental, Warren D.W. Heston, William R. Fair and Eli Gilboa, Memorial Sloan-Kettering Cancer Center, New York, NY 10021 and Duke University Medical Center, Durham, NC 27710
We have shown that vaccination by Interleukin-2 (IL-2) gene-modified irradiated murine bladder cancer cells (MBT-2/IL-2) can cure 60% of the mice with bladder cancers preestablished by orthotopical implantation of MBT-2 cells. These cured mice established permanent immunity against parental cells to reject subsequent challenge of the same cells. MBT-2 cells transfected with granulocyte-macrophage colony-stimulating factor (GM-CSF) gene (MBT-2/GM-CSF) was also successful as a tumor vaccine and cured 40% of the established bladder cancer. Immunohistochemical examination showed infiltration of CD4+ and CD8+ cells in the bladder tumors of vaccinated mice. CD4+ and CD8+ cells were not seen in the tumors of control mice. CTL assay demonstrated that vaccination with either MBT-2/IL-2 or MBT-2/GM-CSF could induce specific immunity in the mice against MBT-2 cells. We are currently investigating the combined vaccination effects of MBT-2/IL-2 and MBT-2/GM-CSF. This combination is expected to provide better effect than that shown by the single cytokine.

DZ 416 ROUTINE ESTABLISHMENT OF DIPLOID FIBROBLAST CULTURES FROM HUMAN ADULTS AS POTENTIAL TARGET CELLS FOR GENE TRANSFER, Hendrik Veelken, Heike Jesuiter, Jan Knoblich, Helene Lohner, Joachim Schultze, Roland Mertelsmann and Albrecht Lindemann, Department of Internal Medicine I (Hematology/Oncology), University of Freiburg, Germany

A routine method to establish cultures of diploid fibroblasts (dFb) from human adults was developed using skin and peritoneal biopsy specimens from 50 cancer patients. After mincing of skin biopsies (mean surface area: 3.1 cm^2), enzymatic dissociation was found to be most efficient for subsequent long-term culture of dFb. 10^7 skin dFb were routinely obtained from patients younger than 70 years after a mean culture time of 33.4 days, with a mean population doubling time (PDT) of 4.7 days. Proliferation of established cultures generally remained stable with the potential to achieve 10^9 and 10^{11} cells after a mean time of 56.9 days (mean PDT=4.0 days) and 93.4 days (mean PDT=5.2 days), respectively. The longest cultures have demonstrated the potential of achieving well over 10^{13} cells without exhausting their proliferative capacity. Alternatively, dFb cultures could be established from peritoneal serosa with a mean time of 29.7 days to reach 10^7 cells (mean PDT=4.7 days).

Cell cycle analyses of representative dFb cultures showed a normal DNA content with 84% of cells in G0/G1, 6% in S, and 10% in G2/M phase. Efficient synchronization was observed after serum deprivation, followed by a single thymidine block. With regard to the use of genetically engineered dFb as a paracrine source of cytokines in cancer immunotherapy, cytokine profiles of untransfected dFb were determined before and after lethal irradiation. Unirradiated dFb produced >500 pg/ml IL-6 but only minimal amounts of IL-1α, TNFα, and GM-CSF. Irradiation significantly increased the secretion of IL-6, TNFα, and GM-CSF but not of IL-1α.

These data demonstrate that long-term dFb cultures can be established routinely from small biopsies of human adults and suggest that these cultures may represent a useful target for gene transfer into autologous cells of individual patients.

DZ 417 GENE THERAPY FOR MELANOMA USING M-CSF AND THE B16F10 CELL LINE, Patrick Walsh, Andrew Dorner, and L. Michael Glode, Department of Dermatology (PW) and Division of Medical Oncology (LMG), University of Colorado, Denver, CO 80262, and The Genetics Institute (AD), Cambridge, MA 02140

Melanoma is an aggressive malignancy with rapidly increasing incidence and no highly effective treatment for metastatic disease. We have developed a gene therapy model for metastatic melanoma using the B16F10 murine melanoma cell line in C57Bl/6 mice and the gene for human macrophage/monocyte colony stimulating factor (hu M-CSF). The B16F10 cell line, which was developed by Isaiah J. Fidler to have very high rates of metastases to the lungs, was transduced with the gene for hu M-CSF using a retroviral vector. Two distinct cell lines were developed and their characteristics are described in the table below. Injection of B16F10 cells into the tail vein of C57Bl/6 mice leads to the establishment of lung metastases and uniform mortality by week 7. Injection of the transduced (F10A2) or vector control (F10A3) cells leads to the establishment of metastases, however, these metastatic lesions are eliminated and the animals have similar survival rates as the non-injected controls. Injection of mixtures of B16F10 and either the transduced or vector control cells produce strikingly different survival rates. The animals that received a mixture of B16F10 and vector control cells developed metastatic disease and had 0% survival at 7 weeks. The group that received a mixture of B16F10 and the transduced, hu M-CSF producing cells had an 80% survival rate at 7 weeks.

CELL	GENE Neo	GENE M-CSF	METS (WEEK)	%SURV(WEEK)
B16F10	-	-	+ (2,3)	0% (7)
F10A2	+	+	+ (2), - (3)	100% (7)
F10A3	+	-	+ (2,3)	80% (7)
MIXTURES OF CELLS:				
B16F10 + F10A2			+ (3)	80% (7)
B16F10 + F10A3			+ (3)	0% (7)

These findings suggest that co-injection of the transduced and parental cells generates a tumoricidal response that is generalized and effective in eliminating the non-transduced tumor cells. This treatment approach holds promise for the treatment of metastatic melanoma in humans.

Gene Transfer in CNS

DZ 500 VARIATION IN RIBOZYME ACTIVITY AGAINST INTERPHOTORECEPTOR RETINOID BINDING PROTEIN (IRBP) MESSENGER RNA BY SITE AND COMPLEMENT LENGTH, Steven L. Bernstein, NEI, NIH, Bethesda, MD 20892.

Determining the optimal length for up- and downstream complement length against a messenger RNA target, and evaluating whether site specificity (outside of the GUX cleavage motif) has any notable effect on ribozyme activity are critical delineators of the usefulness of trans-active hammerhead ribozymes. IRBP is a retina- and pineal specific protein that is the main transporter of retinoids between the retinal pigment epithelium and neurosensory retina. A 110 nt IRBP mRNA fragment was analyzed for possible differences in secondary structure by the GCG-FOLD program. Two sites in the messenger RNA, both containing the GUC cleavage motif, were targeted for ribozyme activity. The DNA template for the mRNA substrate was directionally cloned into Bluescript II KS(+) vector and was subsequently dideoxy sequenced to assure fidelity. The construct was then linearized with Pvu II. Transcription with T_3 RNA polymerase yielded a 312 nt fragment containing the IRBP mRNA substrate. Ribozymes targeted to cleave either at the 17th nt (A) or 55th nt (B) of IRBP were synthesized, either by the oligodeoxynucleotide template-partial duplex transcription method, or by Pvu II linearized templates that were cloned into the KS (+) vector. Ribozymes were tested in-vitro against substrate in buffer containing 50mM tris-15mM $MgCl_2$ at 55°C. The extent of ribozyme activity was site dependant, with marked differences in activity at equivalent complement lengths. Ribozyme activity at both sites decreased as the complement length decreased to 8 nt; further decreases in complement length abolished activity. The ribozyme constructs exhibiting maximal in-vitro activity were ligated to a human β-actin promoter, and used to generate transgenic mouse strains. IRBP mRNA levels were evaluated by the polymerase chain reaction and northern analysis. These results will be discussed as to their significance for gene down regulation and gene therapy.

