GENE THERAPY

Organizers: Richard C. Mulligan, Inder Verma and David Hausman

April 12-18, 1993; Keystone, Colorado

Plenary Sessions *Page*

Poster Sessions

Cell Transplantation

SZ 001 CELLULAR TRANSPLANTATION AND TYPE I DIABETES, Denise L. Faustman, Massachusetts General Hospital-East, Immunobiology Laboratory, Building 149, 13th Street, Charlestown, Massachusetts 02129.

Transplanation of allogeneic or xenogeneic insulin secreting islets of Langerhans is an attractive idea for the treatment of type I diabetes. For this therapy to be effective, transplantation must be performed at an early time point after hyperglycemia in order to be an effective therapy at preventing diabetic complications. Unfortunately, standard transplantation methods utilize toxic immunosuppressive drugs that culminate in induced complications prior to hyperglycemic complications, therefore it would be advantageous to perform islet transplants with reduced immunosuppression.

Immunosuppressive therapy is aimed at inactivating or eliminating the recipient's immune system by systemic treatment of the recipient. We have focused on an alternative hypothesis, i.e., modification of the donor tissue to eliminate host recognition and toxic immunosuppression.

The best described immunogeneic donor antigen for host T cell recognition is polymorphic donor MHC class I expressed on all cells. We have recently demonstrated that disguising these donor proteins by a number of different methods, masking non-lytic antibody fragments or transgeneic donor cells, allows islet xenograft survival without immunosuppression. These successful results suggest that *in vivo*, foreign MHC class I is a powerful antigen and disguising donor class I antigen may be beneficial in prolonging graft survival.

Unfortunately, another barrier for successful type I diabetic islet transplantation exists; recurrent autoimmunity. The previously described "masking" of donor MHC class I antigens on xenogeneic or allogeneic islets appears to not protect against autoimmune mediated islet destruction. Recent evidence from our laboratory suggests that perhaps autoimmunity is secondary to faulty presentation of endogenous antigens in the groove of MHC class I. This pathway for possible self tolerance needs to be corrected for successful islet transplantation in type I diabetic humans.

Bone Marrow

SZ 002 CHARACTERIZATION OF MURINE LONG-TERM HEMOPOIETIC REPOPULATING CELLS AND THE GROWTH FACTORS THAT STIMULATE THEM. Gregory R Johnson and Chung Leung Li. The Walter and Eliza Hall Institute of Medical Research, P.O. Royal Melbourne Hospital, Victoria 3050, Australia.

Because of their low frequency, studies on hemopoietic long-term re-populating cells have been difficult. Normal murine marrow cells have been treated with a panel of antibodies against differentiation antigens present on lymphocytes, monocytes/macrophages and granulocytes to immuno-magnetically remove these cells. This population (Lin^-) was then sorted by FACS for cells expressing low levels of Thy-1, high levels of Ly6A/E(Sca-1) and kit, and for low retention of Rhodamine 123. Sorted cell populations were assayed for their ability to reconstitute hemopoiesis in lethally-irradiated recipients, for CFU-S content and in vitro colony-forming ability. Preliminary experiments revealed no differences between $Thy\text{-}1^-$ and $Thy\text{-}1^{lo}$ populations and this step was not used routinely. All long-term repopulating activity was found in $Ly6A/E^+$ populations.

Lin^-, $Ly6A/E^+$ cells were subdivided into $Rh123^{lo}$ and $Rh123^{med/hi}$ fractions. Both populations contained CFU-S and cells capable of in vitro colony formation. The Lin^-, $Ly6A/E^+$, $Rh123^{lo}$ population required stimulation by multiple hemopoietic growth factors for in vitro colony formation. The two Rh123 subpopulations were transplanted into lethally-irradiated recipients (100 cells per recipient) and only the Lin^-, $Ly6A/E^+$, $Rh123^{lo}$ population showed significant levels of donor engraftment (60-70%) in both myeloid and lymphoid lineages. Engraftment with Lin^-, $Ly6A/E^+$, $Rh123^{med/hi}$ cells was low (2%) and was predominantly lymphoid. Lin^-, $Ly6A/E^+$, $Rh123^{lo}$ cells were further divided on the basis of kit expression. In vitro and in vivo activity was found only in the kit^+ subpopulation. Only 30 Lin^-, $Ly6A/E^+$, $Rh123^{lo}$ kit^+ cells were required for 50% donor reconstitution in long-term repopulating assays and when stimulated by multiple hemopoietic growth factors, 80-100% of the cells formed colonies in vitro.

Studies in vitro showed that the Lin^-, $Ly6A/E^+$, $Rh123^{lo}$ population required multiple growth factors for stimulation, that there was a 3-4 day delay before the cells commenced proliferation and that during the dormant phase the continual presence of growth factors was required. These data suggest that ligand-initiated signalling is different in Lin^-, $Ly6A/E^+$, $Rh123^{lo}$, kit^+ cells and in progenitor cells stimulated by single haemopoietic growth factors.

Studies have also been performed to determine which hemopoietic growth factors (or combinations) in vitro were able to maintain long-term hemopoietic repopulating ability. Although SCF (kit ligand) was active in this assay, combinations of growth factors excluding SCF were also active.

SZ 003 STEM CELL GENE THERAPY FOR ADENOSINE DEAMINASE DEFICIENCY, Dinko Valerio[1], Victor W. van Beusechem[1], Mark P.W. Einerhand[1], Peter M. Hoogerbrugge[1,2], Alexandra, A.J. Migchielsen[1], and Leonie Kaptein[1], [1]Gene Therapy Department of the Institute for Applied Radiobiology and Immunology TNO, P.O.Box 5815, 2280 HV, Rijswijk, The Netherlands, [2]Dept. of Pediatrics, Univ. Hospital, Leiden, The Netherlands.

The preferred target cell in gene therapy for blood disorders is the pluripotent hemopoietic stem cell (PHSC) because of its selfrenewing potential combined with a capacity to contribute to the production of all blood cells over extended periods of time (probably lifelong). To date, the typical example for gene therapy as a whole and for bone marrow gene therapy in particular remains the putative treatment of an inherited form of immunodeficiency caused by a defect in the gene encoding the enzyme adenosine deaminase (ADA). The aim would be to genetically correct blood forming cells from such deficient patients which, following reinfusion into the patient, could regenerate a functional immune system. For the efficient transduction of PHSC we have generated an amphotropic retrovirus carrying the human ADA gene. In vitro experiments with our ADA virus indicated that hemopoietic progenitors from both rhesus monkeys and man can be transduced with high efficiency (up to 40 %) as demonstrated by ADA overexpression in cultured colonies. To assess which factors determine the efficiency of retrovirus-mediated gene transfer into PHSC, murine bone marrow was exposed to the virus-producing cells under varying culture conditions. Large variations in gene transfer efficiency became manifest, mainly dependent on the added growth factors. Under optimal conditions we reproducibly infect 15-20% of the PHSC that contribute to a stably regenerated hemopoietic system (> 6 month post transplantation). In order to extend these studies towards the clinical situation we performed autologous bone marrow transplantations in lethally irradiated rhesus monkeys using retrovirus-infected hemopoietic stem cells. Variables tested to achieve efficient and reproducible gene transfer ex vivo included: growth factor addition, virus titer, virus backbone, the presence of stromal cells and stem cell purity. Upon transplantation of the modified bone marrow, regeneration rates of peripheral blood cells were monitored and indicated that in all instances regenerative capacity of the graft could be conserved. All 14 monkeys transplanted sofar were shown to express the functional hADA enzyme in their peripheral blood cells, as detected by zymogram analysis. The frequency of provirus containing cells in the peripheral blood was determined using a semiquantitative PCR analysis. Depending on the infection procedure used, 8 out of 13 monkeys carried the provirus in their peripheral blood cells for the duration of the experiment (currently 3 monkeys > one year and one monkey > two years after transplantation). Moreover, in all peripheral blood mononuclear cells tested hADA expression could be detected. Genetic modification could also be demonstrated in BMC of various densities, in spleen, in lymph nodes and in cultured T lymphocytes. Based on our data we conclude that safe stem cell gene therapy procedures were derived which reproducibly yield long term genetic modification of the hemopoietic system of non-human primates. The efficacy of our technology can be tested in the clinic, since a stem cell gene therapy protocol for ADA deficient patients has been approved by the Dutch government and the local ethical committee of the University Hospital in Leiden. As a long term goal we plan to develop gene targeting procedures to be used in stem cell gene therapy. Such procedures will need to be tested and validated in a relevant animal model. Therefore, we are also making an effort to generate ADA deficient mice using ES cell technology. See also the abstracts by: Van Beusechem et al., Hoogerbrugge et al., Migchielsen et al., and Kaptein et al.

Lung, Liver, and Cardiovascular System

SZ 004 MICROVASCULAR ENDOTHELIAL CELL GENE TRANSFER IN A RABBIT MODEL, Daniel S. Ory[1], Annette Rivera[1], Beverly Ream[1], John T. Fallon[2], Herman K. Gold[2] and Richard C. Mulligan[1], [1]The Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142, [2]Cardiac Unit, Massachusetts General Hospital, Boston, MA 02114.

Retrovirus-derived vectors were used to develop a gene transfer model for the study of the molecular mechanisms involved in restenosis. Initial studies with a rabbit model involved development of catheter-based methods for modifying local vascular biology by resurfacing with genetically modified microvascular endothelial cells (MVEC). Autologous rabbit MVEC were isolated from adipose tissue and a homogeneous MVEC population was obtained as determined by immunohistochemical staining (Factor VIII positive, α-actin negative, desmin negative) and by FACS analysis of dil-AcLDL uptake. MVEC were cultured in vitro and were infected with supernatants from a high titer (>10^7 cfu/ml) amphotropic viral producer clone (ΨCrip MFGLacZ), which encodes the lacZ reporter gene. X-gal staining indicated in vitro infection efficiencies of 75-85%. Passage 3 to 5 autologous MVEC which expressed lacZ were resuspended in DMEM with 10% autologous serum and used to resurface the external iliac arteries. A modified double-balloon perfusion catheter (10 mm spacing) was used for vessel surface preparation (isotonic, hypoosmotic irrigation) and for subsequent MVEC infusion (4 x 10^6 cells/ml). The external iliac vessels were perfusion-fixed at 5 to 7 days, X-gal stained and examined by scanning electron microscopy (SEM) and light microscopy (LM). Resurfacing efficiencies of 50-70% of the vessel surface area were achieved with the transduced MVEC. SEM of the resurfaced portions of the vessel demonstrated an intact endothelial cell monolayer which oriented to flow and was morphometrically indistinguishable from the native arterial macrovascular endothelial cells. LM confirmed the presence of the endothelial cell monolayer without evidence for significant intimal proliferation. In addition, the vessel media was characterized by normal cellularity and was without deep injury. These initial studies suggest that efficient cell-mediated gene transfer can be performed in the rabbit external iliac arteries with preservation of the normal vascular architecture and with limited vascular damage. Studies are on-going to improve resurfacing efficiency and to assess persistence in vivo of the resurfaced MVEC.

SZ 005 GENE THERAPY FOR HEPATIC DISORDERS, Savio L.C. Woo, Howard Hughes Medical Institute, Baylor College of Medicine, 1 Baylor Plaza, Houston, Texas 77030

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. In our laboratory, two deficient animal models are being tested for the efficacy of gene therapy approaches for their phenotypic correction. Phenylketonuria (PKU) is an inborn error in amino acid metabolism that causes severe and permanent mental retardation in affected children. The genetic disorder is secondary to a deficiency of the hepatic enzyme phenylalanine hydroxylase (PAH) which converts phenylalanine to tyrosine and constitutes the major metabolic pathway for the essential amino acid. We have previously reported the cloning of the human PAH cDNA and the use of it to express the human enzyme in heterologens mammalian cells by retroviral mediated gene transfer. We have also demonstrated that heterologous hepatocytes injected directly into the portal vein or the spleen of cogenic mice migrated to the liver, survived and continued to function as hepatocytes for the life of the recipients. More recently, we have demonstrated that mouse hepatocytes can be transduced in vivo by direct infusion of recombinant retroviral vectors after partial hepatectomy, and the transduced cells persisted in the recipients for at least 6 months. Independently, a PAH deficient mouse model has been created by the Laboratory of William Dove at the University of Wisconsin. Thus, we have initiated collaborative studies to attempt the correction of the deficient phenotype in in the mutant mouse model. The second animal model is the Factor IX-deficient dog in the Laboratory of Kenneth Brinkhous at the University of North Carolina in Chapel Hill. This is a severe bleeding animal model and the construction of recombinant retroviruses expressing human and canine Factor IX in fibroblasts and primary hepatocytes has previously been reported. The ex vivo and in vivo approaches for hepatic gene therapy will be tested in these deficient animal models and if successful, the technologies can be directly applied to the future treatment of a variety of metabolic disorders in man. (The work was supported in part by a Program Project Grant from NIDDK).

The Nervous System

SZ 006 TRANSPLANTATION OF GENETICALLY ENGINEERED POLYMER ENCAPSULATED CELL LINES IN THE NERVOUS SYSTEM, Patrick Aebischer[1,3], Diane Hoffman[1], Xandra O. Breakefield[1], and Priscilla Short[2], [1]Section of Artificial Organs, Biomaterials and Cellular Technology, Brown University, Providence, RI 02912, [2]Department of Neurogenetics, Massachusetts General Hospital, Boston, MA, [3]Division, of Surgical Research, Centre Hospitalier Universitaire Vaudois, Lausanne University, Switzerland.

Neurodegenerative diseases such as Parkinson's or Alzheimer's diseases are characterized by the progressive loss of neuronal elements. This cell loss may be prevented by the localized delivery of neurotrophic factors. Local delivery of appropriate neurotrophic factors may be achieved by neural grafting of cells genetically engineered to release them. Cell lines offer several advantages for potential clinical uses. Cell lines can be banked, screened prior to transplantation for the presence of pathogens, and efficiently engineered to express and release neurotrophic factors using DNA recombination technologies. However, in many experiments, the transplanted cell lines grow beyond the site of implantation and form macroscopic lethal tumors. Thus for potential clinical applications involving transplantation of genetically modified cells, the inherent risk of tumor formation must be minimized. Transplantation of cells isolated within a permselective polymer capsule restricts cell growth to the capsule space while allowing exchange of molecules between the entrapped cells and host tissue. Provided with an encapsulating membrane of appropriate molecular weight cut-off, the transplanted cells can be shielded from the host immune system, allowing transplantation of cell lines across species.
In the present study, rat fibroblasts from the parent cell line or those genetically modified to produce nerve growth factor (NGF) were loaded within a thermoplastic hollow fiber-based capsule.
Only the capsules loaded with the genetically engineered cells released measurable amounts of NGF in vitro. When a NGF releasing capsule was transplanted into the lesioned rat septo-hippocampal system, the encapsulated cells remained viable and released sufficient NGF to prevent the lesion-induced loss of septal choline acetyltransferase (ChAT) expression, whereas the capsules containing the control fibroblast line did not.This effect was observed up to 5 month in vivo. Retrieved capsules after 5 month in vivo were still able to induce neurite outgrowth from PC12 cells indicating a sustained release of NGF from the genetically engineered cells. With both cell types, the cells remained confined to the capsule space. The same concept is being studied with cells genetically engineered to release dopamine in experimental Parkinson's disease models and cells genetically engineered to release enkephalins for the treatment of pain. We conclude that the transplantation of genetically modified cell lines within a polymer capsule provides an effective means by which to attain imperative safety and efficiency levels in gene product delivery for neurological diseases where the deficiency of a specific molecule has been identified.

SZ 007 **IMPROVED REPLICATION-DEFECTIVE HERPES SIMPLEX TYPE 1 VECTORS.** Theodore Friedmann, Atsushi Miyanohara and Paul A. Johnson, Center for Molecular Genetics, Department of Pediatrics, UCSD School of Medicine, La Jolla, CA.

Considerable attention has recently been paid to the use of replication-defective mutants of herpes simplex virus type 1 for efficient gene transfer into the mammalian central nervous system as well as into other target organs. Many prototype vectors have been hampered by the combined technical deficiencies of persistent cytotoxicity and inefficient or transient transgene expression. We have focused on the use of replication-defective mutants of HSV-1 as vectors for gene transfer to neurons and other post-mitotic and quiescent cell types. We have used replication-defective prototype vectors based on a parent IE 3 deletion mutant that cannot express viral early or late genes, into which trangenes have been placed under the control of the heterologous HCMV IE promoter. One such vector, designated CgalΔ3, was able to infect a wide variety of cell types and express the E. coli B-galactosidase gene with high efficiency. However, expression of the transgene was transient, and in vitro studies indicated that many infected cells died as a result of continued expression of the remaining IE genes in the IE 3 deletion mutant (1). Our subsequent studies have focused on (i) reducing viral cytotoxicity and (ii) prolonging transgene expression.

To reduce virus-induced cytotoxicity, we have used the virus mutant *in*1814 containing an insertion mutation in the VP16 gene which abolishes the ability of VP16 to transinduce IE gene expression (2). Since *in*1814 is replication-competent, we have constructed a double mutant containing the VP16 mutation of *in*1814 as well as the IE 3 deletion. This double mutant, designated 14HΔ3, is less cytotoxic and better able to persist in cells in vitro than single mutants deleted for IE3, permitting a significant increase in the proportion of cells that can persist expressing a transgene following infection with the vector.

During HSV latency, expression of most viral genes, except for the latency-associated transcripts (LATs), are turned off . However, it is becoming apparent that only latent viral genomes residing in the trigeminal ganglia express LAT at high levels. In other neurons and non-neuronal cells, LAT expression is often poor or undetectable. Based on the hypothesis that features of the LAT locus may allow LAT expression when transcription of the remaining viral genes is turned off, we have sought to overcome the tissue-specific limitation of LAT expression by introducing heterologous enhancers upstream of the LAT promoter. Our initial in vitro studies have indicated that viral recombinants containing the RSV and/or HBV enhancers upstream of the LAT promoter express the B-gal transgene almost as well as CgalΔ3. We are currently investigating whether transgene expression from such recombinants is prolonged in vivo.