DZ 501 HORMONES REGULATE THE EXPRESSION OF THE GENE FOR THE RECEPTOR FOR ECOTROPIC RETROVIRUSES.

Maria Hatzoglou, Dan Robinson, Hsing-Jieu King and Jin Yun Wu. Department of Nutrition, School of Medicine, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH 44106-4906

The infectibility of the regenerating rat liver by ecotropic retroviruses was studied relative to the expression of the gene for the ecotropic retrovirus receptor (Ecor)/cationic amino acid transporter. It is known that the gene for the receptor is expressed in primary hepatocytes and hepatoma cells but it is absent in the adult liver. Since cell division is required for liver cells to be infected with ecotropic retroviruses, we determined the susceptibility of the regenerating rat liver to infection, at different time points after partial hepatectomy (0-24 hr), in relation to the presence of the receptor mRNA. Infection of the liver occurred only when the liver was exposed to virus 4 hr after partial hepatectomy. This time point of infection paralleled the expression of the gene for the ecotropic retrovirus receptor, which was rapidly induced only in the G1 phase during liver regeneration. However, the expression of the dormant receptor gene in the quiescent liver can be induced by insulin, dexamethasone and arginine, indicating that cell division is not required for expression of the receptor gene in the liver. A diet high in carbohydrate (low in protein) increased significantly the concentration of the receptor mRNA in the liver, indicating a role of hormones in the regulation of expression of this gene *in vivo*. We conclude that the gene for the viral receptor is expressed in the regenerating and quiescent liver when the urea cycle enzymes are down regulated. The infection of the regenerating rat liver by ecotropic retroviruses at the time point of expression of the receptor gene, supports the requirement of expression of this transporter for infection. However, cell cycle dependent factors may play an important role in the infectibility of hepatocytes. Isolation of a 2.85 kb cDNA for the rat Ecor suggested that the rat viral receptor is 97% homologous to the mouse and it contains the envelope binding domain that determines the host range of ecotropic murine retroviruses. This explains the efficient infection of rat cells by ecotropic retroviruses.

DZ 502 METALLOTHIONEIN PROMOTER-INDUCED EXPRESSION OF NERVE GROWTH FACTOR IN PC12 BRAIN GRAFTS: POTENTIAL UTILITY IN GENE THERAPY OF NEURODEGENERATIVE DISEASES, Curtis A. Machida,[1,2] Daniel C. Rohrer,[3] Valerie Nipper,[1] and Gajanan Nilaver.[4] [1]Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006. Departments of [2]Biochemistry and Molecular Biology and [4]Neurology and [3]Division of Neurosurgery, Oregon Health Sciences University, Portland, OR 97201

An optimal cell line for use in transplantation models of Parkinson's and Alzheimer's diseases would: (i) be immortal and pluripotent; (ii) differentiate to functionally replace degenerating pathways; and (iii) induce localized neuronal sprouting of surviving host cells through the release of trophic factors. PC12 cells propagate in culture, neurodifferentiate in the presence of nerve growth factor (NGF), and produce dopamine and/or acetylcholine. However, while NGF-treated PC12 cells can ameliorate neurotransmitter deficits in rats, they degenerate post-implantation without sustained NGF availability. We have introduced an 820 bp cDNA recombinant encoding mouse NGF (mNGF), under control of the human metallothionein IIA zinc-inducible promoter, into PC12 cells via calcium phosphate transfection. Southern blot analysis of polymerase chain reaction (PCR) products has verified incorporation of the transgene into the host genome in 36 stable transfectant cell lines. Northern blot analysis and mNGF immunocytochemistry (ICC) have identified several transfectants exhibiting substantial IIA promoter "off-on" response, and high inducibility of mNGF transcription/translation. Grafts into the striatum of dietary zinc-supplemented rats are being examined by *in situ* hybridization and ICC to verify inducible post-transplant neurodifferentiation. The potential application of these genetically-programmed cells for use in human neurodegenerative diseases is being explored in established rat models of Parkinson's and Alzheimer's diseases.

DZ 503 EVALUATION OF HERPES SIMPLEX VECTORS FOR VIRUS-MEDIATED GENE SUPPRESSION IN THE CNS, Christopher A. Meaney and Joseph C. Glorioso: Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Herpes simplex virus (HSV) derived vectors provide a method for the expression of manipulated genetic material within post-mitotic neuronal cells and may prove useful both in treatment of CNS disorders and for basic studies of nervous system function. The need to prevent expression of mutant proteins in some genetic therapies has lead us to examine HSV-based expression of several genes transcribed by RNA polymerase III because these are expressed at the high levels necessary for antisense RNAs and ribozymes to be effective (Jennings and Molloy 1987, Cotten and Birnstiel 1989). Plasmids containing the VAI gene of Adenovirus, the EBER gene of Epstein Barr virus, and the mammalian neural specific BC1 element were integrated into replication-defective HSV backbones using a phage (CRE/*lox*) recombination system. Northern analysis of cellular RNAs revealed that although EBER and VAI transcripts were seen at all stages of productive infection the BC1 gene likely requires viral replication for expression; only the VAI and EBER RNAs were detected both in cells pretreated with the protein synthesis inhibitor anisomycin and in the presence of the inhibitor of DNA synthesis phosphonoacetic acid (PAA). This pattern of BC1 transcription is similar to that previously described for human Alu sequences integrated into HSV (Panning and Smiley 1989) while expression of the EBER and VAI genes occur independently of viral gene products. HSV vectors are also being evaluated which use the VAI gene to express antisense *c-fos* transcripts. An antisense construct where VAI produces a full length antisense RNA of the *fos* first intron and exon and an RNA terminating prematurely within the intron will degrade *c-fos* mRNA induced in PC12 cells. This and several additional viruses are being examined in culture for their effects on *c-fos* protein production and on mRNAs of related bZIP family members.

DZ 504 Enzyme Replacement With Recombinant β-Glucuronidase Dramatically Reduces Lysosomal Storage In The CNS Of Mice With Mucopolysaccharidosis Type VII, M.S. Sands[1,2], C.A. Vogler[3], J. Grubb[4], A. Higgins[2], A. Torrey[2], B. Levy[3], W.S. Sly[4], E.H. Birkenmeier[2], [1]The University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA 19104, [2]The Jackson Laboratory, Bar Harbor, ME 04609, [3]Dept. of Pathology and [4]The Edward A. Doisy Dept. of Biochemistry and Molecular Biology, St. Louis University School of Medicine, St. Louis, MO 63104

Murine MPS VII has been treated with varying degrees of success by bone marrow transplantation (BMT) in adults and neonates, by hematopoietic stem cell targeted gene therapy, and with organoids producing recombinant β-glucuronidase. However, none of these therapies effectively reduce or prevent the accumulation of lysosomal storage material in the CNS.

Recombinant β-glucuronidase is rapidly cleared from the circulation of newborn MPS VII mice following intravenous injection. Normal or above normal levels of enzyme activity can be detected in most tissues known to express the mannose-6-phosphate receptor. One hour after injection, the brain contains approximately 30% normal levels of enzyme activity. Depending on the tissue, injected enzyme has a half life of 1.5-4.5 days during the first 7 days of age. At six weeks of age, MPS VII mice receiving weekly injections for five weeks starting at birth have 27, 3.5 and 3.3% normal levels of enzyme activity in liver, spleen and kidney, respectively. Liver, spleen and kidney from treated MPS VII mice have little or no microscopic evidence of lysosomal storage and growth of the skull and long bones is markedly improved. Brain has detectable levels of β-glucuronidase and secondary elevations of α-galactosidase and β-hexosaminidase are decreased compared to untreated MPS VII mice. Remarkably, lysosomal storage in cortical and hippocampal neurons is greatly reduced or absent in animals receiving weekly injections from birth. These data indicate that injection of β-glucuronidase starting at birth prevents many pathological changes in most tissues including the CNS and may be an effective long term therapy either alone or in combination with BMT or gene therapy.

DZ 505 TRANSPLANTATION OF β-GLUCURONIDASE (GUSB)-EXPRESSING IMMORTALIZED NEURAL PROGENITORS FOR GENE TRANSFER INTO CNS OF THE MUCOPOLYSACCARIDOSIS (MPS) VII MOUSE, A MODEL OF A NEUROVISCERAL LYSOSOMAL STORAGE DISEASE. Evan Y. Snyder,*#+ Rosanne M. Taylor*@, John H. Wolfe@+; Depts.of Neurology & Pediatrics, Harvard Medical School, Boston, MA 02115# *and* Dept. of Medical Genetics, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA 19104.@

[*contributions of first 2 authors equal; +corresponding authors]

The C17-2 multipotent immortalized neural progenitor cell line has been demonstrated previously by us to engraft and participate in development of the mouse CNS in a nontumorigenic, cytoarchitecturally appropriate manner [Snyder et al, *Cell* 68:33,1992; *Soc. Neurosci. Abstr.* 19:613,1993]. Furthermore, engrafted cells could stably and robustly express an exogenous reporter gene (*lacZ*). These data suggested that using such immortalized progenitors as transuction agents for exogenous factors and as integral members of the CNS cytoarchitecture may be feasible for clinical and research applications. The MPS VII mouse, which lacks GUSB, is an ideal model for neurovisceral lysosomal storage diseases [Wolfe et al, *Nature* 360:749,1992; *PNAS* 87:2877,1990; *Nature Genetics* 1:379,1992]. We report successful diffuse engraftment within MPS VII mice of C17-2 cells expressing GUSB. We developed techniques for diffuse engraftment of GUSB-expressing cells throughout the neuroaxis of newborn mutants. At maturity, recipients were analyzed for lacZ and GUSB expression, GUSB enzymatic activity, lacZ and human GUSB proviral sequences via PCR, and reduction in lysosomal storage at the light & ultrastructural level. All transplanted MPS VII mutants had evidence of engraftment and GUSB activity diffusely throughout the brain. In all animals (n=9 to date), activity was at least 1-4% of normal levels in various regions, and, in some regions of at least 1 animal to date, as much as 20% normal levels. These levels are sufficient to decrease storage based on data in other organ systems [Wolfe et al, ibid]. Neuropathologic analyses are ongoing. Engraftment and expression could be detected at least 8 mos. post-transplant. Engraftment in wild-type mice boosted GUSB levels above normal without adverse effect. Because the blood-brain barrier may impose restrictions to entry of enzyme supplied peripherally (either directly or through genetically-engineered somatic or bone marrow cells) and because bone marrow transplantation entails irradiation which may be inimical to developing CNS, the approach described here may provide a paradigm for therapy of the CNS manifestations of other neurovisceral diseases.

DZ 506 IN VIVO INTERLEUKIN-6 GENE TRANSFER BY PARTICLE BOMBARDMENT REDUCES MURINE TUMOR GROWTH IN MICE. Sun WH, Chen L*, Sun J*, Pugh T, Ershler WB and Yang NS*. Department of Medicine, University of Wisconsin-Madison, and *Department of Mammalian Genetics, Agracetus Inc., Madison WI 53706.

We have previously shown that interleukin-6 (IL-6), one of the major mediators of the inflammatory response, demonstrates potent anti-tumor activity in mice. Administration of recombinant IL-6 was effective in abrogating certain murine tumors and some IL-6 transfected tumor cells fail to give rise to tumors. Here we describe a novel approach to introduce transgenic IL-6 production in subcutaneous tumors and surrounding tissues by *in situ* gene transfer using particle bombardment.

Female C57/BL6 mice (6-8 weeks) were inoculated subcutaneously with MCA-induced murine fibrosarcoma cells ($1 \times 10^5/100\ \mu l$). Twenty four hours later the tumor cell injection sites were bombarded with DNA-coated gold particles (1-3 μM in diameter) using the particle bombardment method. The plasmids contained human IL-6 cDNA cloned in a pBCMG-*neo* expression vector driven by a cytomegalovirus promoter. Subsequent tumor growth was measured daily with tissue calipers and the net tumor weights were recorded at the end of each experiment.

Our data from four independent experiments showed that the tumors bombarded with IL-6 were smaller (approximately 50-70% reduction in size) than tumors receiving control vector DNA (backbone of the pBCMG-*neo* plasmid containing no IL-6 gene). We did not observe any apparent histologic differences between the tumors in the two groups.

The particle bombardment gene transfer offers a promising method of delivering anti-tumor cytokine genes to tumors and may have potential clinical applications in gene therapy of cancers.

DZ 507 ENZYME REPLACEMENT IN MPS VII BRAIN FOLLOWING TRANSPLANTATION OF GENETICALLY MODIFIED FIBROBLASTS
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Mice with ß-glucuronidase (GUSB)-deficient mucopolysaccharidosis (MPS) VII develop visceral and neural lesions of lysosomal storage. MPS VII fibroblasts infected with a double copy retroviral vector (DCHBH) expressed human GUSB cDNA from the native promoter. These corrected cells secreted high levels GUSB and cross-corrected other deficient MPS VII cells. Infected fibroblasts were stereotaxically injected into the cerebral cortex and ventricles of MPS VII mice. After transplantation the total brain GUSB activity was 42% of heterozygous levels at 2 days, 20% at 6 days, 2.5% at 1 month, and was still above background at 2 months. The localized production of GUSB was higher, reaching 78% of heterozygous levels at the injection site. GUSB producing cells were detected histochemically at the sites of injection and throughout the ventricular system for 4-6 weeks following injection, and occasional enzyme-positive cells were detected at injection sites for up to 6 months. Infected fibroblasts were detected by PCR or fibronectin staining for 5-11 months following injection, demonstrating the long-term viability of the grafts. High level expression was found early after engraftment and, although proviral sequences appeared to be down-regulated in vivo, low level expression occurred for at least 6 months. This is a promising method for delivering secreted gene products to the brain, as a treatment for disorders which cause generalized lesions such as the mucopolysaccharidoses. A transfected cell line that appears to express GUSB long-term in vivo is currently being tested to determine if enzyme production from engrafted fibroblasts can be maintained for extended periods of time in the brain.

DZ 508 CAN JC VIRUS BE USED AS A VECTOR FOR GENE DELIVERY TO OLIGODENDROCYTES AND ASTROCYTES OF THE CNS WHITE MATTER? Carlo Tornatore, Karen Meyers and Gene Major, Section on Molecular Virology and Genetics, National Institutes of Health, Bethesda, MD 20892.
JC virus has a host range in the human CNS which is restricted to subcortical oligodendrocytes and astrocytes. Given this tropism for CNS white matter, we asked whether chimeras of JC virus could be used as vectors for gene transfer in leukoencephalopathies. Because of its small size, the only portion of the viral genome which can be disrupted without interfering with the viral structural genes is the T protein coding early region. However, in the absence of T protein, JCV is unable to replicate. We have previously described the successful grafting of immortalized human fetal astrocyte cell line SVG in the primate CNS, a cell line which expresses T antigen and supports JCV replication. This suggests that SVGs could act as both a packaging and delivery system to the host CNS of a chimeric JC virus which lacks the T protein gene. We have constructed a chimera using the prototype MAD-1 isolate, in which the the early region has been replaced by the cDNA for a marker gene (in this case tyrosine hydroxylase) driven by the viral early promoter/enhancer elements. Transfection into both primary human glial cells and the SVG cell line resulted in expression of tyrosine hydroxylase within 3 days, demonstrating that JCV chimeras will express genes substituted into the early region. Experiments will determine the extent of late gene expression, replication and packaging of the chimera into JC viral coat proteins.