(1) Johnson et al. (1992) J. Virol. 66, 2952-2965.
(2) Ace et al. (1989) J. Virol. 63, 2260-2269

SZ 008 GRAFTING GENETICALLY MODIFIED CELLS TO THE BRAIN, F. H. Gage, Department of Neuroscience, University of California, San Diego, La Jolla, CA 92093, USA

Intracerebral grafting has emerged as a useful experimental tool to address a variety of questions in neurobiology. Furthermore, the ability to restore function through neuronal grafting in the CNS has suggested a potential approach to CNS therapy through the selective replacement of cells lost as a result of disease or damage. Independently, the development of molecular genetic tools has begun to suggest genetic approaches to the treatment of human diseases. A combination of these two techniques, namely the intracerebral grafting of genetically modified cells, has been shown to be a useful tool to address biological issues, and an advanced approach toward the restoration of function in the damaged or diseased CNS. Issues and problems associated with the merging of these two approaches will be presented. Neurotrophic factors are molecules that support the survival of selective neuronal processes during development. these molecules have also been shown to protect adult and aged neurons from undergoing degeneration. Furthermore, these factors have been shown to promote the growth of intact and damaged axons. When trophic factors are delivered to the brain via somatic cell transplantation, evidence for functional regeneration has now been established. The anatomical and physiological results of intracerebral grafting of cells genetically modified to secrete neurotrophic factors will be presented.

SZ 009 *Abstract Withdrawn*

HIV

SZ 010 GENE THERAPY AND RIBOZYME FOR HIV INFECTION AND IMMUNE RESTORATION STRATEGIES, Nava Sarver, Developmental Therapeutics Branch, Basic Research and Development Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892.

Scientists at the Developmental Therapeutics Branch (DTB) have implemented scientific initiatives that support cutting-edge strategies and bridge research gaps to block/delay HIV replication, and restore/prevent deterioration of immune functions. Included are diverse gene therapy strategies (transdominant repressors, RNA decoys, ribozymes) as anti HIV agents, with the intent of rapidly translating gene therapy and ribozyme to a clinical mode.

Integral to this anti-viral effort is a concomitant effort to restore immune functions via *ex vivo* genetic manipulation of hematopoietic cells [CD4, CD34, stem cells (SC)]. Questions addressed by DTB-supported investigators include the capacity of bone-marrow and non-bone marrow derived SC to give rise to hematopoietic lineages in HIV+ individuals, the consequences of potential thymic dysfunction on T-cell ontogeny, and the effect of gene transduction on the self renewal and pluripotent capabilities of SC. Complementing the *ex vivo* strategies are studies to augment specific cytotoxic T cells and humoral responses via direct introduction of genetic information into accessible tissues. As this strategy is less time consuming, may be simpler than *ex vivo* procedures, and potentially of considerable clinical value, it is pursued as another targeted form of immune restoration in HIV infection.

Results and lessons learned to date from groups focusing on blocking HIV replication and restoring immune functions via gene therapy strategies, and advances made translating these strategies to a clinical setting will be presented.

Cancer

SZ 011 MULTIDRUG RESISTANCE AS A SELECTABLE MARKER IN GENE THERAPY, Michael M. Gottesman[1] and Ira Pastan[2], [1]Laboratory of Cell Biology, and [2]Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD

The *MDR*1 gene encodes a 170,000 dalton protein called P-glycoprotein which functions as an ATP-dependent multidrug efflux pump which confers resistance to various cytotoxic drugs on mammalian cells. Cross-resistance to anti-cancer drugs including anthracyclines (daunorubicin, doxorubicin), *Vinca* alkaloids (vinblastine, vincristine), epipodophyllotoxins (VP-16, VM-26), taxol and actinomycin D is obtained when vectors encoding an *MDR*1 cDNA are transferred into drug-sensitive cells. We have developed a retroviral vector which can be packaged as a retrovirus which efficiently transforms a variety of human cell types to multidrug resistance. This vector, and other related *MDR*1-expressing vector systems, may be useful for conferring resistance to anti-cancer drugs on human bone marrow, thereby allowing dose escalation of chemotherapy during treatment of cancer, and as a dominant selectable marker for introduction of other genes into drug-sensitive cells and tissues.

The advantages of the *MDR*1 vectors for human gene therapy may be summarized as follows: (1) Retroviral vectors of high titer which transform drug sensitive cells to multidrug resistance are readily obtained; (2) Resistant cell populations can be selected easily *in vitro* using a variety of inexpensive, cytotoxic drugs; alternately, since P-glycoprotein is a plasma membrane protein and monoclonal antibodies (MRK-16, UIC-2) of high affinity and specificity are available, cells expressing P-glycoprotein can be easily identified by FACS analysis or magnetic bead panning technologies. Cells expressing functional P-glycoprotein can also be detected by FACS analysis based on extrusion of fluorescent substrates such as rhodamine 123; (3) In work done collaboratively with the laboratories of Arthur Nienhuis and Arthur Bank, mouse bone marrow stem cells can be readily transformed with an *MDR*1 retrovirus and can be shown to confer taxol resistance *in vivo* to the bone marrow of mice transplanted with the transformed marrow; (4) Resistance conferred by *MDR*1 vectors is readily reversed with a variety of compounds, including verapamil and quinidine, which act as competitive inhibitors of the transporter, thereby making it easy to reverse resistance if necessary in patients; (5) Chimeras between P-glycoprotein and other proteins, such as adenosine deaminase, are bifunctional, i.e., both the selectable P-glycoprotein pump, and the second protein are active, suggesting that a variety of non-selectable markers can be introduced into drug-sensitive cells with high efficiency using chimeric *MDR*1 vectors; (6) Mutants which affect the drug specificity and inhibitor specificity of the transporter are readily obtained, making it possible to design transporters with useful drug preferences and inhibitor resistance or sensitivity. This feature of *MDR*1 vectors will allow the selective differentiation of the exogenous P-glycoprotein from endogenous P-glycoprotein which might be expressed on cancer cells, or P-glycoprotein expressed in its normal location in the kidney, liver, intestine, pancreas, adrenal, and capillary endothelial cells of the brain and testis.

SZ 012 DISSECTION OF THE IMMUNE RESPONSE INDUCED BY TUMORS ENGINEERED TO SECRETE LYMPHOKINES
Drew M. Pardoll MD. PhD.
The Johns Hopkins University, School of Medicine, Baltimore, MD 21205

There is mounting evidence that many if not all tumors possess antigens capable of being recognized by T cells. A number of molecular strategies aimed at enhancing the immunogenicity of tumors have strengthened the hypothesis that the failure of an adequate anti-tumor immune response may be due to defects in immune regulation rather than an absence of tumor specific antigens and therefore an absence of tumor specific T cells. Over the past few years, our group has been analyzing the immune response induced by immunization with tumors engineered to secrete cytokines locally. Immunization of animals bearing small amounts of established renal cell carcinoma were in fact able to be cured of their disease by the systemic immune response induced by syngeneic tumor cells transduced with a murine Interleukin-4 gene. More recently, in collaboration with Richard Mulligan's laboratory, we have evaluated the immune responses generated by tumors transduced with retroviral vectors carrying the GM-CSF gene. In this model of paracrine cytokine production, GM-CSF stood out as the most effective lymphokine in inducing systemic immune responses against the poorly immunogenic F10 variant of B16 melanoma. Hystologic analysis of the inflammatory infiltrate within the GM-CSF transduced tumor cells revealed a large number of activated macrophage-like cells together with a moderate number of granulocytes. The systemic immune response generated by immunization with GM-CSF transduced tumor cells was found to be dependent both on CD4+ T cells and CD8+ T cells. Furthermore, PCR analysis revealed a burst of endogenous IL-2 and IL-4 synthesis by T cells in draining lymph nodes at day seven after immunization. Furthermore, despite the fact that CD8+ tumor specific T cells were generated by this immunization scheme, the rejection of MHC class I+ challenge tumors did not require that the immunizing tumor express MHC class 1 molecules on its surface. Taken together, these findings suggest that immunization with GM-CSF transduced tumor cells alters the presentation of tumor specific antigens such that powerful antigen presenting cells are brought to the tumor site which process and present both MHC class I and MHC class II-restricted antigens. Generation of CD8+ tumor specific T cells by this immunization strategy has allowed us to analyze the complexity of tumor specific peptides recognized by the CTL population. In one colon tumor there was a single predominant tumor specific peptide identified. In the B16 melanoma system, three peaks of peptide activity were identified. Upon dilution, one immunodominant peptide could be discerned among the three specific tumor peptides. Implications of these analyses for the development of strategies for human cancer immunotherapy will be discussed.

SZ 013 GENE THERAPY OF CANCER, S.A. Rosenberg, National Cancer Institute, Bethesda, Maryland 20892

Attempts are underway to develop new immuno- therapeutic approaches to the treatment of patients with advanced cancer. Early efforts of adoptive immunotherapy using lymphokine activated killer (LAK) cells and interleukin-2 (IL-2) resulted in 10% incidence of complete regression and 10 to 25% incidence of partial regression in patients with advanced kidney cancer and melanoma. In subsequent studies, tumor infiltrating lymphocytes (TIL) were shown to recognize unique cancer antigens on murine and human cancers including melanoma, breast cancer, colon cancer and lymphoma. The MHC restricted recognition of human cancer antigens was detected by assaying panels of HLA typed target cells and by transfection into target cells of genes coding for appropriate HLA specificities. In animal studies therapy with TIL was 50 times more potent than with LAK cells and in pilot trials of TIL in melanoma patients 40% underwent objective cancer remission. Indium-111 labelled TIL trafficked to and accumulated in cancer deposits.

In initial studies to genetically modify TIL to improve their therapeutic effectiveness, TIL retrovirally transduced with the gene for NeoR, were administered to 10 patients with advanced cancer and were detected in the circulation 189 days and in the tumor 64 days later. In subsequent studies TIL transduced with the tumor necrosis factor (TNF) gene, secreting over 100 ugTNF/10^6cells/24 hrs have been used to treat seven patients with advanced melanoma. Genes coding for chimeric T cell receptors (TcR) composed of the constant region of the TcR and the variable region of a monoclonal antibody have been transduced into TIL and can redirect TIL lysis to cancer recognized by the monoclonal antibody.

Genetic modification of tumors is also being studied. Antigen processing defects in selected murine and human tumors can be corrected by exposure to interferon-gamma (IFN-gamma) or transduction with the gene for IFN-gamma. Transduction of murine tumors with cytokine genes can increase tumor immunogenicity. These studies led to clinical protocols in which human tumors transduced with the gene for TNF or IL-2 were used to immunize five autologous patients with advanced cancer. Efforts to clone the gene for tumor antigens that are recognized by murine and human TIL are underway.

References

1) Rosenberg, S.A., J Clin Oncol 10:180-199, 1992.
2) Rosenberg, S.A., et al., N Engl J Med 323:570-578, 1990.

Genetics of Disease

SZ 014 THE MOLECULAR ANALYSIS OF MAMMALIAN GENOMES, Hans Lehrach, Imperial Cancer Research Fund, London UK

The human genome project promises for the first time the possibility to get, read and ultimately even to understand most of the information, which a cell or an organism uses to build its machinery, to direct its development and even, at least to some extent, to direct its behaviour. The analysis will require the accommodation of two conflicting goals: (1) The efficient generation and analysis of large amounts of data, requiring a close integration of experiment, automation and computing techniques, and (2) the flexible integration of many types of biological information, generated through many individual efforts in biological research laboratories throughout the world. To accommodate both requirements, we have developed a project to combine efficient, highly automated mapping and sequencing techniques with a program of distributing libraries, clones and information through a reference library system. The status of this work will be described.

Clinical Protocols for Gene Therapy

SZ 015 TRANSFERRED GENE EXPRESSION AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION IN MAN, Malcolm K. Brenner,[1*] Donna R. Rill,[1] Michael J. Buschle,[1] Martha S. Holladay,[1] Helen E. Heslop,[1] Robert C. Moen,[2] Robert A. Krance,[1] Joseph Mirro, Jr.,[1] W. French Anderson,[3] James N. Ihle[1], [1]Department of Hematology/ Oncology, St. Jude Children's Research Hospital, Memphis, TN, 38105, and the Departments of Pediatrics and Medicine, University of Tennessee, Memphis, College of Medicine, Memphis, TN, 38163, [2]Genetic Therapy Incorporated, Clinical Labs, Gaithersburg MD, 20878, [3]National Institutes of Health, National Heart, Lung, and Blood Institute, Bethesda, MD, 20892

Autologous bone marrow transplantation (ABMT) allows cancer patients to be rescued from the effects of supralethal chemotherapy. The incidence of relapse remains high and it has been unclear whether recurrence occurs from residual disease in the patient or in the infused marrow. The concern that patients may have been cured by intensive treatment, only to have their tumor returned by the ABMT, has lead to the adoption of marrow purging. Unfortunately there are no data to show that this treatment is necessary or effective - but ample evidence to show that such marrow treatment slows engraftment and increases the risks of ABMT. We have used retroviral mediated gene transfer (RMGT) of the neomycin resistance gene to mark putative residual malignant cells in the marrow of children receiving ABMT for neuroblastoma and acute myeloid leukemia (AML). Since normal progenitor cells are also marked, this approach can provide information about the efficiency of gene transfer in man and the persistence of these modified cells in vivo.

Since September 1991 we have treated 17 patients. Gene expression in hemopoietic and T and B cell lineages has been at a higher level than predicted from most animal models (2-15%) and has persisted for more than one year. The marrows used for transfer are obtained shortly after multiple courses of chemotherapy and are highly proliferative; this may render them more susceptible to RMGT. Two patients with AML have relapsed. In both, a proportion of the relapse blasts were marker gene positive, showing the infused marrow contributed to disease recurrence. One neuroblastoma patient has relapsed and the marker gene is being sought. Modifications of this marker approach with two distinguishable vectors are now being used to compare the efficacy of available purging techniques. Prolonged gene expression in hemopoietic and lymphoid lineages offers encouragement for gene therapy protocols in which marrow derived cells are the targets.

SZ 016SUES IN THE CLINICAL APPLICATION OF HEPATIC GENE THERAPY. Fred D. Ledley, Departments of Cell Biology and Pediatrics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

Various methods have been described for targeting gene therapy to the liver including both ex vivo and in vivo strategies. The goals of these two strategies are somewhat different, with ex vivo strategies intended to provide permanent expression of a therapeutic product, and current in vivo strategies aimed at using genes as medicines which can be administered repetitively with variations of the dose and schedule to meet the patient's therapeutic need. The problems inherent in the clinical application of these two methods are different; the clinical application of ex vivo gene therapy with the attendent need for surgery and cell transplantation requires confidence that long-term gene expression can be achieved, and the application of genes as medicines requires information about the pharmacokinetics and feasibility of repetitive administration of the gene. We have performed pre-clinical studies with both methods to assess the feasibility and safety of these approaches. In anticipation of ex vivo strategies for gene therapy we have demonstrated that human hepatocytes can be harvested from liver segments preserved in Belzar solution, cultivated in hormonally defined, media, and transplanted into SCID mice. These data suggest that 0.1-1% of hepatocytes in the recipient may contain the recombinant gene, a fraction which is sufficient for some, but not all, clinical indications of gene therapy. These preclinical studies, as well as those of other laboratories, also indicate that this procedure can be performed safely. Studies with asialoglycoprotein (ASO) mediated gene transfer and a gene for methylmalonyl CoA mutase deficiency demonstrate that recombinant gene expression can be achieved reproducibly at therapeutic levels for >24 hours in the liver after IV administration. With repetitive administration, however, high titer ($>10^4$) antibodies were formed against the ASO-PL complex. While these antibodies may not block action of the complex, antibodies may complicate the pharmacology of administration and safety. A novel method of gene therapy using a natural, DNA-binding protein to effect specific receptor mediated DNA-uptake and expression is described. This method is shown effect receptor mediated uptake in hepG2 cells and primary hepatocytes and produce marker gene expression are achieved which are equivalent to those reported with ASO/PL/DNA complexes. The presence of adenovirus in the media further enhances expression of a CAT marker gene. Experiments are underway to assess the utility of this method for in vivo gene transfer and the feasibility of repetitive administration.

Cell Transplantation and Gene Delivery I

SZ 100 THE USE OF KERATINOCYTES FOR THE PHENOTYPIC CORRECTION OF METABOLIC DEFECTS BY SYTEMIC DELIVERY OF THE DEFECTIVE PROTEIN. M. Yvonne Alexander, Caspar J.M. Robinson, Alison. H. Trainer and Rosemary J. Akhurst. Duncan Guthrie Institute of Medical Genetics, University of Glasgow, Glasgow, G3 8SJ, U.K.

Certain acquired and inherited diseases are currently treated by the administration of recombinant or purified proteins; hemophilia A and B, diabetis and pituitary dwarfism. Keratinocytes have potential as a target cell for delivering recombinant proteins into the circulation. Expression vectors have been constructed containing either Factor IX or ornithine transcarbamylase linked to a variety of keratin promoters. The effectiveness of these constructs are being evaluated *in vivo* using transgenic mice. We are also investigating the feasability of an *in vivo* approach to gene therapy using the Oxford gene gun. To this end tungsten particles have been coated with the reporter gene beta-galactosidase, and preliminary results show that these particles are capable of penetrating the epidermal layer in the mouse. Gene transfer and tissue damage was assessed by histochemical staining for beta-galactosidase activity. The extent of the blue staining revealed that a substantial proportion of the bombarded cells and surrouding area had been transfected with the beta-galactosidase gene. Studies are now being carried out to assess the feasability of using the gene gun to provide a mutant spf mouse with the normal functional ornithine transcarbamylase gene it lacks. It may be possible to use the keratinocyte for this *in vivo* approach, to achieve phenotypic correction of Factor IX and ornithine transcarbamylase deficiency by gene replacement therapy.