DZ 509 DIFFERENTIATION OF PHEOCHROMOCYTOMA CELLS *IN VITRO* BY NERVE GROWTH FACTOR EXPRESSED FROM A NOVEL HSV-1 VECTOR, Ming Jing Wang, Paul A. Johnson and Theodore Friedmann, Center for Molecular Genetics, UCSD School of Medicine, La Jolla, CA 92093
Pheochromocytomas belong to class II multiple endocrine neoplasia syndrome (MENII) and originate from the adrenal medulla. PC12 cells, a cell line derived from a rat pheochromocytoma, respond to exogenous nerve growth factor (NGF) and can be differentiated into sympathetic neuron-like morphology with extensive neurite outgrowth *in vitro*. NGF binds to a high affinity receptor, $p140^{prototrk}$, and signal transduction is accomplished by activation of the receptor tyrosine kinase upon ligand binding. A smaller receptor molecule, $p75^{NGFR}$, also binds to NGF at low affinity and in the absence of ligand this receptor may induce cell apoptosis.
We have constructed a series of replication-defective herpes simplex virus type 1 (HSV-1) vectors for gene transfer purposes. Some of these vectors lack HSV-1 immediate early gene 3 (IE3 or ICP4) and contain a VP16 insertion mutation. Upon infection, the double-mutant virus exhibits a marked reduction in cytotoxicity compared with the prototype IE3 single-mutant HSV-1 vector. Activity of a virus-transduced *E. coli* lacZ gene product, β-galactosidase, can be detected in infected cells up to one week after infection with the double-mutant vector. We have introduced the complete cDNA of mouse βNGF under the control of a modified LAT promoter in the double-mutant HSV-1 background. Infection of PC12 cells by the virus resulted in significant cell differentiation as indicated by neurite outgrowth in approximately 20% of the infected cells, compared with a 3% spontaneous neurite formation in uninfected cells. Addition of purified NGF at 50 ng/ml produced 40% neurite outgrowth. Simultaneous expression of lacZ from the same vector was also detected in both differentiated and undifferentiated cells after infection. The usefulness of HSV-1 vectors in the past has been hampered by problems of cytotoxicity and transient expression of inserted genes. The results described here demonstrate that the HSV-1 IE3/VP16 double-mutant can be used to induce a biological process requiring prolonged gene expression and survival of target cells.

DZ 510 MODULATION OF AUTOCRINE GROWTH REGULATION IN HUMAN GLIOMAS BY MOLECULAR THERAPY

W.K. Alfred Yung, Peter A. Steck, and Micheal Hogan Department of Neuro-Oncology, M.D. Anderson Cancer Center, Houston TX 77030

Epidermal Growth Factor Receptor (EGFR) has been shown to be amplified and overexpressed in over 40% of glioblastoma. TGF-a, a ligand to EGFR, has been shown to be over-expressed in malignant gliomas. Several antisense approaches have been utilized in our laboratory to modulate the growth regulatory activity of the EGFR/TGF-a autocrine loop. I) An antisense oligonucleotide (TGF-AC) was synthesized against the translation initiation site of TGF-a mRNA. Growth studies demonstrated a dose dependent growth inhibition of U251 cells by TGF-AC. More importantly, RT-PCR studies showed specific depletion of TGF-a mRNA in TGF-AC treated cells, and most interestingly, EGFR mRNA was also noted to be decreased, clearly indicate the ligand/receptor relationship of TGF-a/EGFR in U251 cells. II) A specific triple-helix forming oligonucleotides (Triplex) was designed against the promotor of the EGFR gene (EG4-ap). Growth studies with U251 and LG glioma cells showed dose dependent growth inhibition with the ED50 at 5mM concentration. Colony forming assay also demonstrated decrease in number and size of colonies by treated cells. RT-PCR analysis for EGFR mRNA showed specific inihibition of EGFR transcription by EG4-ap. Most excitingly, receptor binding analysis clearly showed depletion of receptor expression on the cell surface, suggesting that EG4-ap triplex was capable of inhibiting EGFR mRNA transcription, which in turn lead to decrease in receptor protein expression and growth inhibition. These studies illustrate several potentially exciting molecular agents/approaches that can be applied in the clinic for the treatment of malignant brain tumors.

DZ 511 HIGH-EFFICIENCY GENE TRANSFER AND HIGH-LEVEL EXPRESSION OF WILD-TYPE p53 IN HUMAN LUNG CANCER CELLS MEDIATED BY RECOMBINANT ADENOVIRUS.

Wei-Wei Zhang, Xiangming Fang, Wojciech Mazur, Brent A. French, Renee N. Georges, and Jack A. Roth. The Department of Thoracic and Cardiovascular Surgery, The Department of Hematology, The University of Texas M. D. Anderson Cancer Center and The Department of Medicine, Section of Cardiology, Baylor College of Medicine, Houston, Texas.

A replication-defective and helper-independent recombinant p53 adenovirus (Ad5CMV-p53) was generated. Ad5CMV-p53 carries an expression cassette that contains cytomegalovirus (CMV) promoter, human wild-type p53 cDNA, and SV40 polyA signal. Three human non-small cell lung cancer cell lines represented by their differences in p53 status were used for evaluation of Ad5CMV-p53. In the H358 cell line, which has a homozygous p53 deletion, a 100% transfer efficiency of the p53 gene was detected by immunohistochemistry when the cells were infected with Ad5CMV-p53 at 30-50 PFU/cell. Western blots showed a high level of p53 expression. The protein expression peaked at day 3 postinfection and lasted at least for 15 days. Growth of the infected H358 cells was inhibited 79% when compared to that of noninfected cells or the cells infected with the control virus. Growth of human lung cancer cell line H322, which has a point mutation in p53, was inhibited 72% by Ad5CMV-p53, while growth of human lung cancer H460 cells containing wild-type p53 was less affected (28% inhibition). Tumorigenicity tests in nude mice demonstrated that infection of Ad5CMV-p53 prevented the H358 cells from forming tumors. In a mouse model of orthotopic human lung cancer, intratracheal instillation of Ad5CMV-p53 inhibited tumor formation by H226Br cells that were inoculated intratracheally 3 days prior to the virus treatment. These results suggest that adenovirus is an efficient vector for mediating transfer and expression of tumor suppressor genes in human cancer cells and that Ad5CMV-p53 may be further developed into a therapeutic agent for cancer gene therapy.

Tissue Specific Gene Expression; AIDS Gene Therapy

DZ 600 RETROVIRAL TRANSFER OF THE LACZ GENE INTO HUMAN CD34+ CELLS: PROSPECTS IN THE ANALYSIS OF THE AUTOLOGOUS STEM CELL TRANSPLANTATION.

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The analysis of the behaviour of reimplanted genetically modified cells represents a major goal in gene therapy. In order to get a precise view of autologous hematopoietic stem cell grafts we decided to mark human hematopoietic cells by transferring the bacterial beta-galactosidasis gene into CD34+ cells with retroviral vectors prior to reimplanting them into the patient. Several experimental considerations, including easier detection of the expression, argues for the use of the nlsLacZ gene versus the LacZ gene. X-gal staining of infected CD34+ cellular populations revealed that 1 to 10% of the cells could express the nlsLacZ gene after a 48 hours cocultivation of the target cells with a ψ-crip derived packaging cell line which produces the vector MFG-nlsLacZ. This percentage was maintained throughout the proliferation and the differentiation of the cells until the end of the cellular culture. Blue colonies obtained by plating X-gal+ infected cells in methylcellulose confirmed the absence of cytotoxicity of the expression of the nlsLacZ gene in CD34+ precursors derived cells. In parallel and as a model, TF-1, a growth factor dependent human myeloid cell line, was infected with the same vectors and we obtained up to 80% of transduction were obtained assessed by FDG-FACS analysis or by X-gal staining. The analysis of cellular populations containing 100% TF 1 blue cells reveals that neither the proliferation rate nor the differentiation were modified by the expression of the LacZ gene. These results lead us to propose a clinical trial: CD34+ cells will be purified from peripheral blood of patients stimulated by G-CSF. 50% of them (2 10^6/kg) will be cocultivated in presence of a growth factor cocktail with the packaging cell line. After reinjection, expression and presence of the nlsLacZ gene in hematopoietic tissues and blood will be followed by X-Gal staining and PCR analysis. This protocol is presently submitted to the relevant committees for approval.

DZ 601 ANALYSIS OF RETROVIRALLY TRANSDUCED ANTI HIV-1 GENES IN LONG-TERM BONE MARROW CULTURES,

Ingrid Bahner, Chen Zhou, QianLin Hao and Donald B. Kohn, Department of Research Immunology, Childrens Hospital of Los Angeles, Los Angeles, CA 90027

One possible gene therapy approach for AIDS may involve bone marrow transplant of autologous or allogeneic stemcells, transduced to express one or a combination of HIV resistance genes. We and others have recently reported that the rev trans dominant mutant M10 is a very efficient HIV-IIIb inhibitory gene when retrovirally transduced into the CEM T cell line. We have also demonstrated that a hammerhead tat/rev ribozyme and a RRE decoy construct, both expressed in the retroviral vector LN, are effective against HIV replication in the same cell line. These initial promising results have been confirmed with the clinical isolate SF 2 in CEM cells. We are now testing the above vectors in primary long term human bone marrow cultures. Briefly, a CD34+ sorted cell pool is transduced with the above retroviral vectors and long term cultures are established by plating the transduced cell pool on bone marrow stromal monolayer and selecting for expression of the neo gene. Preliminary results suggest that the transduction of human hematopoietic progenitor cells with the various vector constructs has no cytotoxic side effects on hematopoesis as measured by CFU plating of vector transduced and control-transduced CD34+ cells. After the selection period, progeny cells are challenged with the monocytotrophic strain JR-FL. We are further investigating the effects of virus infected bone marrow cells on the stroma cells and their ability to support normal hematopoesis. When HIV-infected bone marrow cells are grown on stroma the number of progenitor cells is reduced by 80% as compared to HIV infected bone marrow cells grown in a growth factor cocktail without stroma. We hypothesize that the abnormal hematopoesis observed in patients with AIDS may be caused by stroma cells that have been infected with virus or absorbed the virally encoded tat protein. We therefore are testing the ability of stroma transduced with the above HIV resistance gene(s), including a tat inhibiting vector, to support normal hematopoesis.

DZ 602 IMMUNE RESPONSE IN CYNAMOLOGOUS MONKEYS VACCINATED WITH RECOMBINANT DNA CONTAINING THE GENE FOR HUMAN IMMUNODEFICEINCY VIRUS TYPE 1 ENVELOPE, Jean D. Boyer*, Bin Wang*, Lori Gilbert*, Kesen Dang*, Michael Merva*, Chris Phan*, Kenneth Ugen*, Leslie Cooney+, Rich Carrano+, William V. Williams** and David B. Weiner*, *Department of Pathology, University of Pennsylvania, Philadelphia PA,19104, **Department of Medicine, Hospital of the University of Pennsylvania, Philadelphia PA, 19104, Apollon, Malvern PA, 19355.

Direct gene inoculation *in vivo* has been shown to lead to immune responses against the encoded proteins. This technique, therefore, may be a viable vaccination option with certain advantages over standard vaccine approaches. For instance, only the genes which contain the immunodominant regions may be cloned into the expression vectors while eliminating any genes that might allow the vector to revert to a replication competent virus. Our lab has cloned the gene for HIV-1 envelope, gp160, was into an expression vector containing the rous sarcoma virus (RSV) enhancer, the mouse mammary tumor virus long terminal repeat (MMTV LTR) promoter and the SV40 polyadenylation and splicing sites. Cynamologous monkeys were immunized once every two weeks with 100 µg of the recombinant DNA and blood samples were collected before each injection and tested for the presence of an anti-HIV immune response. Sera samples showed the presence of anti-envelope antibodies against gp120 and gp41. Peripheral blood lymphocytes were isolated and used to show that T cells also recognize HIV-1 envelope.

DZ 603 **A MODIFIED RETROVIRAL VECTOR EXPRESSING AT HIGH LEVEL IN EMBRYONIC CARCINOMA (EC) CELL LINES.** P.M. Challita, M. Dawson, L. Hao, D. Skelton, C. Cook, L.S. Sender, and D.B. Kohn. Childrens Hospital and Department of Microbiology, University of Southern California Medical School, Los Angeles, CA 90033.

MoMuLV-based retroviral vectors are the most efficient gene transfer vehicles into large number of cells for the purpose of gene therapy. However, the MoMuLV-LTR has been observed to be inactive and become *de novo* methylated when transduced into EC cell lines and *in vivo* murine hematopoietic stem cells (HSC). In an attempt to overcome the inactivity of the LTR, we have derived a novel retroviral vector (MP-ncr-dl-neo) containing several modifications in the transcriptional unit. The vector is based on the myeloproliferative sarcoma virus (MPSV)-LTR and contains a substitution of the wild-type primer binding site (PBS) with that isolated from the dl587rev retroviral strain. It has also been engineered to delete a negative control element located at the 5' end of the U3 region. The MP-ncr-dl-neo vector was tested for expression in EC cell lines by titering and Northern blot analysis. It shows a 20-40 fold increase in titer over the level observed from a standard vector (LN) containing the MoMuLV-LTR and PBS, and 10-fold over the level obtained from the same vector lacking the deletion of the negative control element (MP-dl-neo). Currently, the methylation status of the modified vector in EC and ES cell lines is under study. We are also analyzing the modified vector in murine HSC by performing gene transfer/bone marrow transplantation. The construction of a retroviral vector with consistent expression after transduction of pluripotent stem cells has would be important for the application of gene therapy *via* bone marrow.

DZ 604 HUMAN LYMPHOCYTES INTRACELLULARLY EXPRESSING AN ANTI-GP120 SINGLE-CHAIN ANTIBODY ARE RESISTANT TO HIV-1 INFECTION, Si-Yi Chen, Jessamyn Bagley, and Wayne A. Marasco. Division of Human Retrovirology, Dana-Farber Cancer Institute, Department of Medicine and Pathology, Harvard Medical School, Boston MA 02115.