SZ 101 INTRAARTICULAR EXPRESSION OF THE INTERLEUKIN-1 RECEPTOR ANTAGONIST PROTEIN BY EX-VIVO GENE TRANSFER, G. Bandara, G.M. Mueller, J. Galea-Lauri, M.H. Tindal, H.I. Georgescu, G.L. Hung, J.C. Glorioso, P.D. Robbins, C.H. Evans Departments of Orthopaedic Surgery and Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

Gene therapy offers a radical new approach to the treatment of arthritis. Towards this end we are developing in vivo and ex vivo methods of gene transfer to joints. This abstract describes an ex vivo approach in which genes are transduced into lapine synovial fibroblasts. The cells are genetically altered and then transplanted into the knee joints of recipient rabbits. Synoviocytes were infected with a BAG retroviral vector carrying the neo and lac Z marker genes. Following infection and selection in G418, all cells were neo$^+$ and nearly all were also lac Z$^+$. Cells were additionally infected with a MFG retrovirus carrying a cDNA coding for the human interleukin-1 receptor antagonist protein (IRAP or IL-1ra); such synoviocytes secreted over 100ng/day of biologically active human IRAP/10^6cells. Other populations of synovial fibroblasts were grown in the presence of BrdU to permit their immunolocalization after transplantation. Suspensions of lac Z$^+$, neo$^+$, IRAP$^+$ synovial fibroblasts and BrdU-labelled synoviocytes were transplanted into recipient knee joints by intraarticular injection. Seven days following transplant, colonies of lac Z$^+$, neo$^+$, IRAP$^+$ cells could be recovered from recipient synovia; immunohistochemistry demonstrated the colonization of the synovia by BrdU-positive cells. Assay of joint lavages confirmed the in vivo expression of several ng per knee of biologically active human IRAP for up to 11 days post-transplant; this concentration of IRAP is likely to be therapeutic. Expression of IRAP was however, transient with loss of activity by 14 days post-transplant. This is the first report of the intraarticular expression of a potentially therapeutic protein by gene transfer technology.

Supported in part by a UERP grant from Procter & Gamble and by Grant No. RO1 DK46640 from NIDDK.

SZ 102 TOWARDS GENE THERAPY FOR HAEMOPHILIA B USING NORMAL HUMAN KERATINOCYTES, George G. Brownlee, Ann J. Gerrard, David L. Hudson and Fiona Watt, Sir William Dunn School of Pathology, University of Oxford, OX1 3RE, U.K. and I.C.R.F. Lincoln's Inn Fields, London, U.K.

Only gene therapy offers the prospect of a permanent cure for haemophilia. For this purpose in the case of haemophilia B we are investigating the possibility of grafting keratinocytes, which are genetically altered so as to secrete recombinant factor IX. Initially we established that subcutaneously injected human factor IX is reasonably quickly and efficiently transported to the bloodstream in mice(1). Then we showed that normal human keratinocytes can synthesise recombinant human factor IX in tissue culture if they are transduced by infection with a defective factor IX retrovirus. The factor IX produced is not fully biologically active in a clotting assay, but at least 50% of it is active. Subsequent grafting into nude mice showed that human factor IX continued to be synthesised by the transduced keratinocytes *in vivo* and was detected in blood for 1 week at 1-3 ng/ml, which is equivalent to about 0.1% of a therapeutic dose in patients(2). New experiments are now under way to attempt to (a) improve the yield and lengthen the time over which factor IX is secreted *in vivo* and (b) to increase the biological activity of the factor IX by co-expressing the recently cloned γ-glutamyl carboxylase.

(1) Gerrard, A.J., Austen, D.E.G. & Brownlee, G.G. (1992) Br. J. Haemat. 81, 610-613.
(2) Gerrard, A.J., Hudson, D.L., Brownlee, G.G. & Watt, F.M., Nature (Genetics), in press.

SZ 103 AN IMMUNOISOLATION DEVICE FOR IMPLANTATION OF GENETICALLY ENGINEERED CELLS: LONG TERM EXPRESSION OF FACTOR IX IN RATS. V. Carr-Brendel[1],J. Lozier[2],T.J.Thomas[1],B. Saeed[1], S.Young[1],J. Crudele[1],L. Martinson[1],B. Roche[1], D. Boggs[1], R. Pauley[1], D. Maryanov[1], S. Josephs[1], K. High[2], B. Johnson[1], and J. Brauker[1]. [1]Applied Sciences, Baxter Healthcare, Round Lake, IL 60073 [2]Dept. of Medicine, Div. of Hematology, University of North Carolina, Chapel Hill, NC 27599-7035

Immunoisolation is the encapsulation of therapeutic tissue within a membrane that protects the tissue from a host's immune response, and protects the host from direct contact with genetically engineered cells. Baxter has developed a bilaminar membrane-based immunoisolation device for implantation of genetically engineered allogeneic or autologous cells. When implanted into a rat, the device was shown to promote vascular structures at the membrane interface, altering the fibrotic capsule normally associated with an implanted material. Additionally, the device was able to protect allogeneic lung tissue (Sprague Dawley implanted into Lewis rat) from rejection for a year, but the same tissues were rejected in 2 weeks if holes greater than 0.45μm were poked in the membrane to allow access of host cells. Dog fibroblasts were transduced with a retroviral vector containing the cDNA encoding either human or canine factor IX. After screening the cells in vitro in both a functional assay (25-50% coagulation) and an ELISA for human (25-140 ng/24 hour/10^6 cells) or canine(1 μg/ 24 hour/10^6 cells) factor IX, 10^5 cells were implanted per device (10 devices/rat) into athymic rats. Expression of canine factor IX was detected in rat plasma by ELISA (0.001-0.004 units/ml) for 60 days post implant. Histological sections of the cells within the device were immunostained, and were positive for human factor IX expression. These data demonstrate that encapsulated genetically engineered cells survive and express factor IX. Moreover, an immunoisolation device increases the safety of cellular implants. The cells are segregated from the host tissues, and may be easily retreived if their function decreased.

SZ 104 CORRECTION OF LYSOSOMAL STORAGE IN ß-GLUCURONIDASE-DEFICIENT MICE BY GENETICALLY-MODIFIED CELLULAR IMPLANTS.

Olivier Danos, Philippe Moullier, Nadia Naffakh, Valérie Maréchal and Jean-Michel Heard. Laboratoire Rétrovirus & Transfert Génétique, Institut Pasteur, Paris, France.

The *gus*$^{mps/mps}$ mutant mouse has no detectable ß-glucuronidase activity and provides a model for human mucopolysaccharidosis type VII (MPS VII) an autosomal recessive lysosomal storage disease involving severe skeletal deformities and multiple organ dysfunctions. Free circulating ß-glucuronidase can bind specific receptors at the surface of cells and become internalized and transported to the lysosomes. This provides a rationale for correcting the mutant phenotype using an implant of genetically-modified autologous cells which continuously delivers the enzyme to the organism. Primary skin fibroblasts, bone marrow cells or myoblasts were obtained from MPS VII mice, used as targets for retrovirus-mediated transfer of the human ß-glucuronidase cDNA and reimplanted into three groups of 4-6 week-old MPS VII recipients. The first group (n=15), was implanted with collagen lattices containing enzyme-secreting fibroblasts placed into the peritoneal cavity. The second group (n=10) was sublethally irradiated (4.5 Gy) and grafted with genetically-modified hematopoietic cells. In the third group (n=9), ß-glucuronidase containing myoblasts were injected into the tibialis anterior, during a mechanically induced muscle regenaration. Following treatment, a drop in urinary glycosaminoglycan concentration was observed in the three experimental groups and was stable over time. Operated MPS VII mice were sacrificed at various times between 2 and 22 weeks following transplantation and ß-glucuronidase activity was found in liver, spleen, bone marrow, lung, and brain homogenates. Histochemical staining revealed the human enzyme activity in the macrophages of all examined tissues. When compared to untreated animals, histological examinations evidenced a dramatic morphological correction of the liver and spleen of treated animals, with normalization of organ architecture and the disappearance of stored material in liver Kupffer cells and spleen macrophages. A comparative evaluation of the three approaches will be presented and discussed in the prospect of gene therapy for human MPS.

SZ 105 RETROVIRAL MEDIATED G-CSF EXPRESSION IN TRANSPLANTED RAT VASCULAR SMOOTH MUSCLE CELLS.

S.W.Han*, N.Ramesh*, S.Lau,* M.M.Clowes#, A.W.Clowes# and W.R.A.Osborne*, #Dept of Surgery, *Dept of Pediatrics, University of Washington, Seattle, Washington 98195.

Cyclic Hematopoiesis is a fatal stem cell defect characterized by recurrent fluctuations in neutrophil counts. Patients can be treated by administration of recombinant granulocyte colony stimulating factor(G-CSF). Gene transfer therapy for this disorder was studied in rats by transplantation of vascular smooth muscle cells genetically modified to express G-CSF. Cultured rat arterial smooth muscle cells were infected with LGSN, a retroviral vector encoding canine G-CSF cDNA and a selectable Neo gene. Infected, selected cells, shown to secrete G-CSF by a sensitive ELISA, were returned to the left common carotid artery following balloon catheter injury. Pre-treatment neutrophil counts were 1640 PMN/µl and were elevated for up to 45 days post-transplantation with a mean of 2960 PMN/µl and a peak of 3590 PMN/µl, and then declined towards baseline value. Antibodies to canine G-CSF were detected in sera of treated rats, suggesting a mechanism for the decrease in neutrophil counts. Cultured explants of transplanted carotids secreted canine G-CSF indicating that vector inactivation was probably not involved in the decreased biological response. DNA extracts of seeded carotids were positive for retroviral vector sequences by Southern and PCR analysis. To overcome the antibody mediated loss of secreted cytokine activity we have cloned rat G-CSF cDNA. These studies demonstrate that vascular smooth muscle cells provide a suitable target tissue for gene therapy involving a secreted protein.

SZ 106 AN ANIMAL MODEL SYSTEM FOR THE THERAPY OF GROWTH HORMONE DEFICIENCY,

[1]Lih-Hwa Hwang, [2]Bing-Fang Chen, [3]Shui-Tsung Chen, [3]Wen-Chang Chang, and [1]Ding-Shinn Chen, [1]Hepatitis Research Center, National Taiwan University Hospital; [2]Graduate Institute of Microbiology, College of Medicine, National Taiwan University; and [3]Institute of Biochemical Sciences, Academia Sinica; Taipei, Taiwan, R.O.C.

We have employed the hypophysectomized rat as an animal model system to explore the feasibility of using the fibroblast cells for gene therapy. To this aim, we have evaluated several different constructions of retroviral vector, including the traditional vector, the U3-based vector, and the internal ribosome entry site (IRES)-directed bicistronic retroviral vector. A porcine growth hormone (pGH) cDNA and a neo^r gene were used for the efficiency comparison of these vectors for coexpressing two genes. Our results demonstrated that the pGH cDNA was expressed most efficiently from the bicistronic vector, i.e., the PSN vector which was composed of the IRES derived from swine vesicular disease virus, in NIH 3T3 cells. We therefore adopted the PSN vector to infect the primary rat embryo fibroblast cells (REF), and estimated the secretion rate of pGH from the REF to be around 1 - 2 µg /10^6 cells/day. The hypophysectomized rats were surgically removed their pituitary glands and used as an *in vivo* bioassay system for the porcine growth hormone. They were injected intraperitoneally with 1 x 10^7 cells/rat of the PSN virus-infected REF or the neo^r virus-infected REF (as a control). The body weights and the tibia growth of the rats transplanted with the PSN virus-infected REF were significantly increased as compared to that transplanted with the neo^r virus-infected REF after two weeks. Observation of a longer duration of the transplanted cells in the animal is being undertaken. The significance of all these results will be discussed.

SZ 107 HUMAN FACTOR IX EXPRESSION BY HUMAN MYELOMA CELL LINES; MODEL OF POTENTIAL THERAPY FOR HEMOPHILIA B,

Russette M. Lyons, Brian D. White, June M. Saunders, Ted Smith and Michael Kalelo, Molecular and Cell Biology Lab, Genetic Therapy, Inc. Gaithersburg, MD 20878

Lymphocytes are an attractive cell target for many gene therapy applications and have been used successfully in the treatment of two patients with severe combined immune deficiency (SCID) resulting from adenosine deaminase (ADA) deficiency. This early success prompted an investigation of the potential for Factor IX expression by human T lymphocytes for the treatment of hemophilia B. Hemophilia B is caused by a deficiency of Factor IX, a post-translationally modified protein involved in the clotting cascade. Two approaches were taken to introduce the human Factor IX gene into human T cells to determine their ability to express and appropriately process Factor IX. T cells were electroporated with either a CMV or HTLV-I promoted Factor IX expression plasmid. Minimal levels of Factor IX were detected by ELISA; little or no processing was detected. Using a retroviral vector, LIXSN, primary human T cells were transduced with an efficiency of 1-3%. A cell population which was 30% positive for the presence of the vector was obtained by selection in G418. Factor IX could not be detected in medium from this selected cell population. Several human B cell lines were also transduced with the LIXSN retroviral vector with a transduction efficiency of 10-30%. Myeloma cell lines, representative of differentiated immunoglobulin-secreting plasma cells, secreted significant levels of Factor IX (170 ng/10^6 cells/24 hr) into the medium. Lymphoblastoid cell lines did not secrete detectable Factor IX. Vectors are currently being developed for increased Factor IX expression in B cell lines and subsequently, primary human B cells. Models to evaluate the *in vivo* differentiation of transduced primary human B cells and production of therapeutic levels of active Factor IX are being pursued.

SZ 108 EFFICIENT IN VIVO GENE TRANSFER INTO PORCINE CORONARY ARTERIES USING RECOMBINANT ADENOVIRAL VECTORS

Wojciech Mazur, Mir-Nadir Ali, James P. Finnigan, Douglas M. Cromeens, Albert E. Raizner and Brent A. French, Department of Medicine, Baylor College of Medicine, Houston, TX 77030

Restenosis resulting from intimal hyperplasia of smooth muscle cells (SMCs) is a clinical problem affecting 30-50% of patients undergoing coronary angioplasty. This renarrowing of the coronary arteries may be amenable to genetic therapy since: i) genes can be transferred directly into coronary vessels using specialized catheters, ii) a number of proteins have been shown to inhibit smooth muscle cell proliferation, and iii) even a temporary inhibition of SMC proliferation should limit the formation of restenotic lesions. Several laboratories have demonstrated direct gene transfer into the arteries of intact animals, but applicability has been limited by the subphysiological (picogram) levels of recombinant gene product which have been reported to date. We here demonstrate that replication-deficient adenoviral vectors are far more efficient than Lipofectin at mediating direct gene transfer into living coronary arteries, resulting in the production of significant (nanogram) quantities of recombinant gene product. For each transfected artery, 50 µg of reporter vector DNA (pCMVLacZ or pRSVLuc) was complexed with 150 µg of Lipofectin (BRL) and diluted to 4 mls with Opti-MEM I (BRL). Viral infections were performed with 4 ml dilutions of recombinant adenoviruses harboring analogous expression cassettes: $5x10^9$ pfu per artery of AdCMVLacZ (generously provided by A. Bett and F.L. Graham) or $2x10^{11}$ pfu per artery of AdRSVLuc. Under fluoroscopy, these solutions were infused at a pressure of 8 atmospheres into the coronary arteries of intubated Hanford miniature swine using perforated balloon catheters. Levels of reporter gene activity were determined 3 days following infusion catheterization using a chemiluminescent assay for β-galactosidase and a luminescent assay for luciferase. The results of this comparison are shown below, where each value in the 2x2 matrix represents the mean amount of reporter gene product recovered from 3 to 6 coronary arteries ± standard deviation.

	CMVLacZ (ng)	RSVLuc (pg)
Lipofectin:	1.52 ± 0.68	0.11 ± 0.10
Adenovirus:	106 ± 96	35.9 ± 27.7

Thus, with both reporter systems, markedly higher levels of recombinant gene product were obtained from adenoviral lysates than from lipofections using 50 µgs of highly purified plasmid DNA. In addition, it should be possible to obtain even higher levels of reporter gene product from adenoviral vectors by simply concentrating the recombinant virus. Additional studies will be required to determine the relative contributions of promoter activity, message stability, and protein degradation to the increased expression of the CMVLacZ cassette over the RSVLuc cassette; but both reporter systems demonstrate the clear superiority of adenoviral vectors over more conventional methods of gene transfer.

SZ 109 EFFICIENT ADENOVIRAL GENE TRANSDUCTION IN HUMAN AND MOUSE HEPATOCYTES *IN VITRO* AND IN MOUSE LIVER *IN VIVO*,

M. Morsy[1], A. Bett[2], G. Alford[1], F. Graham[2], and C. T. Caskey[1,3], [1]Baylor College of Medicine, Houston, TX 77030, [2]McMaster University, Hamilton, Ontario, CANADA, L8S 4K1, [3]Howard Hughes Medical Institute at Baylor College of Medicine, Houston, TX 77030

We have shown that human hepatocytes are highly receptive to recombinant adenoviral infection. One hundred percent infection and induction of ß-galactosidase expression was achieved in human hepatocytes using AdHCMVsp1LacZ at 200 and 400 multiplicities of infection (moi). Infection of human hepatocytes at such a high moi did not affect endogenous ornithine transcarbamylase (OTC) levels, nor was there evidence of cytopathic effects over the period of 2 weeks in culture. Furthermore, *in vivo* experiments with adult mice showed that greater than 40% of hepatocytes expressed ß-galactosidase at 72 hours. Wild type levels of OTC enzyme activity were also achieved using AdSRαhOTC in primary hepatocytes derived from the OTC-deficient sparse fur (spf) mouse model.