A single-chain antibody, derived from a human monoclonal antibody that recognizes the CD4 binding region of the human immunodeficiency virus type 1 (HIV-1) envelop protein, has been designed for intracellular expression in mammalian cells. The single-chain antibody is composed of an immunoglobulin heavy-chain leader sequence and heavy and light-chain variable regions that are joined by an interchain linker. This antibody is stably expressed and retained in the endoplasmic reticulum and is not toxic to the transformed COS cells. The antibody binds to the nascent gp160 and blocks its maturation, which results in the inhibition of syncytia formation and production of infectious virus. Furthermore, an expression vector was constructed in which the gene encoding the single-chain antibody is expressed under control of HIV-1 LTR. A transduced human T-cell line showed an inducible expression of the antibody in the presence of tat protein or when infected with HIV-1. A significant inhibition of surface expression of the envelope protein and syncytium formation was observed in the transformed T-cells. The virus infection in the cell culture is significantly inhibited. Thus, intracellularly expressed antibodies may have a potential application in gene therapy of AIDS and other diseases.

DZ 605 **INDUCTION OF GENES IN CELLS BY 3-DEAZA-ADENOSINE ANALOGS AND THEIR POSSIBLE CORRELATION WITH ANTI-HIV ACTIVITIES**, P. K. Chiang, B. P. Doctor, D. S. Burke, G. C. Zeng, R. L. Copeland, R. K. Gordon, and D. L. Mayers, Walter Reed Army Institute of Research, Washington, DC 20307-5100

3-Deaza-adenosine (DZA) analogs are potent inhibitors of S-adenosylhomocysteine (AdoHcy) hydrolase. In cells treated with DZA analogs, AdoHcy accumulates, leading to an inhibition of methylation reactions. DZA analogs have been shown to activate the gene expression of collagen IV in F9 cells [J. Biol. Chem. 267, 4988 (1992)]. Additionally, confluent 3T3-L1 fibroblasts treated with DZA differentiate into adipocytes [Science 211, 1164 (1981)]. DZA produced in the confluent 3T3-L1 cells a rapid but transient expression of mRNA for the proto-oncogenes c-*fos* and c-*jun* within 30-60 min, after which the mRNA of both proto-oncogenes became undetectable. In comparison c-*myc* proto-oncogene became detectable after 2 h, and then declined after 4 h. Furthermore, in cells transfected with c-*fos*-CAT, the CAT activity was activated by DZA in a dose-dependent manner. Electrophoretic mobility shift assays showed an increase in the levels of transcription factors AP-1 and AP-2 1 h following treatment with DZA. When tested against human immune deficiency virus type-1 (HIV-1) in vitro, DZA analogs exhibit potent anti-HIV-1 activity, as measured by the reduction of syncytia formation and p24 antigen in cells. Moreover, the 3-deaza nucleosides are effective against AZT-resistant HIV isolates. The relationship between gene activation by DZA analogs and anti-HIV-1 activity is being explored.

DZ 606 CLONING AND CHARACTERIZATION OF A ROUS SARCOMA VIRUS ENHANCER BINDING FACTOR AND EVIDENCE FOR ITS ROLE IN HOST TISSUE PERMISSIVENESS, Peter J. Cleavinger, Jagan C. Kandala, Matt C. Harline, Boo Shin, Jan Svoboda, Ashok Nambiar, and Ramareddy V. Guntaka, Department of Molecular Microbiology and Immunology, University of Missouri, School of Medicine, Columbia, MO 65212

Earlier work from our laboratory indicated that at least four factors from avian cells bind to distinct sequences in the enhancer region (-234 to -54) of Rous sarcoma virus LTR (Kenny and Guntaka, 1990, *Virology*, **176**:483-493). We have now isolated three different, yet related, clones from a chick embryo cDNA library by expression cloning based upon their ability to bind the RSV LTR. All three are related to the Y box (inverted CCAAT) binding proteins and share extensive amino acid homology over a span of 90 to 100 amino acids. One of these factors, RSV-EFII, has been expressed in *E. coli* as a fusion protein and analyzed by gel mobility shift assay. Results indicate that it interacts strongly with the E4 region as well as E3-1 and E1 (-149 to -126). Although EFII shares homology to the Y box binding protein family, it failed to interact strongly with the inverted CCAAT boxes located at -65 to -69 and -129 to -133. Northern analysis indicates variable tissue distribution greatest in muscle tissue and cultured fibroblasts, paralleling the spectrum of host permissiveness of RSV. Furthermore, when EF-II is expressed *in vivo*, it is shown to transactivate transcription from the RSV LTR. Taken together these data indicate that the cellular factor EF-II participates in the tissue specific expression of Rous sarcoma virus.

DZ 607 REDIRECTING THE CYTOTOXIC CELLS OF THE IMMUNE SYSTEM USING RETROVIRAL TRANSDUCTION OF CHIMERIC ANTIGEN RECEPTOR GENES. T. Dull, M. Finer, K. Cooke, L Qin, D. Ruff, K. Zsebo and M. Roberts.

We have developed a strategy to redirect the specificity of the hematopoietic system's cytotoxic cells toward targets such as virally infected or tumor cells. MHC unrestricted chimeric antigen receptor molecules (UR), encoding a single chain antibody fused to the CD3 complex associated- z chain, have been efficiently introduced into either human CD8+ T cells (up to 40% transduction), murine hematopoietic stem cells and human CD34+ cells (up to 10% and 45% transduction, respectively) using the novel *kat* transient retroviral production system. The *kat* system enables delivery of UR constructs without the need to generate stable retroviral producer clones for each construct. *kat* vectors expressed UR constructs at levels equivalent to those observed in NIH 3T3 cells, sufficient to purify transduced cells to homogeneity by immunoaffinity chromatography. Introduction of universal receptors into CD8+ T cells *in vitro* results in the activation of a number of T cell effector functions including cytokine secretion, proliferation and cytolysis, in response to target cells expressing the appropriate target antigen. The ability to redirect the cytotoxicity of myeloid or NK cells derived from transduced murine stem cells *in vivo*, and granulocytes derived from transduced human CD34+ cells *in vitro* are being evaluated.

DZ 608 **MOLECULAR ANALYSIS OF THE B CELL-SPECIFIC IMMUNOGLOBULIN λ LIGHT CHAIN GENE ENHANCERS,** Charles F. Eisenbeis, Harinder Singh, and Ursula Storb, Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637

The murine immunoglobulin λ light chain gene locus contains a pair of highly homologous, B cell-specific transcriptional enhancers, each associated with, and lying 3' of a Jλ-Cλ cluster. In the human, there is a single enhancer in the λ locus which lies 3' of the cluster of seven Jλ-Cλ genes. The human λ enhancer shares greater than 70% homology with its murine counterparts over its functional core. In an effort to understand the cell type- and developmental stage-specific expression of the λ genes, we have undertaken a molecular analysis of the regulatory activity of the murine λ enhancers. Putative sites of protein binding have been identified within these enhancers. Two of these sites, λA and λB, bind B cell-specific factors, and are absolutely required for enhancer function. A B cell-restricted complex which forms specifically on λB contains both PU.1 (a member of the Ets family whose expression is limited to cells of the hematopoietic system with the exception of T cells) and a second factor. These proteins bind cooperatively to a bipartite site within the λB sequence; the second factor, which cannot bind autonomously, relies on PU.1 to recruit it to its adjacent but distinct binding site. Transient transfection assays using a CAT reporter have shown that both halves of this bipartite site are required for enhancer function. We are presently attempting to identify additional proteins which comprise the B cell-specific complexes which form on the λA and λB sites. Our hope is to elucidate the molecular basis for the cell type-specific function of the λ enhancers, and to understand the role these enhancers play in the development of both normal and leukemic lymphocytes.
(This work was supported by ACS grant IM-69901 to U.S.)