To our knowledge, this is the first demonstration of efficient adenoviral-mediated exogenous gene activity in human hepatocytes, with 100% induction of ß-galactosidase and OTC activity *in vitro* and greater than 40% transduction of ß-galactosidase activity *in vivo*. These data strongly suggest that the adenoviral system is powerful in transducing high levels of gene expression in the liver.

SZ 110 HUMAN FACTOR IX EXPRESSION IN RAT VASCULAR SMOOTH MUSCLE CELLS IN VITRO AND IN VIVO

Ted Smith, June Saunders, Gene Liau*
Genetic Therapy, Inc., Gaithersburg, Md. 20878
*American Red Cross, Rockville, Md. 20855

We are interested in developing a gene therapy protocol for Hemophilia B, which is an X-linked recessive disorder caused by a deficiency in the level of the blood coagulation factor IX. Although factor IX is normally produced by the liver, any cell which secretes biologically active gene product may be suitable for gene therapy. We have been examining vascular smooth muscle cells (VSMC) as a target cell and have generated preliminary data on expression of human factor IX from rat VSMC. Cells were isolated from the thoracic aorta of Fischer 344 rats and authenticated by their expression of smooth muscle α-actin mRNA. These cells were transduced with the LIXSN retroviral vector, which contains human factor IX cDNA driven by the LTR promoter and the neo gene driven by an internal SV40 promoter. Transduced cells were selected for G418 resistance and assayed for expression of factor IX. Analysis by ELISA demonstrated that these cells secreted 1200 ng/10^6 cells/24 hr. of factor IX and an enzyme linked coagulation assay indicated that nearly all of the factor IX produced was biologically active. A Western analysis yielded two bands with molecular mass of 67 kDa and 70 kDa. The 67 kDa polypeptide co-migrated with factor IX isolated from human plasma. The 70 kDa polypeptide may represent factor IX which still contains the propeptide. Rat VSMC containing the LIXSN vector and expressing recombinant factor IX were implanted subcutaneously into rats on bovine collagen sponges. Approximately 1 x 10^6 cells were implanted per animal into 3 animals. Plasma samples were analyzed by ELISA for human factor IX. The data show peak levels (30-125 ng/ml) three weeks after implantation. The levels declined to background by 50 days post-implantation. Currently, we are developing vectors and implant strategies designed to maintain long term in vivo expression.

SZ 111 **MULTIPOTENT NEURAL PROGENITOR CELL LINES CAN ENGRAFT & PARTICIPATE IN DEVELOPMENT OF MULTIPLE STRUCTURES AT MULTIPLE STAGES THROUGHOUT MOUSE NEURAXIS -- A POTENTIAL STRATEGY FOR GENE TRANSFER INTO DEVELOPING MAMMALIAN CNS,**

Evan Y. Snyder*, J. Macklis, B. Yandava, V. Nguyen, S. Arnold, Z.-H. Pan, E. Hartweig, C. Cepko, Depts.of Neurology, Pediatrics, & Genetics, Harvard Medical School & Children's Hospital, Boston, MA 02115 (*corresponding author)

Previously, we reported the generation of multipotent neural cell lines by retrovirus-mediated v-*myc* transfer into postnatal murine cerebellar (CB) progenitors [*Cell* 68:33,1992]. When transplanted into CB of newborn mice, these cells integrated into host brain in a nontumorigenic, cytoarchitecturally appropriate manner & recapitulated their multipotency in vivo: cells from the same clonal line differentiated into neurons or glia in a manner appropriate to their site of engraftment, presumably responding to microenvironmental cues. Some transplant-derived neurons received appropriate synapses. Engrafted cells, labeled by *lacZ* expression, could be identified up to 22 mos. postengraftment, indicating also stable long term, site-specific expression of an exogenous gene. To determine unequivocally that engrafted cells were of donor origin, we demonstrated that the unique viral integration site in donor cell lines was identical to that in engrafted cells. Because these studies did not answer whether these immortalized cells were committed/restricted to CB fate or represented CNS progenitors with broader potential, we now report engraftment of lines in regions & at developmental periods other than those from which they were generated. When examined at adulthood, following transplantation into various germinal zones at various times, these same immortalized CB progenitors participated in development of multiple structures throughout the neuraxis & at multiple stages spanning from midgestation (E12) to 2 wks. postnatal. These cells again demonstrated their multipotency by differentiating into multiple cell types in these various CNS loci, apparently responding to signals of the respective region at the particular developmental stage. *LacZ* expression by these cells was often robust. The great number & dispersion of cells often seen at adulthood suggested continued mitosis & migration posttransplant & prior to end-differentiation, though, as before, no tumors were seen. Immunocytologic, ultrastructural, & electrophysiologic assessment of engrafted cells is ongoing. These data suggest that using such immortalized multipotent progenitors as transduction agents for exogenous factors, or as integral members of CNS cytoarchitecture, may be feasible for both clinical & research applications. Such lines may also provide models for commitment, differentiation, & plasticity of neural progenitors.

SZ 112 A SINGLE PROTEIN CATALYZES BOTH *N*-DEACETYLATION AND *N*-SULFATION DURING THE BIOSYNTHESIS OF HEPARAN SULFATE.
Stuart J. Swiedler, Zheng Wei, and Masayuki Ishihara,
Glycomed, Inc., Alameda, CA 94501.
Ariel Orellana and Carlos B. Hirschberg,
Dept. of Biochemistry and Molecular Biology,
U. of Massachusetts Medical Center,
Worcester, MA 01655.

Heparan sulfate (HS) is a highly sulfated carbohydrate polymer that binds to and modulates the activities of numerous proteins. We are evaluating a recombinant-based strategy to control the formation of these protein-binding domains. Our goal is to influence the action of growth factors such as basic fibroblast growth factor (bFGF) whose binding to HS is a prerequisite for their entry into cells. The formation of these binding domains in HS is dependent on a series of biosynthetic reactions that modify the polysaccharide backbone; the initiating and rate-limiting steps of the process are the *N*-deacetylation and *N*-sulfation of *N*-acetylglucosamine residues. Using a recombinant cDNA-based approach, we now report that, in rat liver, heparan sulfate biosynthesis utilizes a single protein that carries out both reactions. Data will be presented that addresses whether or not controlling levels of this enzyme through genetic manipulation will directly influence the formation of binding domains in HS directed to bFGF.

SZ 113 **Keratinocyte gene therapy: The use of genetically altered keratinocytes for local or systemic delivery of a new gene product.**
Lorne B. Taichman and Elizabeth S. Fenjves. Dept. of Oral Biology and Pathology, State University of New York, Stony Brook, NY 11794.

Epidermal keratinocytes can be manipulated in culture and used as autologous grafts, consequently, they may be suitable targets for gene therapy. Previous work done in our laboratory suggests that grafts of genetically altered keratinocytes can be used as a vehicle for secreting a new gene product into local tissues or systemically. These studies have shown, 1) that apolipoprotein E (apoE), naturally secreted by keratinocytes, reaches the circulation of nude mice bearing grafts of human epidermal keratinocytes and 2) that SCC9 cells, a permanent keratinocyte line, can be transfected with the gene for apoE and induced to secrete high levels of apoE both in vitro and in vivo. To determine whether normal strains of keratinocytes can be induced to secrete an exogenous protein, apoE was tagged with an epitope from hemagglutinin of haemophillus influenza (HA1), and transfected into human keratinocytes in culture. Secretion of the engineered apoE (HA1-apoE) was monitored in submerged cultures, lifted rafts, and in nude mice bearing epidermal grafts of transfected keratinocytes. HA1-apoE was observed in the media of both culture systems at levels of 60 ng/ml. Experiments are currently underway to determine whether HA1-apoE is found in the systemic circulation of graft-bearing animals. The results of these experiments will help determine the feasibility of keratinocyte gene therapy for systemic delivery.

SZ 114 GENE THERAPY OF THE CNS LESIONS OF MURINE MUCOPOLYSACCHARIDOSIS VII
RM Taylor and JH Wolfe, Medical Genetics, Veterinary School, University of Pennsylvania, Philadelphia, PA 19104

Mucopolysaccharidosis (MPS) VII is a neurovisceral lysosomal storage disease caused by deficient ß-Glucuronidase (GUSB) activity, which occurs in humans, mice and dogs. Murine fibroblasts were infected with a double copy retroviral vector, DCH ßH, containing the human GUSB cDNA and its promoter with a selectable Neo sequence. After selection in G418 these cells were stereotaxically injected into the cerebrum of mice with MPS VII. The survival of corrected fibroblasts from a long term cell line (3521-DCH ßH) was assayed using a histochemical stain which specifically detects GUSB activity. GUSB positive cells were detected in all mice following injection. Clusters of cells were found at the injection site and scattered cells were found in many other locations, including the meninges, the ventricles and in cortical sites distant from the injection. High level GUSB activity was found for at least 8 weeks after injection. The 3521-DCH ßH cell line has formed a tumor in only 1 of 19 control mice followed up to 8 months after injection. These experiments are currently being extended to early passage MPS VII fibroblasts corrected with DCH ßH to examine the expression of GUSB after injection. This preliminary study shows that retrovirally corrected fibroblasts can engraft and provide a sustained source of lysosomal enzyme following intra-cerebral injection.

SZ 115 IMPLANTATION AND SURVIVAL OF A HUMAN FETAL ASTROCYTE CELL LINE IN THE BASAL GANGLIA OF THE NON-HUMAN PRIMATE, RHESUS MONKEY.
Carlo S. Tornatore[1], Kris Bankiewicz [2], Daniel Lieberman[2], and Eugene O. Major[1]. [1]Laboratory of Viral and Molecular Pathogenesis and [2]Surgical Neurology Branch, NINDS, NIH, Bethesda, MD,20892.

The grafting of fetal tissue in the treatment of neurodegenerative diseases would be greatly facilitated if a viable human fetal cell line could be substituted for primary fetal tissue. A permanent, immortalized human fetal astrocyte cell line (SVG) has been established (Major E. et al. PNAS 1985) which is anchorage dependent for growth, does not form tumors in nude mice and maintains characteristics of fetal astrocytes such as GFAP expression. To determine the viability of the SVGs as a neural xenograft, the cells were implanted bilaterally into the basal ganglia of six rhesus monkeys. One month post-operatively two of the monkeys were sacrificed and in both cases the SVG cells could be easily identified in the CNS parenchyma both morphologically and by identification of an SVG marker protein, T antigen. The cells remained confined to the site of implantation without evidence of inflammation, graft rejection or tumor formation. The remaining monkeys have been followed using Magnetic Resonance Imaging of the CNS and in no case has a tumor forming mass been identified. This study demonstrates that an immortalized human fetal astrocyte cell line can be successfully grafted and survive in the CNS of the primate. The potential of these cells in the treatment of human neurodegenerative diseases is discussed.

SZ 116 **GENE THERAPY FOR THE PERIPHERAL NERVOUS SYSTEM: RAT NEURITOGENIC T CELL LINE CARRY MOUSE NERVE GROWTH FACTOR GENE** **Y. Zhang, J. Gehrmann, R. Gold, R. Kramer and H. Wekerle Max-Planck Institute for Psychiatry, Am Klopferspitz 18A, 8033 Martinsried, FRG**

The tissues of the nervous system are secluded from blood circulation by a tight endothelial border. In contrast to macromolecules and most blood cells, activated T lymphocytes are able to cross the blood tissue barriers. We used activated T cells specific for peripheral nerve myelin to import therapeutically relevant gene products into the peripheral nervous system (PNS). The T cell line (R4) used was isolated from Lewis rats immunised against the PNS myelin protein, P2. These cells express the CD4+ CD8- phenotype. Upon fresh activation, R4 cells transfer autoimmune demyelinating inflammation (experimental autoimmune neuritis, EAN) to naive syngeneic recipients. R4 cells secrete the cytokine profile typical for the Th1 subset, but no NGF. We infected the P2 specific T cell line with a retroviral vector carrying the mouse NGF gene. *In vitro*, the NGF transduced R4 cells produce high doses of NGF, when activated by presentation of the relevant P2 epitope. *In vivo*, the NGF-engineered R4 cells could be traced to the PNS using *in situ* hybridization. Of interest, the disease mediated by gene tranduced R4 T cells was remarkably attenuated both clinically and histpathologically than the one caused by wild type T cell line. These results raise hopes to use tissue specific auto-reactive T cells as carriers for delivering therapeutically useful agents into organs, which otherwise would be difficult to reach.

This work was supported by a grant from Volkswagen Foundation

Manipulation of Hematopoietic Cells

SZ 200 A β THALASSAEMIA GENE THERAPY STRATEGY. Dubravka Drabek[1], Mark Einerhand[2], Frank Grosveld[1] and Michael Antoniou[1]. [1]Laboratory of Gene Structure and Expression, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA. UK. [2]TWO Institute of Applied Radiobiology and Immunology (ITRI), P.O.Box 5815, 2280 HV Rijswijk, Lange Kleiweg 151, The Netherlands.

Gene therapy for β thalassaemia requires the sustained, high level expression of β-globin in the erythroid lineage after introduction of this gene into the patient's bone marrow stem cells. This pattern of expression can only be achieved by linkage of the locus control region (LCR) to the β-globin gene.

Studies in murine erythroleukaemia cells have allowed us to characterize the minimal requirements for high level, position independent expression of the human β-globin gene when integrated into the host genome. We have found that the spacing of the LCR elements between themselves and the promoter are crucial for efficient transcriptional activation. Data will be presented where a 2.5kb fragment consisting of the LCR and a mini-β-globin gene was incorporated into an Adeno-associated virus (AAV) vector for delivery to haematopoietic stem cells.

SZ 201 EVALUATION OF THE POTENTIAL OF GENE THERAPY FOR GAUCHER DISEASE, Barranger J.A., Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA 15261. Gaucher disease is the most common lysosomal storage disorder. Among Eastern European Jews, the carrier frequency is 10%. Study of the biology of the disease has revealed that the macrophage is central to the pathogenesis of the disorder. Furthermore, strategies designed to correct glucocerebrosidase deficiency in the macrophage have resulted in successful, but limited therapeutic modalities. Gene therapy is potentially a cure for the disease. Recent studies in mice demonstrate that retroviral mediated gene transfer to hemopoietic stem cells is highly efficient and results in sustained expression of the gene at high levels. Studies in dogs, monkeys, and human $CD34^+$, lin^- cells have shown gene transfer efficiencies in these larger animal species of 10 to 50%. Deficiency of glucocerebrosidase has been reversed in macrophages from Gaucher patients. These studies strengthen the rationale for gene therapy for Gaucher disease.

SZ 202 **AN IMPROVED SELECTION METHOD FOR RETROVIRAL GENE TRANSFER TO LONG-TERM REPOPULATING HEMATOPOIETIC STEM CELLS**, Antonio Bernad[1], Florencio Varas[1], Jesus M. Gallego[1,2], Jose M. Almendral[1,2] and Juan A. Bueren[1]. [1]Department of Cellular and Molecular Biology, (CIEMAT) 28040 Madrid. [2]Department of Molecular Biology (CBM) 28049 Madrid. Spain.

In vitro expansion of retrovirally transduced bone marrow (BM), in combination with a drug selection protocol, was aimed at transfering exogenous genes into the mouse hematopoietic system.

In order to optimize conditions capable of expanding hematopoietic precursors, BM harvested 4 days after 5 FU treatment of donor mice was incubated with different combinations of growth factors (SCF plus IL3 or IL6 plus IL3). We found that a 3 day incubation in SCF/IL-3 media yielded the highest expansion of BM precursors (CFU-GM and CFU-S_{12}), conditions that also preserved the long-term repopulating cells. However, these conditions were incapable of enhancing the efficiency of retroviral infection with respect to non incubated BM. Thus, in the derived protocol, BM cells harvested 4 days after 5FU treatment, once infected by supernatants containing the pXT1 retroviral vector ($1\text{-}2x10^6$ cfu neo^r/ml; moi 5), were incubated with the combination of SCF plus IL3 (Wehi-CM) for 48h and then selected for G-418 resistance. The analysis of the BM collected at this time revealed a high proportion of precursors (780 CFU-GM and 50 CFU-S_{12} per 10^5 cells) all of them genetically marked.

The efficiency of this procedure in transducing more primitive repopulating cells was evidenced by the analysis of mice reconstituted with neo^r marrow, 3 and 5 months after transplantation. Different linfohematopoietic organs (BM,spleen and thymus) from the recipient animals were essentially reconstituted by transduced cells. Moreover, the high proportion of CFU-GM, CFU-S_{12} and precursors of CFU-S_{12} (pre-CFU-S) containing and/or expressing the neo^r gene demonstrated that expansion-selection procedure is compatible with the preservation of stem cell functions. These observations open new pespectives for the transplantation of genetically transduced BM populations purged of non-infected precursors.

SZ 203 GENE TRANSFER INTO PERIPHERAL BLOOD HEMATOPOIETIC PROGENITORS FROM CANCER PATIENTS TREATED WITH HIGH-DOSE CHEMOTHERAPY AND GROWTH FACTOR(S). M Bregni, M Magni, S Siena, M Di Nicola, F Peccatori, F Ravagnani, G Bonadonna, and AM Gianni. C. Gandini Transplantation Unit, Division of Medical Oncology and Immunohematology, Istituto Nazionale Tumori, Milano, Italy

Scarce availability and relative refractoriness to efficient vector infection are major obstacles to clinical application of gene transfer into bone marrow hematopoietic progenitors (HP). In contrast, large amounts of HP can be retrieved from peripheral blood of cancer patients in the recovery phase that follows high-dose cyclophosphamide (HD-CTX) therapy and hematopoietic growth factors (HGF). We have utilized N2 retroviral vector to transfer neomycin resistance (neoR) gene into peripheral blood HP. HP were harvested by leukapheresis from peripheral blood of 7 cancer patients (1 NHL, 6 breast cancer) in the recovery phase following HD-CTX and iv infusion of the following HGF: rhIL-3 (1 pt); rhGM-CSF (1 pt); rhG-CSF (1 pt); rhIL-3 and rhGM-CSF in combination (4 pts). Leukapheresis cells comprised a substantial fraction of CD34+ cells (median 7.3% of nucleated cells). Light-density cells were precultured for 48 hr with rhIL-3±rhIL-6±rhSCF, then repeatedly exposed to filtered fresh supernatant from the helper-free packaging cell line PA317-N2 with a virus:target ratio of 5:1. An overall neoR gene transfer rate of 93% into 53/56 day-14 myeloid colonies from infected cells was assessed by PCR analysis. As controls, we infected with the same method peripheral blood and bone marrow cells collected from patients before HD-CTX therapy and HGF infusion. Peripheral blood cells failed to originate colonies. Bone marrow infection resulted in only 22% PCR-positive colonies, as expected from experiments not involving cocultivation. Ongoing experiments include: i) retroviral infection of immunoselected peripheral blood CD34+ cells; ii) PCR analysis of gene transfer into peripheral blood CFU-mix, BFU-E, BFU-meg; iii) PCR analysis of colonies generated by peripheral blood long-term culture initiating cells.

SZ 204 **SILENCING OF RETROVIRAL VECTORS AFTER TRANSDUCTION OF MURINE HEMATOPOIETIC STEM CELLS IS ASSOCIATED WITH METHYLATION.**

Pia M. Challita*, Carolyn Cook+, Leonard S. Sender+, Chen Zhou*, and Donald B. Kohn*. *Childrens Hospital and Department of Microbiology, University of Southern California Medical School, Los Angeles, CA 90033, +Department of Pediatrics, University of Louisville School of Medicine, Louisville, KY 40292.

Successful gene therapy/bone marrow tranplantation (BMT) requires gene transfer into pluripotent hematopoietic stem cells (HSC) and persistent expression of the exogenous gene in the resultant mature hematopoietic cells. Our assay for gene transduction of pluripotent HSC using retroviral vectors in the murine gene transfer/BMT model is the spleen foci generated after 2°BMT (or 2°CFU-S). We are able to introduce the exogenous gene into a high percentage of the murine HSC. However, our data show that the MoMuLV-LTR is silenced after transduction of HSC; the provirus does not express vector transcripts in more than 75% of the 2°CFU-S. We determined the methylation status of the provirus in the non-expressing 2°CFU-S by southern blot analysis using the methylation-sensitive restriction enzyme SmaI. The studies demonstrated a high percentage of provirus methylation in the 2°CFU-S as opposed to the 1°CFU-S.

In order to understand the contribution of methylation to the silencing of the MoMuLV-LTR in HSC, we modified the MoMuLV-based retroviral vector by inserting, in the 5'end of the LTR, a CpG-rich sequence derived from the Thy-1 housekeeping gene. This modification lead to an extensive protection of the vector LTR from *de novo* methylation in embryonic stem cell lines. We are now in the process of studying the methylation and expression of the novel vector in the murine HSC. This analysis will allow us to understand the role of DNA methylation in the silencing of transduced genes in HSC and may provide a tool for more efficient long-term expression by retroviral vectors for the purpose of gene therapy.

SZ 205 **AMPHOTROPIC VIRUS BINDING TO CD34+ HUMAN BONE MARROW PROGENITORS IS INCREASED BY GROWTH FACTOR STIMULATION,** **Gay M. Crooks, Mary B. Hanley, Russ M. Lyons, Robert C. Moen and Donald B. Kohn, Division of Research Immunology/BMT, Childrens Hospital Los Angeles, Los Angeles, CA 90048, and Genetic Therapy Incorporated, Gaithersburg, MD**

Retroviral vectors are currently the most widely used method of gene transfer for the purposes of gene therapy. Amphotropic retroviruses are able to infect human cells by attaching to a specific receptor on the membrane of target cells. This amphotropic receptor has neither been identified nor its gene cloned. Thus, the influence of receptor number and affinity on gene transfer efficiency is difficult to study. We have applied an assay of retrovirus binding to study the regulation of the amphotropic receptor on human hematopoietic progenitors (CD34+ cells). This assay uses a rat monoclonal antibody 83A25 which recognizes the envelope glycoprotein (gp70) common to all Moloney murine leukemia viruses. Virus binding to target cells is detected by indirect fluorescent antibody staining with 83A25 and FACS analysis. No significant retroviral binding to fresh CD34+ cells can be measured. Prestimulating CD34+ cells in a combination of IL3, IL6 and Steel factor increases viral binding and efficiency of gene transfer. Although gene transfer is enhanced when CD34+ cells are cocultivated with BM stroma, no associated increase in virus binding is seen. These studies suggest that growth factors may upregulate or alter the affinity of the amphotropic receptor on CD34+ cells.

SZ 206 GENE TRANSFER INTO CANINE PLURIPOTENT HEMATOPOIETIC PROGENITORS IN LONG-TERM MARROW CULTURES. I. D. Dubé, S. Kamel-Reid, S. A. Kruth, D. Bienzle, A.C.G. Abrams-Ogg, R. F. Carter, J. E. Dick, J. Ackland-Snow, and R.M. Jacobs. Universities of Toronto and Guelph and Toronto General Hospital, Toronto, Canada.

Despite the ability to transfer genes into short-lived and lineage-restricted hematopoietic cells, there remains a need to develop reliable methods for gene transfer into totipotent hematopoietic stem cells in non-murine systems. We recently described a novel 21-day, three-cycle transduction protocol for retrovirally-mediated transfer of a reporter gene (*neo*) into hematopoietic cells maintained in long-term marrow cultures (LTMCs) *(Blood 79:356-364:1992)*. In the present work, we infused limiting dilutions of *neo*-marked LTMC cells (4×10^3 to 4.5×10^7) into 15 autologous recipient dogs. To specifically assess the re-populating potential of LTMC cells in the presence of competition from endogenous stem cells, recipients were not subjected to any type of marrow ablative conditioning. Hematopoiesis in dogs was monitored by Southern and PCR analyses, CFU-GM and BFU-E assays (+/- G418), and assays of activated T-cells at time points after infusion to determine the contribution made by LTMC-derived cells and the extent to which such cells gave rise to differentiated progeny that expressed the *neo* gene. In all dogs, the expression of the reporter gene, *neo*, was detected in about 20%-30% of hematopoietic progenitor cells (CFUs) within the first 6 months post-infusion and then, for most dogs, levels declined to the 1%-5% range by 12-15 months. In two dogs, among those receiving higher numbers of infused cells, *neo* continued to be detected in 20%-30% of CFUs at 12-15 months. Follow-up studies on these, and three previously reported dogs, indicate that our method facilitates gene transfer into LTMC cells that are able to engraft, proliferate, differentiate, and self-renew in the *in vivo* hematopoietic micro-environment without the need for positive selection or marrow ablative conditioning. This raises the exciting possibility that LTMCs may contain ideal target cells for clinical trials involving gene therapy into hematopoietic cells.

SZ 207 HEMATOPOIETIC STEM CELLS (HSC) WITH LONG TERM RECONSTITUTING POTENTIAL STRONGLY ATTACH TO VIRUS PRODUCING CELLS. Dominique Duménil, Anne Dubart. INSERM U 362, Institut Gustave Roussy, Villejuif, France.

Analysis of the effects of transgene overexpression in HSC is best performed on the clonal progeny of infected HSC to avoid dilution by uninfected cells. In an attempt to develop such a strategy, we infected 5-FU murine bone marrow cells by coculturing them with Neo virus-producing cells during 7 days. Non adherent cells as well as cells integrated in the adherent layer were then injected separately into irradiated recipient mice and spleen colonies (SC) generated by day 12 CFU-S were counted 12 days later : one third of the primary day 12 CFU-S was recovered in the adherent layer, and two-third in the non-adherent fraction. However, CFU-S from both fractions clearly differ in their reconstituting potential : thus 42 SC generated by adherent CFU-S were injected individually into secondary irradiated recipients and 24/42 (57%) allowed 4 month survival in secondary irradiated mice. None of the 36 SC derived from non-adherent primary CFU-S induced long-term survival. In animals grafted with adherent CFU-S, the proportion of donor-type cells was over 75% in 6/7 animals grafted in sex-mismatched condition as assessed with a Y chromosome specific probe 4 months after the graft. However, DNA analysis of bone marrow cells from the 24 secondary reconstituted mice failed to detect the neo gene, which indicates that reconstitution was accounted for by non-infected cells. In contrast, 27 of the 36 primary SC generated by non-adherent cells had integrated the neo gene. Our results thus confirm heterogeneity in the day 12 CFU-S compartment with respect to long-term reconstitution capacity. More importantly, it is clear (1) first that the most primitive stem cells, i.e. which generate CFU-S capable of reconstituting hematopoiesis on a clonal basis, strongly adhere to the virus-producing cells, and (2) second that these cells are not infected, probably because they are in a quiescent state during the coculture. It will be therefore very important to define in vitro conditions which will trigger the proliferative state of these HSC and therefore make them susceptible to viral infection.

SZ 208 A NEW, HIGHLY EFFICIENT RETROVIRAL MEDIATED GENE TRANSFER SYSTEM. Wilfred T.V. Germeraad, Shinji Fujimoto, Norio Asami, Daiki Okuda and Yoshimoto Katsura, Chest Disease Research Institute, Kyoto University, Kyoto, Japan.

In order to improve the infection efficiency of gene transfer, we deviced a new culture method in which bone marrow cells and the retrovirus producer cell line (GP+E86 W5 [W5 is a modified N2 vector]) are cultured together in a well, but physically separated by a porous membrane. In the first variation, BM cells were cultured on a stroma cell layer in the absence or presence of low doses of various growth factors for 3 days and then transduced with the W5 recombinant virus for another 3 days. Spleen colonies of lethally irradiated recipients were enumerated and DNA was extracted from entire spleens. PCR analysis indicated that a succesful transduction of the gene into these progenitors had occured only when BM cells were cultured in the presence of IL-1, 3, 6 and SCF. In the second variation we used a LTBMC (Dexter culture) as this allowed for a safe and long infection period by simply changing the membrane with new producer cells when they reached confluence. After the transduction phase of 14 - 21 days, cells were cultured with G418 for 10 days and subsequently injected into lethally irradiated mice. When individually excised colonies were analysed, the provirus was present in every colony, so a 100 % infectivity to CFU-S can be achieved using this method. In this system there is no need for addition of any growth factor. Currently, long term repopulating assays of transduced bone marrow cells from LTBMC are being carried out.

SZ 209 COMPARISON OF VARIOUS PROCEDURES FOR TRANSFER OF THE ADENOSINE DEAMINASE GENE INTO HUMAN AND MONKEY HEMOPOIETIC PROGENITORS.

PM.Hoogerbrugge[1,2], VW.v.Beusechem[1], J.Waayer[2], LCM. Kaptein[1], JAK. Bart[1], I. Riviere[3], RC.Mulligan[3] and D.Valerio[1]. [1]Dept. of Gene Therapy, Inst. for Applied Radiobiol. and Immunol. TNO, Rijswijk; [2]Dept. of Pediatrics, Univ. Hosp. Leiden, The Netherlands; [3]Whitehead Inst. for Biomedical Research, Cambridge, MA, USA.

In vitro studies have shown that both (1) co-cultivation of bone marrow cells with recombinant retrovirus-producing cells, and (2) cultivation of bone marrow cells in the presence of virus-containing medium on bone marrow stromal cells results in efficient gene transfer. So far, long-term *in vivo* expression of newly introduced genes has only been reported following transplantation of bone marrow co-cultivated with virus producing cells (v Beusechem et al., Proc. Natl. Acad. Sci. USA, 1992: 89:7640). Inherent to this method is the infusion of irradiated, virus-producing fibroblasts. So far, this never resulted in toxic side effects, but the infusion of virus producing cells can be prevented by using supernatant only.

In the present study, we compare the gene transfer efficiency of both methods, using three ADA-virus producing cell lines (POC-1, POAM-P1 and MFG). The titers of these lines differed approximately 2 log. Co-cultivation of bone marrow and virus-producing cells was performed in the presence of IL-3. No growth factors were added to the stromal cell cultures.

Because we observed that after more than 3 days of culture the number of myeloid progenitor colonies (CFU-C's) declined, all cultures were performed for 3 days. Following co-cultivation of human bone marrow with POC-1, POAM-P1 and MFG, the percentages CFU-C's that overexpressed ADA ranged from 10 to 30%. Next, virus containing supernatants from POC-1 and MFG were added to bone marrow cells cultured over an irradiated stromal layer. This resulted in transduction frequencies of 15% and 30 %, respectively.

Based on our *in vitro* data, we have now also transplanted monkeys with bone marrow following transduction on a stromal layer. Data of these studies, which are currently in progress, will be presented.

SZ 210 TRANSDUCTION EFFICIENCY AND HUMAN ADENOSINE DEAMINASE EXPRESSION IN RHESUS MONKEYS TRANSPLANTED WITH AUTOLOGOUS BONE MARROW CELLS INFECTED WITH TWO DIFFERENT RETROVIRUSES.

Leonie C.M. Kaptein[1], Isabelle Riviere[3], Victor W. van Beusechem[1], Peter M. Hoogerbrugge[1,2],Trudy A. Bakx[1], Richard Mulligan[3], Dinko Valerio[1]. [1]Dept. of Gene Therapy, Inst. for Applied Radiobiology and Immunology TNO, Rijswijk, the Netherlands, [2]Dept. of Pediatrics, Univ. Hospital, Leiden, the Netherlands, [3]Whitehead Institute for Biomedical Research, Cambridge, MA.

Retroviruses are widely used for gene transfer into cells. Studies aimed at improvement of their use concern increasing the transduction of target cells and regulation of the expression levels of the introduced gene in the target cells.

In this study we compare two different retrovirus producing cell lines for their efficacy in transducing pluripotent hemopoietic stem cells of non-human primates and their expression levels in the hemopoietic system. The two cell lines are POC-1 and MFG-ADA, both containing the human adenosine deaminase (hADA) cDNA gene within their provirus. Rhesus monkey bone marrow cells were co-cultured with either the POC-1 or the MFG-ADA producing cell lines and are subsequently reinfused into lethally irradiated monkeys.

Recently, we have shown that the use of POC-1 cells results in approximately 0.1% of peripheral blood mononuclear cells and granulocytes containing the provirus up to at least 1 year after autologous transplantation (v. Beusechem, Proc. Natl. Acad. Sci. USA (1992) 89: 7640-44).

Here we present the transduction of monkey bone marrow cells with MFG-ADA. Two monkeys treated with MFG-ADA are at the moment at day-108 and day-25 after bone marrow transplantation. Preliminary data on the first monkey suggest that its erythrocyte samples contain higher levels of hADA activity than was observed in previous experiments using POC-1. Whether this is due to a higher infection frequency and/or a higher expression level remains to be resolved. The frequency of transduced peripheral blood cells is currently being analyzed by PCR.

SZ 211 **GENE THERAPY FOR GAUCHER DISEASE: GLUCOCEREBROSIDASE GENE TRANSDUCTION OF GAUCHER MARROW BY RETROVIRAL VECTORS.**

Donald B. Kohn, Jan A. Nolta, Xiao-Jin Yu, and Mary Beth Hanley. Childrens Hospital of Los Angeles, Departments of Pediatrics and Microbiology, University of Southern California School of Medicine.

Gaucher disease, a lysosomal glycolipid storage disorder, results from genetic deficiency of an acidic glucosidase, glucocerebrosidase (GC). The beneficial effects of allogeneic bone marrow transplantation (BMT) for Gaucher disease suggest that GC gene transduction and the transplantation of autologous hematopoietic stem cells (gene therapy) may similarly alleviate symptoms. We have constructed a retroviral vector, L-GC, produced by a clone of the amphotropic packaging cell line PA317, which transduces the normal human GC cDNA with high efficiency. Total marrow mononuclear cells and CD34-enriched cells from a four year female with type 3 Gaucher disease were transduced by the L-GC vector and studied for up to five months in long-term bone marrow culture (LTBMC). Prestimulation of marrow with IL-3/IL-6/SCF, followed by co-cultivation or supernatant transduction, produces gene transfer into 40-45% of the long-lived progenitor cells. Normal levels of GC expression in progeny cells produced by the LTBMC (primarily mature myelomonocytic) are seen by Northern blot, Western blot and GC enzyme assay throughout the duration of culture. To extend the analysis of gene transduction of pluripotent Gaucher stem cells, we have developed a novel model for sustained human myelopoiesis in immunodeficient (*bnx*) mice. $CD34^+$ cells from Gaucher marrow were transduced with the L-GC vector and co-transplanted with autologous marrow stromal cells that were engineered to produce human IL-3. After 2-4 months, the mice contain 10-15% human hematopoietic cells in the marrow and spleen. The presence of the L-GC vector and expression of human GC in circulating mature myeloid cells is demonstrated by PCR and immunohistochemistry. These studies demonstrate that retroviral vectors can efficiently transfer the GC gene into long-lived hematopoietic progenitor cells from the bone marrow of patients with Gaucher disease, and express physiologically relevant levels of GC enzyme activity.

SZ 212 **RECONSTITUTION OF THE MURINE RETICULO-ENDOTHELIAL SYSTEM WITH HUMAN GLUCOCEREBROSIDASE-TRANSDUCED BONE MARROW-DERIVED MACROPHAGES.**

W.J. Krall, P.M. Challita, and D.B. Kohn, Childrens Hospital Los Angeles, and Department of Pediatrics and Microbiology, University of Southern California School of Medicine, Los Angeles, CA 90027.