DZ 609 CHARACTERIZATION OF A CARDIAC-SPECIFIC AND DEVELOPMENTALLY UPREGULATED PROMOTER IN TRANSGENIC MICE, ¶*Wolfgang-Michael Franz, ¶Hugo A. Katus, §Gottfried Brem, *Reinhard Kandolf, *Max-Planck-Institute for Biochemistry, Martinsried, §Inst. f. Molek. Tierzucht, LMU, München, ¶Dept.of Cardiology, Univ.of Heidelberg, D-69115 Germany

Based on hybridization studies indicating constitutive expression levels of the endogenous myosin-light-chain-2 (MLC-2) gene in embryonic, fetal, and adult myocardium, a model system for selective targeting of genes to the heart of transgenic mice has been developed. A 2.1 kb DNA fragment of the 5' flanking region of the rat cardiac MLC-2 gene was fused to the firefly luciferase reporter gene and introduced into fertilized mouse oocytes. In four independent transgenic mouse lines, the expression of the MLC-2 luciferase fusiongene was found exclusively in heart muscle. In contrast to the endogenous MLC-2 gene, which is constitutively expressed in ventricles and outflow tract during cardiac embryogenesis and coexpressed in slow twitch skeletal muscle in adult mice, two properties of the 2.1 kb MLC-2 promoter were observed: (i) a cardiac-muscle restricted activity in embryonal, fetal and adult mice with no coexpression in any other tissues and (ii) a 10-fold upregulated expression activity during the embryonal period of ventricular loop and septum formation. These data suggest, that (i) the 2.1 kb DNA fragment of the cardiac MLC-2 gene contains the regulatory elements required for selective gene expression in cardiac myocytes *in vivo* and (ii) a growth-dependent control mechanism on either transcriptional or post-transcriptional level may exist during embryonal heart formation. This ventricular-specific 2.1 kb MLC-2 promoter will serve as a valuable tool to facilitate the overexpression of foreign gene products in the developing and mature heart muscle and may be of particular use for somatic gene delivery systems.

DZ 610 DIRECT COMPARISON OF AN MFG-BASED VECTOR TO AN N2-BASED VECTOR IN PRIMARY HEMATOPOIETIC CELLS.

W. Krall, P.M. Challita, J.A. Nolta, I. Riviere, P. Lehn, E.I. Ginns, R.C. Mulligan and D.B. Kohn. Childrens Hospital Los Angeles, the NIMH, and the Whitehead Institute.

The MFG retroviral vector was designed to produce optimal expression of inserted cDNA. The exact benefits of these modifications on gene expression in primary hematopoietic tissues have not been directly compared in a quantitative manner to that by a "standard" vector. We have, therefore, compared the expression by a pair of vectors, one based upon the N2 vector (G2) and one on the MFG vector (MFG-GC), which use the normal human glucocerebrosidase (GC) cDNA (relevant to Gaucher disease) as the reporter. In the 3T3-based packaging cells, the level of GC enzymatic activity from MFG-GC was 5.5-fold higher than from G2, despite a higher vector copy number in the G2 clone (6 copies vs 1). In the murine gene transfer/BMT model, each vector was used to transduce post-5-FU marrow by co-cultivation in the presence if IL-3, IL-6, SCF and LIF. A portion of the marrow was cultured for 2 days and then cytospin preparations were stained by immunohistochemistry using a monoclonal antibody specific for human GC protein. The staining of the marrow transduced by MFG-C was more intense than from the G2 transduced marrow. Each vector yielded >90% DNA-positive 1° CFU-S. The average level of GC enzymatic activity in 5 1° CFU-S transduced by G2 was 1.4-fold greater than endogenous, whereas that in 5 MFG-GC foci was 4.9-fold above endogenous. The level and splicing forms of GC RNA, immunoreactive protein and enzymatic activity is being studied in the hematopoietic organs after long-term reconstitution, in 2° CFU-S (where G2 is usually found to be transcriptionally inactive) and in human Gaucher bone marrow. These studies demonstrate that for the human GC cDNA, the MFG vector is advantageous for producing higher levels of enzymatically active glucocerebrosdiase.

DZ 611 RETROVIRAL-MEDIATED GENE EXPRESSION IN HEMATOPOIETIC CELLS USING HEMATOPOIETIC AND VIRAL PROMOTERS.

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Gene transfer into hematopoietic stem cells with expression targeted to the relevant maturing progeny has clinical applications for gene therapy of genetic diseases such as Gaucher disease. We hypothesize that if promoters of myeloid specific antigens, such as CD11b, that are upregulated with myeloid differentiation are used to direct expression of an exogenous gene, the expression of this gene would also be upregulated. MoMuLV based vectors using promoters from the myeloid differentiation antigen CD11b (LN11bGC), leukocyte antigen CD18 (LN18GC) and hematopoietic stem cell antigen CD34 (LN34GC) were compared to vectors with viral promoters (CMV:LNCGC, SV40:N2SVGC and MoMuLV LTR:LGCSN), to direct expression of the reporter gene glucocerebrosidase (GC). The LN vector was used as a negative control. HL-60 cells, a myeloid leukemia cell line, were transduced with the above vectors, selected in G418, differentiated into granulocytes using DMSO or monocyte/macrophages using PMA and GC activity measured. The endogenous GC activity was 92, 112 and 156 nmol/min/mg protein in undifferentiated, DMSO induced and PMA induced HL60 cells respectively. The net GC activity above the endogenous (LN-HL60 cells) activity was as below:

Net GC Activity (fold increase over corresponding LN)

	UNDIFFERENTIATED	DMSO	PMA
LGCSN	160	219 (3.0)	356 (3.3)
LNCGC	10 0 (1.0)	204(2.3)	
N2SVGC	11	59(1.5)	10(1.0)
LN11bGC	41	251(3.2)	165(2.0)
LN18GC	22	35(1.3)	-28(0.8)
LN34GC	24	26(1.2)	3(1.0)

Thus, CD11b promoter driven GC activity increases significantly with granulocytic and monocyte/macrophage differentiation, although is not initially as high as MoMuLV LTR in undifferentiated cells. We have transduced primary human marrow CD34+ cells by these vectors, differentiated them into granulocytes or macrophages using specific growth factors and are measuring GC expression.

DZ 612 STUDIES ON THE CELL SPECIFIC EXPRESSION OF THE PCB-BP (CC10) GENE

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The gene for the small secretory protein PCB-BP (Polychlorinated Biphenyl Binding Protein), also known as CC10 (10 kDa Clara Cell protein), is predominantly expressed in the nonciliated bronchiolar Clara cells in the airway epithelium of rodents (Lund et al., 1988, Hackett et al., 1992). High level synthesis and secretion makes it an abundant protein in the airway epithelium lining fluid and it is estimated to constitute 0.1-0.5% of total protein in bronchoalveolar lavage fluid.
The aim of our study has been to investigate the mechanisms behind this high level, cell specific expression and to explore the possibility to use the promoter of the PCB-BP gene to direct high level expression and secretion of proteins of possible therapeutic importance to the Clara cells *in vivo*.
Promoter elements from the rat PCB-BP gene thought to encompass the elements necessary for the high level, cell specific expression (Stripp et al., 1992) have been isolated and linked to a ß-Gal reporter gene. Results on the properties of these constructs in cell lines of human lung origin as well as in primary cultures of lung cells will be presented.
To investigate the possibility of using the PCB-BP promoter in gene therapy applications we have made a construct to express a fusion protein containing the PCB-BP signal peptide fused to the mature alpha-1 antitrypsin to give effective synthesis and secretion of the antitrypsin under the control of the cell specific PCB-BP promoter.