Lack of the lysosomal enzyme, glucocerebrosidase (GC), causes Gaucher's Disease in which tissue macrophages engorged with lipid accumulate in the spleen, liver, bone marrow, and in some cases, the central nervous system. A gene therapy approach employing retroviral transduction of autologous bone marrow with the human GC cDNA followed by bone marrow transplantation (BMT) is currently being studied in our laboratory. Amelioration of the clinical symptoms of Gaucher's Disease may require replacement of the diseased reticuloendothelial system with incoming GC-transduced macrophages. In addition, restriction of GC expression to the myeloid lineage may be desirable in order to avoid side effects associated with unregulated enzyme producion. We have sought to address these issues in the murine BMT model by constructing retroviral vectors in which GC expression is directed by the viral LTR, or by one of several internal elements including the CD11b, CD18, and GMCSF promoters. Growth factor-stimulated donor bone marrow was transduced with these vectors by co-cultivation with virus-producing fibroblasts prior to syngeneic BMT in C57B6 recipient mice. GC transduction and expression is presently being studied in 12-14 day CFU-S, and in the tissues of long-term reconstituted animals by polymerase chain reaction and Northern Blot analysis. Concomitantly, tissues including the spleen, thymus, bone marrow, liver, lung, kidney, brain and spinal cord, are being examined for evidence of GC expression in macrophages by immunohistochemical techniques using the human GC-specific antibody, 8E4. These experiments seek to identify promoter elements which confer tissue-specific expression of glucocerebrosidase, and will reveal the predicted time course for macrophage replacement in individuals receiving bone marrow transplant-mediated gene therapy.

SZ 213 ISOLATION AND EXPANSION OF $CD34^+$ CELLS IN THE AIS CELLector™ CD34 YIELDS LARGE INCREASE IN COMMITTED PROGENITOR CELL NUMBER,

Jane Lebkowski, Lisa Schain, Mark Harvey, Annemarie Moseley, Thomas B. Okarma, Applied Immune Sciences, Inc., Santa Clara, CA 95054

Expansion of $CD34^+$ cells ex vivo could yield a ready source of committed human progenitors for patient infusion after intensive chemotherapy for the rapid recovery of peripheral cell counts. To this end we isolated $CD34^+$ cells from normal human bone marrow using polystyrene devices containing covalently immobilized soybean agglutinin and the CD34 monoclonal antibody, ICH3. $CD34^+$ cells isolated using this method are > 85% pure. Since the $CD34^+$ cell capture device is also a culture vessel, media containing IL1, IL3, IL6, SCF, G-CSF, GM-CSF, and Erythropoietin was added to the isolated adherent $CD34^+$ cells, and directly cultured at 37°C. During the initial 10-14 days of culture, the cell number expanded up to 100 fold. After this time period, cell number remained constant and eventually declined. During the rapid cell expansion, $CD34^+$ cell frequency declined from 85% at onset to 10% at day 6 to eventually undetectable levels at later time points. The total number of $CD34^+$ cells slightly expanded during the early phases of the culture, but later dramatically declined. Similar observations were made for CFU-GM, BFU-E and CFU-GEMM numbers. After 6-8 days in culture progenitors expanded maximally, being 6-20 fold increased in number. At later time points CFU-GM, BFU-E and CFU-GEMM numbers declined as the dividing cells differentiated. Experiments demonstrating the gene transduction of such cultured cells will be presented. The data demonstrate the feasibility of large scale culture and expansion of progenitors from isolated $CD34^+$ cells and their potential use for patient transfusion.

SZ 214 **X-LINKED CHRONIC GRANULOMATOUS DISEASE: CORRECTION OF NADPH OXIDASE DEFECTS BY RETROVIRUS-MEDIATED GENE TRANSFER,** Roland J. Levinsky, Mohamed H. Parkar, Mary K.L. Collins*, Christine Kinnon and Colin D. Porter, Institute of Child Health, London WC1N 1EH, UK and *Chester Beatty Research Laboratories, London SW3 6JB, UK.

Chronic granulomatous disease (CGD) is an inherited immunodeficiency disorder resulting from the inability of patient phagocytes to produce superoxide anions due to defective NADPH oxidase. The disease is confined to the hematopoietic system and this makes it a candidate disorder for treatment by bone marrow transplantation and retrovirus-mediated somatic gene therapy.

Defects in an X-linked gene, which encodes gp91-*phox*, the 91KD ß chain of cytochrome b_{-245}, account for two-thirds of CGD patients. We have used retrovirus-mediated expression of gp91-*phox* to functionally reconstitute NADPH oxidase activity in EBV-transformed B cell lines from three unrelated X-CGD patients. We will present data that demonstrates that the retrovirus containing the X-CGD cDNA is stably integrated into these cells. Western blot analysis of membrane preparations from these cells using a monoclonal antibody specific for gp91-*phox*, detected a protein of the correct size, which was apparently correctly glycosylated and targetted to the membrane. Restoration of NADPH oxidase function in the transduced X-CGD B cell lines expressing gp91-*phox* was demonstrated using a sensitive luminol-based chemiluminescence assay. The functionally reconstituted oxidase in these cells is regulated by protein kinase C, with the magnitude of the response comparable to the level of protein expression.

These studies are the first to demonstrate expression of gp91-*phox* and thus indicate the feasibility of the treatment of X-CGD by somatic gene therapy techniques.

SZ 215 TRANSDUCTION OF HUMAN BONE MARROW BY ADENOVIRAL VECTOR, Kohnoske Mitani[1], Frank L. Graham[2] and C. Thomas Caskey[1], [1]Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030 and [2]Department of Biology, McMaster University, Ontario L8S 4K1, Canada

Recombinant adenoviral vectors have been shown to be a potential new tool for a variety of human gene therapy protocols. We examined the effectiveness of an adenovirus vector for gene transfer into human bone marrow (BM). The adenovirus vector Ad-ADA encodes human ADA cDNA under control of the human polypeptide elongation factor-1α promoter. Mononuclear cells from one ADA-deficient and two normal human BM samples were infected by this vector with different multiplicities of infection (m.o.i.) and kept in myeloid long-term culture. Retroviral gene transfer was also performed with the ADA-deficient bone marrow as a control. The infected cells were harvested at different times and the expression of the vector encoded ADA in crude cell extracts of non-adherent cells was analyzed by an ADA conversion assay. The expression of Ad-ADA was higher than that from a retroviral vector at one week post-infection. The expression of the retrovirus vector was persistent at week 10, as expected. In two out of four (one from each ADA-deficient and normal BM) experiments, the ADA activity decreased with passage, probably because integration of adenovirus into the host genome is very rare. Unexpectedly, sustained expression from Ad-ADA was observed in the other two experiments. In all cases, strong toxicity of the virus to stromal cells was observed. At the end of the experiments (8-11 weeks post-infection), culture medium was harvested and used for a plaque forming assay to measure infectious virus. Only from BM cultures which showed sustained expression of ADA, free virus was detected on 293 cells; the titer was 150 pfu/ml and 700 pfu/ml from ADA-deficient and normal BM, respectively. Therefore, long-term expression of ADA correlated with the existence of the free virus in media. Three independent virus clones recovered after long-term culture of the infected ADA-deficient BM were analyzed. Their inability to plaque on Hela cells and the results of Southern hybridization strongly suggested that the recovered viruses were Ad-ADA. Our results suggest a potential use of adenoviral vectors for gene therapy which does not require long-term expression such as some kinds of cancer gene therapy. These results also might raise a concern regarding the safety aspect of adenovirus vectors as a tool for human gene therapy.

SZ 216 GENERATION AND COMPARATIVE PHARMACOLOGICAL ANALYSIS OF TRANSGENIC MICE EXPRESSING VARIANT METHOTREXATE-RESISTANT MURINE DIHYDROFOLATE REDUCTASES, Jill A. Morris and R. Scott McIvor, Institute of Human Genetics, Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN 55455.

Leukemic relapse in patients having undergone cytoablation followed by allogeneic bone marrow transplantation represents a significant challenge for clinicians. The extreme sensitivity of rapidly-dividing grafted bone marrow cells to methotrexate precludes treatment of these relapsing patients to eliminate the recurrent disease. One possible therapeutic approach would be to introduce a methotrexate-resistant dihydrofolate reductase (Mtx^r-DHFR) sequence into donor marrow cells to provide protection upon subsequent exposure to methotrexate. We have established transgenic mice using Mtx^r-DHFR transcription units (Tg-*Arg22* and Tg-*Trp31*) as a source of donor marrow expressing Mtx^r-DHFR enzyme. Marrow from these animals will be used as donor marrow in syngeneic transplants followed by Mtx administration to the recipients to mimic the clinical scenario and to examine the level of protection afforded to the grafted marrow. The transgenes contain mutant DHFR cDNA sequences (*Arg22* and *Trp31*) including the first two introns under transcriptional regulation of the DHFR promoter. Three transgenic founder lines have been established for the Tg-*Trp31* transcription unit and two are known to transmit their transgene to offspring intact. Two transgenic founder lines have been established for the Tg-*Arg22* transcription unit. These five lines are presently being examined for expression in different tissues including hematopoietic tissues.

SZ 217 SOMATIC GENE THERAPY FOR DIABETES WITH AN IMMUNOLOGICAL SAFETY SYSTEM FOR COMPLETE REMOVAL OF TRANSPLANTED CELLS, Hiromitsu Nakauchi[1)], Yasushi Kawakami[2)], Takashi Yamaoka[2)], Rei Hirochika[1)], Kamejiro Yamashita[2)], Mitsuo Itakura[3)]. [1)]Lab. Cell Growth and Differentiation, RIKEN, Tsukuba, Japan, [2)]Devision of Endocrinology and Metabolism, Instutute of Clinical Medicine, The University of Tsukuba, Tsukuba, Japan, [3)]the Otsuka Department of Clinical and Molecular Nutrition, School of Medicine, The University of Tokushima, Tokushima, Japan.

To develop somatic gene therapy for diabetes, we studied an animal model with proinsulin-producing fibroblasts with an immunological safety system. Cultured mouse fibroblasts of the Ltk$^-$cell line were transfected first with the efficient human proinsulin expression vector pBMG-Neo-Ins. Initially, 2 x 10^6 cells with a proinsulin-production rate of 91 ng. 24 $h^{-1}.10^6$ $cells^{-1}$ were transplanted i.p. into streptozocin-induced diabetic C3H mice. The blood glucose concentrations improved between the first and the 28th day, but the animals died of hypoglycemia between the 29th and 46th days. The proinsulin-producing Ltk$^-$ cells were further transfected with a second plasmid, pHEBo-CD8.2, encoding BALB/c mouse T-cell differentiation antigen. The CD8.2 allotype is different from CD8.1 allotype by only one amino acid substitution and should be only slightly antigenic to the recipient C3H mice. Somatic gene therapy with these doubly transfected cells followed by the consecutive administration of a monoclonal antibody to CD8.2 resulted in an initial decrease of blood glucose concentrations followed by the permanent recurrence of hyperglycemia, thus proving the complete removal of the transplanted cells. Cultured fibroblasts were thus proven capable of supplying sufficient proinsulin to lower the blood glucose concentrations in diabetic animals. The immunological safety system with a combination of artificial expression of cell surface antigen and the administration of the specific monoclonal antibody was an effective safety system for somatic gene therapy.

SZ 218 RETROVIRAL TRANSDUCTION AND EXPRESSION OF PURINE NUCLEOSIDE PHOSPHORYLASE IN LYMPHOCYTES; PRECLINICAL STUDIES TOWARDS GENE THERAPY FOR PNP-DEFICIENCY, David M. Nelson, Mark D. Foresman, Kim A. Butters, Nancy L. Reinsmoen, and R. Scott McIvor, Departments of Genetics and Cell Biology, Laboratory Medicine and Pathology, and Surgery, University of Minnesota, Minneapolis, MN 55455

Purine nucleoside phosphorylase (PNP) deficiency is associated with a fatal T-cell immunodeficiency in children for which no effective clinical recourse is currently available other than bone marrow transplantation. PNP deficiency is thus a candidate condition for gene therapy by introduction of functional PNP sequences into either T-cells or progenitor cells in the bone marrow. Our work has focused on using retroviral mediated gene transfer to effect expression of functional PNP in lymphoid cells. PNP retroviral vectors were first used to infect PNP-deficient murine lymphoid cells (S49-NSU1) as an *in vitro* model for gene therapy. Expression of functional PNP and abrogation of the deoxyguanosine toxicity implicated in PNP disease by our vectors demonstrated correction of the metabolic defect. We have extended this work to demonstrate retroviral transduction and expression of functional PNP in normal human peripheral blood lymphocytes using vectors expressing murine PNP which was electrophoretically distinguished from the human enzyme by isoelectric focusing. Retroviral mediated PNP gene transfer into lymphocytes from PNP-deficient patients is planned as further preclinical work-up for gene therapy of PNP-deficiency.

SZ 219 TRANSDUCTION OF G-CSF PRIMED PERIPHERAL BLOOD $CD34^+$ CELLS WITH THE GENE FOR GLUCOCEREBROSIDASE. Nimgaonkar M., Bahnson A., Boggs S., Robbins P.D., Patrene K., Fei Y., Ball E.D. and Barranger J.A, Division of Hematology and Bone Marrow Transplant and Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA 15261.

Gaucher disease, caused by glucocerebrosidase (GC) deficiency, is presently treated by either enzyme replacement or allogenic bone marrow transplantation (BMT), both of which are less than ideal. Transferring the gene for GC to peripheral blood (PB) $CD34^+$ cells and their transplantation could be a safe and effective alternative (gene therapy). We have examined the transduction of PB $CD34^+$ from G-CSF primed patients undergoing autologous BMT. Enrichment was performed using an avidin biotin immunoadsorption technique (CellPro™). Pre- and post-enrichment samples were analyzed for $CD34^+$lineage$^-$ (i.e. $CD3^-/CD19^-$) cells using a FACScan flow cytometer. Enrichment was 20 fold (4.12% vs 85.0% $CD34^+lin^-$, respectively).

Enriched $CD34^+$ cells were incubated for 24 hours in a combination of growth factors: PIXY/SCF, IL6/IL3/SCF and GM-CSF/IL3/IL6/SCF. These cells were then infected with supernatant from an amphotropic producer of MFG-GC over a period of 6 days. Similarly cultured noninfected $CD34^+$ cells were used as controls. Every 48 hours for 14 days, differentiation antigens CD15/CD11b/CD14 and CD33 were analyzed on these cells by flow cytometry.

Infected $CD34^+$ cells showed a 200 U/mg increase GC activity above endogenous levels (controls = 250 U/mg). GC transduction was confirmed by Southern blot. There was no significant difference in transduction efficiency ammong the cells cultured in different cytokine mixtures. The percentage of $CD34^+lin^-$ cells in the cultures decreased because of a corresponding increase in differentiated cells. Although there was a 20 fold increase in total cell numbers, the absolute number of $CD34^+$ cells remained constant over 11 days. These data suggest 1) it is possible to transduce G-CSF primed PB $CD34^+$ cells 2) the MFG-GC vector is expressed at potentially therapeutic levels in these cells after expansion *in vitro* and 3) the optimal time for stem cell transduction is in the first 4 days post harvest when the fraction of $CD34^+$ cells is maximal.

SZ 220 SURVIVAL AND DIFFERENTIATION OF PRIMITIVE HUMAN CD34+ HEMATOPOIETIC CELLS IN LIQUID CULTURES AND IN SCID MICE. Ritchey, D*, Hall, E*, Kaushal, S*, Greenhouse, J*, La Russa, V^, Kessler, S#, Gartner, S*, Liu, Y*, Sitz, K^, Perera, P§, Kim, J^, St. Louis, D*, Vahey, M^, Yu, Z*, Xu, J*, Redfield, R^, Burke, D^, Mosca, J*. *Henry M. Jackson Foundation, Rockville, MD 20850, ^WRAIR, Rockville, MD, §NIH, Bethesda, MD 20894, #NMRI, Bethesda, MD 20814.

It has been shown that highly purified CD34+ cells from human bone marrow can serve as a source of target cells for virus infection. In this study, we sought to determine *in vitro* and *in vivo* models which will ultimately be used to produce and maintain target cells for retroviral transfer of genetic material into primitive progenitor cells. Our objectives were 1) determine if highly purified CD34+ cells could be maintained in a non-proliferative state and retain their responsiveness to hematopoietic growth factors and 2) determine if human primitive lympho-hematopoietic CD34+ cells could engraft SCID mice.

Fresh CD34+ marrow isolates were grown in primary liquid cultures in the presence or absence of human recombinant (hr) growth factors (IL1alpha, IL3, IL6, SCF, GM-CSF) for 7 days. Cytokine addition resulted in a 7-fold increase in cell number over cytokine-deprived cultures. Secondary cultures of cells were stimulated after 7 days by the addition of cytokines. Cells from these secondary cultures demonstrated a proliferative response including GFU-GM similar to that observed if cytokines were administered at day 0, suggesting that these cells could be maintained in a non-proliferative state (Go), awaiting the appropriate signals for entry into the cell cycle. The survival of CD34+ cells for 7 days in the presence or absence of cytokines were measured by their capacity to generate CFU-GM in agar cultures. There was a 2 fold increase in GFU-M and a 10 fold increase in GFU-G colonies after 7 days in these primary cultures containing cytokines when compared to cultures deprived of cytokines. FACS analysis of 7 day old primary cultures of CD34+ cells, in the presence of cytokines, shows a relative depletion of cells expressing the CD34+ (99% day 0, 5% day 7) with an increase of cells which express the CD33+ antigen (78%). Primitive cells capable of reconstituting the lympho-hematopoietic system were assayed from 10^4 - 10^6 CD34+ cells which were injected ip into 1-2 week old SCID mice (hu-BM-SCID). Engraftment of these primitive cells was monitored by their capacity to give rise to cells expressing the human L1 gene in the peripheral blood of mice 5 weeks after transplantation. The human L1 gene was detected by DNA PCR suggesting the continuous production of human cells in hu-BM-SCID mice after transplantation.