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DZ 613 TAT-MEDIATED ACTIVATION OF AN ISRE CONTAINING HIV-1 LTR AND ITS POTENTIAL USE FOR IFN GENE THERAPY;

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We have previously shown that the T cells containing the integrated HIV-1 LTR IFNA hybrid plasmid were nonpermissive to HIV-1 infection. These cells produced only very low levels of IFNA constitutively, however, in the presence of the Tat protein, IFN synthesis was increased by 100-fold. However, the transcriptional activation of HIV-1 LTR can also occur in an Tat-independent fashion. We have attempted in this study to construct a plasmid that would respond only to the Tat transactivation, but not to the stimulation by cytokines, heterologous viruses, and mitogens and have shown that deletion of the NF-κB elements in the human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR) completely abolished Tat-mediated transactivation in three human T cell lines, A3.01, Jurkat, and CEM-174, and also dramatically reduced the magnitude of activation in a monocyte line, U937. In contrast, when the NF-κB elements in the HIV LTR IFNA2 hybrid gene were replaced by an IFN-stimulated response element (ISRE)-containing DNA fragment derived from the 5' flanking region of the IFN-stimulated gene 15 (ISG15), Tat-induced transactivation was restored. Thus, by substituting the NF-κB motifs of HIV-1 LTR with the cis-acting element, ISRE, it is possible to achieve effective Tat transactivation and high levels of Tat-induced IFN synthesis and reduce the nonspecific activation of HIV-1 LTR. These results suggest that it is attainable to target IFN synthesis selectively to HIV-1 infected cells.

DZ 614 RETROVIRAL VECTORS FOR ENHANCED GENE EXPRESSION IN T LYMPHOCYTES,
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Recombinant retroviruses have been used effectively to introduce and express exogenous in many cell types. Transduction of T-cells is well documented but high level gene expression has proven elusive. To address this problem, a series of retroviral vectors were constructed to utilize promoter and /or enhancer fragments from genes which are normally restricted to expression in T-cells. These fragments include elements from the CD2, p56lck and CD7 genes. The chloramphenicol acetyl transferase gene (CAT) serves as the reporter for all of the vectors. Virions were packaged using GP+E-86 and PA317 cell lines and used to transduce the Jurkat T-cell line. Cell lysates were assayed for CAT enzyme activity by CAT diffusion assay. Transient transfections of vectors into Jurkat cells are being employed to help assess the tissue specific potential of these constructs without the complications arising from the retroviral lifestyle and chromosomal positional effects. The parental vectors express CAT activity at higher levels than do the CD2 lcr containing vectors examined so far. Preliminary Southern blot data suggests that the introduction of the entire 2.0kb CD2 lcr fragment may lead to destabilization of the viral genome and the loss of genetic information. Further probing of the deletions should provide insight into the mechanism of splicing/loss and may help to characterize DNA fragments which are stable in retroviral vectors and which confer copy dependent tissue specific expression of exogenous genes. When we have identified vector designs which give enhanced gene expression in Jurkat cells, we will extend the studies to primary peripheral blood T-cells.

DZ 615 CONTROLLING SIGNAL TRANSDUCTION AND CELL DEATH WITH SYNTHETIC LIGANDS,
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Dimerization and oligomerization are general biological control mechanisms contributing to the activation of cell membrane receptors, transcription factors, vesicle fusion proteins, and other classes of intra- and extra-cellular proteins. A general procedure that uses lipid soluble, synthetic ligands was devised that permits controlled intracellular dimerization or oligomerization of proteins. These dimeric ligands are based on the immunosuppresive drug FK506; however, during dimerization interaction with calcineurin, and therefore immunosuppression, has been completely eliminated. The cytoplasmic domains of several signaling receptors, including CD3 ζ from the T cell receptor complex, MB1 from the B cell receptor complex and FAS, have been fused to the FK506 binding protein, FKBP12, and these chimeric proteins have been expressed both transiently and stably in various T cell lines. This approach was used to induce intracellular homodimerization of cell surface receptors lacking their transmembrane and extracellular regions but utilizing a myristylation site to confer membrane attachment. In each case, addition of the dimerization reagent to cells in culture resulted in signal transmission and either specific target gene activation or apoptosis depending on the receptor domains used. The ability of the dimerization reagent to activate a signaling cascade is complemented by the ability of monomeric forms of the reagent to rapidly inactivate the pathway. Ligand-regulated activation and termination of signaling pathways controlling gene transcription or ligand-regulated apoptosis should find application in both basic research and medicine.

DZ 616 TISSUE SPECIFIC EXPRESSION OF THE MUC1 GENE PRODUCT - A CANDIDATE ANTIGEN FOR IMMUNOTHERAPY OF BREAST AND OTHER CARCINOMAS
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The MUC1 gene is expressed in most glandular epithelial cells and the product (PEM) is overexpressed and aberrantly glycosylated in breast and other carcinomas. We are 1) evaluating the use of the coding region of MUC1 gene as a DNA-based vaccine in Cancer Therapy, and 2) analyzing the elements in the MUC1 promoter responsible for tissue specific expression. A *transgenic mouse* expressing MUC1 in a tissue specific fashion has been developed and provides a suitable model for testing vaccine formulations based on MUC1 or PEM. *In vitro* studies using 5'MUC1 sequences fused to a reporter gene have identified positive regulatory elements in the promoter as well as a sequence which acts in non-expressing cells to silence the positive elements. The MUC1 promoter may be useful in directing expression of genes in some carcinomas.

Late Abstract

COEXPRESSION OF IL-2 AND IL-4 IN MURINE MELANOMA CELLS DELAYS TUMOUR FORMATION MORE EFFECTIVELY THAN THE EXPRESSION OF EITHER ALONE. Paul Towner, Joop Gäken, Simon Hollingsworth, Pragna Patel, David Darling. King's College School of Medicine and Dentistry, Denmark Hill, Camberwell, London, UK.

Retroviral vectors encoding IL-2 and IL-4 were introduced into the murine melanoma cell line B16 F10 and the spontaneous mouse breast tumour cell line NC. The expression of IL-2 or IL-4, or the combination of both were characterised in the two cell types. The cells were subsequently introduced into syngeneic mice to assess their ability to form tumours. In contrast to previous reports the expression of either IL-2 or IL-4 by these tumour cells did not result in long lasting tumour immunity, although the onset of tumour formation was significantly delayed. Optimum delay of tumour formation was dependant on the coexpression of IL-2 and IL-4, although the combination did not result in a synergistic effect. Preliminary results suggest that cells explanted from the tumours still secrete IL-2 and IL-4, although at a reduced rate compared to cells kept in *in vitro* culture. The rate of appearance of tumours arising from cells expressing IL-2 and IL-4 is not simply related to a decrease in the levels of cytokine. Introduction of irradiated cells expressing either IL-2 or IL-4, or both together, into syngeneic mice followed by a subsequent challenge with unmodified live tumour cells gave similar results. A combination of IL-2 and IL-4 resulted in the longest delay of tumour formation, whilst IL-2 alone showed little effect. We are currently assessing the effect of B7 (CD28 counter receptor) expression either alone or in conjunction with these and other cytokines on tumour rejection.