SZ 221 STEM CELL FACTOR ENHANCES HEMATOPOIESIS OF ISOLATED $CD34^+$ CELLS IN LONG TERM BONE MARROW CULTURE, Lisa Schain, Mark Harvey, Thomas B. Okarma, and Jane Lebkowski, Applied Immune Sciences, Inc., Santa Clara, CA 95054

Recombinant Human Stem Cell Factor (SCF) has been shown to synergize with other growth factors to increase the number, size and replating efficiency of hematopoietic progenitors. To understand how SCF affects long term hematopoiesis, isolated $CD34^+$ cells were seeded onto irradiated allogeneic stroma and half of the cultures were supplemented with 10 ng/ml rSCF. Cultures supplemented with SCF produced approximately 5 fold more nonadherent cells at any given time point and consistently proliferated 1-3 weeks longer than conventional cultures. When CFU-GM levels were monitored, SCF again produced 2-5 fold more CFU-GM in the nonadherent population at any time point. As a result of this increased production of CFU-GM an overall 5 fold increase in cumulative levels of CFU-GM was observed with SCF. SCF also appeared to stimulate the activity of the primitive long term bone marrow culture initiating cell (LTBMCIC) as evidenced by the 5-8 fold increase in CFU-GM observed after 5 weeks of stromal culture with the growth factor. These data suggest that SCF acts to stimulate the activity of both committed and primitive stem cells. These observations could have important implications for the long term culture of stem cells for patient infusion, autografting, and gene therapy. Our results using this culture system for gene transfer into isolated $CD34^+$ cells will be discussed.

SZ 222 CORRECTION OF CHRONIC GRANULOMATOUS DISEASE (CGD) BY GENE TRANSFER INTO PERIPHERAL BLOOD HEMATOPOIETIC PROGENITORS (PBHP), Sudhir Sekhsaria, John I. Gallin, Richard C. Mulligan[§] and Harry L. Malech, Laboratory of Host Defenses, NIAID, NIH, Bethesda, MD 20892 and the [§]Whitehead Institute, Cambridge, MA 02142

PBHP may be a useful target for correction of inherited diseases by gene transfer. CGD patients have life-threatening infections due to lack of superoxide (O_2^-) generation by phagocytes. One third of CGD patients have a defect in the $p47^{phox}$ oxidase factor gene. We made ψ-crip lines which package an amphotropic retrovirus construct, mfg-p47, encoding $p47^{phox}$. Supernatant from these clones efficiently transduced $p47^{phox}$ protein production in 3T3 fibroblasts and reconstituted O_2^- generation in EBV transformed B cell lines derived from each of 4 $p47^{phox}$-deficient CGD (p47-CGD) patients. CD34 positive PBHP were selected from peripheral blood of p47-CGD patients by apheresis and monoclonal antibody affinity column. PBHP were grown in a combination of early acting growth factors for 48 hrs prior to transduction on two consecutive days with mfg-p47 retrovirus. Transduced PBHP were grown in semisolid agarose or in liquid cultures with early factors and G-CSF to produce myeloid differentiation. Controls were cultures of normal PBHP and p47-CGD PBHP with sham transduction. O_2^- production by myeloid colonies in agarose was examined by stimulated nitroblue tetrazolium dye (NBT) reduction. O_2^- production by PBHP from liquid cultures was determined by a chemiluminescence assay. The mfg-p47 transduction of PBHP from 5 different p47-CGD patients markedly increased NBT positive myeloid colonies to 17% of colonies compared to a mean of 4.7% with sham transductions ($p < 0.004$). PBHP from 6 different p47-CGD patients transduced with mfg-p47 and differentiated in liquid cultures demonstrated a significant increase in O_2^- production to an average of 17% of normal control from a baseline level of 3% ($p<0.005$). All patients showed a response in all assays. It is important to note that there was no selection for transduced PBHP as the mfg-p47 vector carries no selective marker. We show that the CGD genetic defect can be corrected by retroviral gene transfer into p47-CGD primary hematopoietic progenitors from peripheral blood. The use of PBHP as a target of gene therapy offers potential advantages in the development of treatments for inherited disorders affecting bone marrow.

SZ 223 ABLATION OF ALLOREACTIVITY IN VITRO BY GANCICLOVIR (GCV) TREATMENT OF HERPES SIMPLEX THYMIDINE KINASE (HS-tk)-TRANSDUCED T CELLS, Pierre Tiberghien[1], Craig W. Reynolds[2], Jonathan R. Keller[1], William J. Murphy[2], Russette Lyons[3], Yawen Chiang[3], Dan L. Longo[2], Francis W. Ruscetti[2]. [1]PRI/DynCorp, Inc., BCDP, [2]BRMP-LLB, NCI-FCRDC, Frederick, MD, 21702 and [3]GTI, Gaithersburg, MD, 20878.

Allogeneic bone marrow transplantation (BMT) is associated with a severe complication: Graft-versus-Host Disease (GvHD). While effectively preventing GvHD, ex-vivo T lymphocyte marrow depletion unfortunately increases graft rejection and reduces the graft-versus-leukemia (GvL) effect. The ex-vivo transfer of a HS-tk suicide gene in T cells before their infusion with the hematopoietic stem cells could allow for selective in vivo depletion of these T cells with GCV if significant GvHD was to occur. Thus, one could preserve the early beneficial effects of the T cells on engraftment and the GvL effect in patients not experiencing severe GvHD. In order to obtain T cells specifically depleted by GCV, we transduced primary T lymphocytes with a retroviral vector containing the HS-tk and Neomycin resistance (NeoR) genes. Gene transfer was performed by coculturing PHA/IL-2-stimulated T cells on an irradiated retroviral vector producer cell line (PA317/G1TkSvNa) or by incubating the T-cells in supernatant from this producer. In both cases, subsequent culture in G418 and IL-2 allowed for the selection of G418-resistant cells. GCV treatment of these cells resulted in > 80% growth inhibition while GCV treatment of control cells had no effect. The G418-selected cells were capable of reactivity in mixed lymphocyte cultures and GCV completely inhibited this allogeneic reactivity. Combining transduced and non-transduced T cells did not reveal a bystander effect. Therefore, the transfer of the HS-tk and NeoR genes into primary T cells can result in specific GCV-induced inhibition of in vitro alloreactivity. Animal models to further analyze the potential for clinical applications are currently being pursued.

SZ 224 RETROVIRAL VECTOR-MEDIATED TRANSFER OF THE HUMAN ADENOSINE DEAMINASE GENE INTO RHESUS MONKEY $CD34^+CD11b^-$ BONE MARROW STEM CELLS, Victor W. van Beusechem, Julia A.K. Bart-Baumeister, Trudy A. Bakx, Leonie C.M. Kaptein and Dinko Valerio, Dept. of Gene Therapy, TNO-Institute of Applied Radiobiology and Immunology, 2280 HV Rijswijk, The Netherlands.

The pluripotent hemopoietic stem cell (PHSC) residing in the bone marrow is regarded as the ultimate target of choice for gene transfer in genetic approaches to the treatment of a variety of congenital and acquired diseases involving the hemopoietic system. Recently, we have reported long-term genetic modification of the hemopoietic system of rhesus monkeys following co-cultivation of their bone marrow cells (BMC) with the cell line POC-1, which produces recombinant retroviruses carrying the human adenosine deaminase (ADA) gene (Van Beusechem *et al.*, Proc. Natl. Acad. Sci. USA 89(1992):7640). In that study, approx. 0.1% of PHSCs became transduced. Data will be presented here that show that the transduction efficiency was not influenced by the retrovirus titer. In order to elucidate the factors that do influence the transduction efficiency of primate PHSCs, we devised more well-defined cultures using enriched PHSCs. The enrichment was performed by depleting low-density BMC for CD11b expressing cells followed by positive selection for CD34 expressing cells by means of immunomagnetic bead separation. This resulted in a 20-45 times enrichment of GM-CFU and a 3.0-4.5 log depletion of T lymphocytes, as compared to post-Ficoll BMC. Following 90 hr co-cultivation of $CD34^+CD11b^-$ BMC from two monkeys with irradiated POC-1 cells and subsequent autologous transplantation, provirus-carrying mononuclear cells and granulocytes could be detected in the circulation of these animals for as long as they were analyzed (currently 266 and 280 days post transplantation in an ongoing study). A control monkey, which received separately cultured $CD34^+CD11b^-$ BMC and irradiated POC-1 cells remained negative. After 9 months, provirus-carrying T lymphocytes were isolated from the peripheral blood of the two transduced monkeys and *in vitro* expanded. The T cells from one of these animals could be selected *in vitro* for ADA-overexpression. Clonal analysis of these transduced T cells is currently being undertaken.

SZ 225 RETROVIRAL-MEDIATED TRANSFER AND EXPRESSION OF HUMAN IDURONATE-2-SULFATASE cDNA IN LYMPHOID CELLS: METABOLIC CORRECTION AND CROSS-CORRECTION OF MUCOPOLYSACCHARIDOSIS II (HUNTER SYNDROME), Chester B. Whitley, Stephen E. Braun, Paul L. Crotty, Rose A. Anderson, Jon J. Jonsson and R. Scott McIvor. University of Minnesota Medical School, Minneapolis, MN 55455

Hunter syndrome is a severe X-linked disease resulting from deficiency of iduronate-2-sulfatase (IDS) enzyme activity and the consequent systemic accumulation of glycosaminoglycans (GAG). To evaluate the potential effectiveness of gene therapy, a retroviral vector L2SN was constructed containing the IDS coding region. High-titer L2SN virus supernatant ($2x10^6$ cfu/ml) was generated by PA317 cells. IDS-deficient lymphoblastoid cell lines (LCL) from a patient with Hunter syndrome were transduced and selected with G418. IDS activity in L2SN-transduced Hunter LCL was found to be 10-fold greater (mean 8,766 U/mg/hr; SD 3,468) than normal LCL (mean 829; SD 131) or normal uncultured leukocytes (mean 807; SD 252) verifying biologic function of the IDS coding sequence, and demonstrating robust expression from the retroviral LTR. L2SN-transduced Hunter LCL failed to show progressive accumulation of $^{35}SO_4$-GAG indicating normal substrate catabolism in transduced cells. In metabolic cross-correction experiments, L2SN-transduced Hunter LCL cleared $^{35}SO_4$-GAG from co-cultivated Hunter fibroblasts; importantly, L2SN-transduced Hunter LCL reduced $^{35}SO_4$-GAG more effectively than normal LCL. Subsequently, L2SN was used to transduce peripheral blood lymphocytes from a patient with Hunter syndrome producing a heterogeneous cell population with increased IDS enzymatic activity. We speculate that high-level IDS expression, and metabolic correction, *in vitro* predict successful application of retroviral-mediated gene transfer to lymphohematopoietic cells for treatment of Hunter syndrome. (Supported by NIH DK39891 and the Daniel Molinaro Foundation)

Gene Delivery II

SZ 300 DIFFERENTIAL TRANSDUCIBILITY OF HUMAN HEPATOCYTES BY AMPHOTROPIC AND XENOTROPIC RETROVIRUS. **R. Mark Adams, Humberto Soriano, Mary Wang, David Steffen, and Fred Ledley, Departments of Cell Biology and Pediatrics, Baylor College of Medicine, Houston, TX 77030**

In conjunction with the initiation of clinical trials aimed at assessing an *ex vivo* strategy for hepatic gene therapy, we have performed studies aimed at optimizing the efficiency of transduction of *human* hepatocytes with retroviral vectors. We have previously shown that the transduction of human hepatocytes is significantly less efficient than the transduction of murine cells using essentially identical conditions and that xenotropic vectors consistently produced greater transduction efficiencies. We now demonstrate that transduction of human hepatocytes with amphotropic and xenotropic vectors varies not only in magnitude but in the temporal pattern of transduction susceptibility and the correlation with cellular proliferation. Human hepatocytes were harvested from organs preserved in Belzer(UW) solution and were cultivated in serum-free, tyrosine-free, hormonally defined medium (SUM/CHOW/human) optimized to maintain hepatocyte morphology. Cells exhibit proliferation to confluence in culture and express hepatocyte-specific markers for more than 3-5 weeks. The efficiency of transduction with an amphotropic vector (LNL6) was 5-fold lower than the transduction efficiency using an analogous xenotropic vector, despite the fact that the titer of the xenotropic vector was 10-fold lower on ratl or HeLa cells. Transduction with amphotropic vectors was maximal at 24-30 hours and decreased substantially by 48 hours. Transduction with xenotropic vectors had a broad window of maximal susceptibility between 48-60 hours and paralleled the fraction of replicating cells determined by parallel *in situ* [^{3}H]-thymidine incorporation. The efficiency of transduction remained 2-3 fold lower than the fraction of cells exhibiting [^{3}H]-thymidine incorporation. The distinct temporal pattern of transduction by the xenotropic and amphotropic vectors suggests that the amphotropic and xenotropic receptors may be differently regulated in primary human hepatocytes in the hours following harvest and that the expression of these receptors, rather than the fraction of cells dividing, may limit transduction under our cultivation conditions.

SZ 301 *IN VIVO* ADENOVIRUS-MEDIATED GENE TRANSFER TO RHESUS MONKEY AIRWAY EPITHELIUM

Abraham Bout[1,2], Michel Perricaudet[3], Gary Baskin[4], Bob J. Scholte[2], Andrea Pavirani[5] and Dinko Valerio[1]. [1]Department of Gene Therapy and [4]Department of Chronic and Infectious Diseases, Institute of Applied Radiobiology and Immunology, TNO, Rijswijk and [2]Department of Cell Biology I, Erasmus University, Rotterdam, the Netherlands; [3]Laboratoire des Virus Oncogenes, CNRS, UA 1301, Institut Gustave Roussy, Villejuif and [5]Transgene S.A., Strasbourg, France

Gene therapy of lung disorders such as Cystic Fibrosis require *in vivo* gene transfer techniques. One possibility in this respect is to use recombinant adenoviruses, which have been proven to be capable of infecting cotton rat airway epithelium *in vivo*. To assess efficacy and safety in a model more relevant for the clinical situation, we have tested the use of a recombinant human adenovirus in non-human primates. We have instilled recombinant adenovirus that harbors the *Escherichia coli lacZ* gene as a reporter (Ad-RSV-ßgal), via a broncho fiberscope in the lungs of rhesus monkeys. Six days after administration the animals were sacrificed. The monkeys displayed no abnormal behavior, no change in temperature or weight loss and their hematological parameters remained normal. Slices of trachea, main bronchi and lungs were stained with X-GAL to monitor *lacZ* expression. Macro- and microscopic examination of the tissues revealed that lacZ positive cells occurred in a patchy distribution at all levels of the trachea, bronchi and bronchioles, including terminal bronchioles. Within patches of blue cells we found up to 50 % of the cells lining the airways to be positive. Blue cells were predominantly, but not exclusively, present in areas where the epithelium was slightly thickened and had a reduced number of goblet cells. Similar areas of epithelium were seen in control tissue, and are therefore not caused by the administered adenovirus. Light microscopical morphologic typing showed that mainly ciliated cells were positive, but positive basal and mucous goblet cells were also observed, although less frequently. All organs examined, including the lungs and trachea, displayed no significant pathological changes. The observed efficient infection of rhesus monkey airway epithelium with minimal toxicity underlines the potential of adenoviruses as tools for gene therapy of CF. Ad-CFTR recombinant adenovirus has been added to rhesus monkeys endo-tracheally. Results of these experiments will be presented. Further preclinical tests on rhesus monkeys with recombinant human adenoviruses (Ad-RSV-ßgal and Ad-CFTR) will be performed to assess chronicity of expression, immune response, long-term toxicity and repeated virus deliveries. Results of such studies will provide us with data indispensable to evaluating the clinical applicability of recombinant adenoviruses.

SZ 302 SYNTHETIC RETROTRANSPOSON VL30 DERIVED VECTORS FOR GENE THERAPY. Asit K. Chakraborty, Mary Ann Zink, Bruce M. Boman, and Clague P. Hodgson. *Creighton Cancer Center, Dept of Biomedical Sciences, Creighton School of Medicine, Omaha, NE. 68178.*

Retroviral vectors have proven useful for gene therapy but suffer from several drawbacks, including recombination, pathogenesis, and *in vivo* shutdown of transcription. We have used gene amplification together with synthetic oligonucleotides to construct vectors based upon mouse VL30, a family of retrotransposons found in the mouse genome. The vectors were designed for transcription of genes from either the long terminal repeat or from internal promoters. Essential *cis*-acting sequences were included, together with variable amounts of suspected packaging sequences and multiple cloning sites for insertion of one or more genes. A gene-amplified phosphotransferase gene (*neo*) was included for selection experiments. Three out of four vectors efficiently expressed and faithfully transduced the *neo* gene, which was transcribed from the VL30 LTR. Initial titers from filtered ψ2 cell media were 10^{-5} IFU/ml. The new vectors contain from 1 to 1.2 kb of vector RNA sequences plus inserted genes and have about 50bp of scattered homology to MLV helper, limiting opportunities for homologous recombination with helper virus. Moderate to high vector RNA levels expressed in NIH3T3 cells were similar in abundance to that of endogenous VL30 RNA. DNA blot analysis revealled one to three copies of vector DNA integrated at random in cloned cell lines expressing *neo* phenotype, and no rearrangements were observed. ψ2 cells expressing the vectors did not give rise to replication-competent virus, in contrast to a control retroviral vector transmitted by ψ2. Finally, the results indicated that entirely synthetic products of gene amplification will suffice to make biologically active vectors. VL30 vectors are expressed in a number of human cell types and should be useful for gene therapy.

SZ 303 VL30 VECTORING SYSTEMS: SOME SURPRISES IN THEIR REGULATION IN EMBRYONIC STEM CELLS,

Dominic E. Cosgrove, Asit K. Chakraborty,[+] James A. Grunkemeyer, and Clague P. Hodgson[+], Boys Town National Research Hospital Department of Genetics, Omaha, NE 68131; [+]Creighton University Cancer Center, Omaha NE 68178

Retroviral vectors are often transcriptionally silent after being introduced into ES cells or embryos, mimicking retroviral shutdown *in vivo*. Murine retrotransposon VL30 vectors were used to infect the ESD3 embryonic stem cell line to assess their activity relative to that observed in differentiated tissues and transformed cell lines. The vectors contained a marker for resistance to neomycin like drugs. Following selection in G418, several clones were isolated and analyzed. Southern blot analysis confirmed a single copy of the vector in each clone analyzed. Northern analysis of RNA from these clones using a probe specific for the neomycin resistance marker revealed the expected 2.3 kb message was truncated to 1.8 kb. When these same blots were reprobed with DNA specific for VL30, it was observed that transcripts from both the endogenous VL30 genes and the vector were indeed truncated by approximately 500 bases relative to the control cell line. The molecular explanation for the shorter transcript is currently under investigation. In addition, a 12 kb VL30-related transcript was observed in both the infected and uninfected ES cells that is totally absent from the control cell line. Such a transcript has previously been reported only in brain. In summary, the results suggest atypical regulation of endogenous VL30 sequences in ES cells, and also demonstrated the ability to express a foreign selectable gene for use in ES cell transgenics.

SZ 304 ADENOVIRUS ENHANCEMENT OF TARGETED DNA DELIVERY INTO PRIMARY HEPATOCYTES THROUGH RECEPTOR MEDIATED ENDOCYTOSIS, 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 previously shown that a direct DNA delivery system, when combined with the endosomal lysis ability of adenovirus, can result in DNA being delivered into primary hepatocytes that results in high level expression of a reporter gene. The liver specific ligand, Asialoorosomucoid (ASOR) was conjugated to Poly-L-Lysine (PLL) in the presence of the water soluble carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). The resulting ASOR/PLL conjugate was incubated with DNA, forming an ASOR/DNA complex. DNA delivery by the complex into primary hepatocytes through binding with the hepatic asialoglycoprotein receptor resulted in ß-Gal activity that was greatly enhanced by the co-internalization of the complex with a replication defective adenovirus. After 24 hours, 100% of the hepatocytes can be positively stained for ß-Galactosidase activity. Quantitative analysis of ß-Gal activity, showed a 1000-fold enhancement of activity over that from the complex alone. At high viral titers and with longer incubations, cytopathic effect of the adenovirus is noted. To generate a much more suitable ASOR/DNA complex for enhanced DNA delivery at lower adenoviral titers, we have coupled the complex directly to the adenovirus. This Adenovirus/DNA complex results in quantitative staining of hepatocytes at much reduced adenoviral titers and at the same time, little or no cytopathic effect from the adenovirus is seen with prolonged incubations. This methodology shows great promise for efficient gene delivery to the liver for the correction of hepatic deficiencies in the future.

SZ 305 ADENOVIRAL MEDIATED GENE TRANSFER TO CELLULAR COMPONENTS OF THE CENTRAL NERVOUS SYSTEM IN VIVO, Beverly L. Davidson and Blake J. Roessler, Division of Rheumatology, Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, MI 48109-0680

Current methodologies designed to deliver genes to the central nervous system (CNS) for treatment of inborn or acquired disorders with a CNS component have to date been largely ineffective. In most cases the efficiency of gene transfer is low, and the time course of expression of the transgene is limited. We have developed a model system of direct gene transfer to the brain using recombinant adenovirus. Using *E. coli* β-galactosidase as a transgene, we have been able to detect gene transfer in the striatum of mice for periods exceeding two months, with high levels of expression in neurons, astrocytes, and oligodendrocytes. Specific identification of lacZ positive brain regions and cell types was determined by a combination of light microscopy, immunohistochemistry, and electron microscopy. In addition to *E. coli* β-galactosidase, we have used recombinant adenoviral vectors containing the rat *hprt* cDNA for brain directed gene transfer experiments in a murine model of HPRT deficiency. Post-injection analysis included enzyme activity assays, nucleotide pool determination, and quantitative determination of components involved in dopaminergic metabolism. These data, and preliminary data regarding HPRT gene transfer to the caudate putamen of non-human primates, will be discussed.

SZ 306 **NOVEL GENE EXPRESSION SYSTEM FOR CARDIO-VASCULAR GENE THERAPY**

Ciao-Chang Fu, Jung-Kuang Hsieh, Ulla Tuulikki-Dennehy, Catherine Demoliou-Mason and Vija Vir Kakkar, Thrombosis Research Institute, Manresa Road, London SW3 6LR, UK

The vascular smooth muscle cells (VSMC) migration, proliferation and phenotype modulation constitute important steps in intimal hyperplasia and the pathogenesis of atherosclerosis. With the aim to establish human somatic cell gene therapy in cardiovascular diseases, we have undertaken the development of specific gene expression in human VSMC by using eucaryotic cellular promoter systems. This strategy has the potential of targeting the tissue of interest and offers the advantage of long term expression since it obviates the possibility of promoter inactivation that occurs with viral promoters in vivo.

We have identified and characterized recently a novel strong promoter from mammalian VSMC. Using the Chloramphenicol Acetyltransferase (CAT) gene we have demonstrated that this promoter can drive CAT-gene expression to levels comperable to those of viral promoter vectors like RSV in primary and subcultured human VSMC. Using combinations of this novel promoter and minimized human tRNA intragenic promoter we have established a novel eukaryotic gene expression system.

The potential therapeutic application of this novel gene expression system was tested in culture, using a human VSMC model of growth factor (i.e. PDGF)-modulated cell proliferation and transfection studies of cells with "anti-proliferation" gene constructs the expression of which is under the direction of these new vectors.

SZ 307 IN VIVO GENE TRANSFER TO SYNOVIUM USING CATIONIC LIPOSOMES J. Galea-Lauri, G.M. Mueller, G. Bandara, H.I. Georgescu, G. Hung, X. Gao, J.C. Glorioso, L. Huang, P.D. Robbins and C.H. Evans, Departments of Orthopaedic Surgery, Pharmacology and Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

Transfer of suitable genes to the synovial lining of joints offers new therapeutic possibilities for the treatment of arthritis. Both ex vivo and in vivo approaches to transfering genes to synovium have been suggested (Bandara et al. DNA and Cell Biol. 11:227-231, 1992). Here we describe the use of cationic liposomes to transfer the lac Z gene to synoviocytes both in vitro and in vivo. Primary cultures of rabbit synovial fibroblasts (type B synoviocytes) and the HIG-82 line of rabbit synoviocytes were exposed to various concentrations of "DC-chol" liposomes (10-300nmoles) combined with a CMV-Lac Z reporter plasmid (1-10μg DNA) in serumless medium in 24-well or 6-well plates. Five hours later, growth medium was restored to the wells. Following 41 hours further incubation, cells were stained for expression of β-galactosidase. Best results were obtained in 24 well plates with 15nmoles liposomes and 2μg DNA, a combination which produced approximately 20% Lac Z$^+$ cells. For in vivo lipofection, 1ml of a solution containing 200nmoles liposomes and 6μg DNA was injected intraarticularly into the knee joints of recipient rabbits. Control knees were injected with naked DNA, liposomes alone or carrier solution. Rabbits were killed 44 hours later and tissues examined for the expression of the lac Z gene. Staining of intact knee tissues revealed that empty lipsomes alone provoked intense blue staining of synovia. Only when cells were enzymically digested from the synovia and plated in cell culture was it possible to demonsfrate unequivocally the presence of lac Z$^+$ cells in tissue recovered from knees injected with liposomes and DNA, under conditions where there was no staining of cells recovered from control knees.

Supported in part by grant RO1 DK46640 from NIDDK.

SZ 308 GENE DELIVERY SYSTEMS INCORPORATING BACTERIAL INVASION PROTEINS, Stephen L. Hart, Richard P. Harbottle, Eddie F.V. Kinrade, Charles Coutelle and Bob Williamson, Department of Biochemistry and Molecular Genetics, St. Mary's Hospital Medical School, London W21PG

The most efficient internalisation pathway of the intracellular, pathogenic bacteria *Yersinia pseudotuberculosis* is mediated by *inv* encoded invasin (103-kD). Cell invasion occurs by a phagocytosis-like mechanism, even in cells that are not normally phagocytic, such as fibroblasts and epithelia. Invasin is responsible for bacterial attachment and internalisation, binding to β1 integrin receptors on the eukaryotic cell surface. The high binding affinity of this interaction triggers the internalisation mechanism. Internalisation of *Listeria monocytogenes* is mediated by internalin, a protein of 744 amino acids encoded by *inla* that shows no similarity to invasin, or any other protein. The nature of the internalin receptor and the internalisation mechanism are not known. Our aim is to exploit the proteins internalin and invasin in gene delivery systems either displayed on bacteriophage fd or in protein-DNA complexes. At the tip of one end of fd phage there are four or five copies of a minor coat protein (cpIII) which target and bind the phage to bacterial pili. A derivative of fd, fdDOG, facilitates the construction of cpIII-fusion proteins so that foreign proteins can be displayed on the phage surface as amino-terminal extensions of cpIII. CpIII-internalin and cpIII-invasin fusion constructs were made in fdDOG to form fdINL and fdINV respectively. Immunofluorescence microscopy observations suggested that phage constructs incubated with cultured Caco-2 epithelial cells attached to the cell surface but were only poorly internalised. The amino acid sequence RGD is a feature of most integrin-binding proteins (other than invasin). Phage constructs were made containing the RGD sequence, which was displayed over the phage surface in fusions with the major coat protein (cpVIII) with the aim of facilitating internalisation by providing more integrin binding sites.

Protein-DNA complexes incorporating receptor ligands such as transferrin and asialoglycoprotein conjugated with polylysine have been used to deliver genes to cells expressing the appropriate receptors. We are investigating the incorporation of invasin or internalin in protein-DNA complexes as an alternative cell-targeting strategy.

SZ 309 UNIQUE PATTERN OF EXPRESSION IN THE RAT LIVER ACINUS OF GENES TRANSFERRED IN THE FETAL LIVER VIA RETROVIRUSES; Maria Hatzoglou, Jin Yin Wu, Wouter Lamers and Antoon Morman, Department of Nutrition, Case Western Reserve University, Cleveland, OH, 44106

Transfer of genes into the fetal liver is a promising approach for correction of inborn errors in metabolism identified in prenatal life. We demonstrate that infection of the fetal rat liver with replication incompetent ecotropic retroviruses carrying a chemeric bovine growth hormone (bGH) gene, results in the stable expression of the gene in the hepatocytes of the rat in adult life. The bGH gene was linked to the promoter for the phosphoenolpyruvate carboxykinase (PEPCK) gene (-450 to +73) which is highly active in the periportal hepatocytes within the context of the architecture of the liver lobulus. Subsequent partial hepatectomy stimulated expression of the bGH gene in the liver of rats infected as fetuses. Expression of the gene was heterogeneously distributed through the liver parenchymal cells with higher expression in the pericentral compartment. This zonation of expression was not expected, since the endogenous PEPCK gene, is expressed in periportal hepatocytes. We suggest that proviral sequences influence the expression of the PEPCKbGH gene in populations of parenchymal cells in which the PEPCK promoter is not normally active. We also determined the successibility of the regenerating rat liver to infection, at different time points (0-48 hrs) after partial hepatectomy. A small number of hepatocytes expressed the β galactosidase gene which was included in the provirus, when the regenerating liver was infected 4 hrs after partial hepatectomy. This time point of infection paralleled the expression of the gene for the ecotropic retrovirus receptor, which was rapidly induced in the regenerating liver at the G_1 phase and returned to undetectable levels at 24 hrs after partial hepatectomy. We suggest that cell cycle dependent factors may play an important role in the infectibility of hepatocytes.

SZ 310 DIRECT in vivo GENE TRANSFER BY HVJ-LIPOSOMES Yasufumi Kaneda[1], Naruya Tomita[2], Jitsuo Higaki[2],Ryuichi Morishita[2], andToshio Ogihara[2] (Institute for Molecular and Cellular Biology [1], Department of Geriatrics,School of Medicine[2], Osaka University, Suita, Osaka 565, Japan)

We have developed the efficient method to transfer foreign genes directly into organs of adult animals. In our system, DNAs and nuclear protein, HMG-1, were entrapped into liposomes and the liposomes were treated with HVJ (Sendai virus) to form HVJ-liposomes. The priorities of our method are the use of HVJ to introduce DNA directly into the cytoplasm by virus-induced fusion and the use of nuclear proteins to transport DNA rapidly into the nucleus for its high expression. By HVJ-liposomes, SV40 large T Ag DNA, human insulin DNA and HB virus DNA were successfully introduced and expressed in rodent livers. The expression of the genes in the liver lasted 7-10 days by one shot. When human renin gene was introduced into rat livers, active human renin was secreted into rat sera and the level of rat angiotensin II in the sera greatly increased. By the injection of human renin gene into the rat liver, blood pressure of the rat was elevated from 110 mmHg to 140 mmHg. This high blood pressure was significantly correlated with the concentration of human active renin and of rat angiotensin II in the serum. The hypertension was suppressed by the injection of a new inhibitor specific for human renin. Thus, the direct gene transfer by HVJ-liposomes will be able to provide a new way for the construction of animal models and may be useful for the postnatal gene therapy. So far, we succeeded in the expression of various genes in various organs. We are also going to present a recent progress of our gene transfer in vivo.

SZ 311 TARGETED GENE DELIVERY BY RETROVIRUSES WITH PEPTIDE HORMONE SEQUENCES INCORPORATED INTO THE ENVELOPE, Noriyuki Kasahara, Andrée Dozy, Y. W. Kan, Department of Laboratory Medicine and Howard Hughes Medical Institute, University of California San Francisco, CA 94143

We have tested a model system for tissue-specific gene delivery by incorporating a ligand into the viral envelope, which then targets the virus to cells expressing the receptor for that ligand. The peptide hormone erythropoietin (EPO) was used to replace sequences that had been deleted from the amino-terminal end and the central region of the Moloney Murine Leukemia Virus envelope. These recombinant EPO-envelope constructs were stably transfected into the amphotropic viral packaging cell line PA317. Western blot and flow cytometric analyses, using anti-EPO antibodies, confirmed that recombinant EPO-envelope proteins were being expressed and were detectable on the surfaces of the packaging cells. Stable transfectants producing high levels of the recombinant EPO-envelope proteins were used to package replication-defective retroviral vectors encoding the neomycin resistance (neo^R) gene, and the resultant viruses were tested for infectivity on target cells with or without the EPO-receptor. All cells exposed to virus were subjected to selection with neomycin, and the surviving colonies were examined and counted after Giemsa staining. The efficiency of infection was increased between 3- and 10-fold when viruses expressing the recombinant EPO-envelope protein were targeted to cells bearing the EPO-receptor; as compared to cells without the receptor. These results indicate that retroviral vectors can be engineered to target genes into specific cell types *in vivo*.

SZ 312 DIRECT DELIVERY OF RETROVIRAL VECTORS TO CANINE HEPATOCYTES IN VIVO. Mark A. Kay[2,3], Steven Rothenberg[4], William Pokorny[4] and Savio L.C. Woo[1,2,3]. (1) Howard Hughes Medical Institute, Departments of (2) Cell Biology, (3) Molecular Genetics and (4) Surgery. Baylor College of Medicine, Houston, TX 77030.

We have previously reported the application of an ex vivo method of hepatic gene transfer in the canine animal model which involves the isolation of hepatocytes after partial hepatectomy, followed by in vitro gene transduction and autologous transplantation of genetically reconstituted cells into the animals. More recently, we devised a simple method for direct transduction of mouse hepatocytes in vivo that involves a partial hepatectomy followed by the portal vein infusion of recombinant retroviral vectors. We have investigated a similar method in the canine model. This involves an 80% partial hepatectomy followed by the portal vein infusion of recombinant retroviral vectors. When a b-gal containing vector was used in two animals, up to 1% of the hepatocytes were transduced as determined by x-gal staining. We have then used the LN/Alb-hAAT vector for direct transduction of hepatocytes in 10 dogs. This vector contains the human alpha-1-antitrypsin (hAAT) cDNA under the transcriptional control of the albumin promoter-enhancer. Three categories of expression patterns have been observed. The first consists of serum hAAT concentrations of 0.5 to 2 ug/ml and lasted 20 to 40 . The second group of animals expressed similar levels of hAAT but the concentrations of hAAT have declined to the range of 20 to 100 ng/ml over 6 months. In two animals, constitutive expression of 1 to 15 ug/ml of hAAT have been achieved over 8 months. Control animals infused with virus without undergoing prior hepatectomy do not express hAAT. Thus, direct transduction of hepatocytes in a large animal model is possible but there appears to be variability in expression patterns between animals. The mechanism that causes variable expression between animals must be better understood for the method to be applicable in hepatic gene therapy in man.

SZ 313 DEVELOPMENT OF A GENE THERAPY FOR MARFAN SYNDROME: RIBOZYME MEDIATED SPECIFIC DOWN-REGULATION OF THE FIBRILLIN 15 GENE PRODUCT, Michael W. Kilpatrick (1,3), Gordon Carmichael (2) and Petros Tsipouras (1). Departments of Pediatrics (1) and Microbiology (2) University of Connecticut Health Center, Farmington, CT 06032 and Department of Clinical Genetics, University of Birmingham, UK (3).
The identification of mutations in the fibrillin gene on chromosome 15 (Fib15) in individuals with Marfan syndrome makes it possible to begin to unravel the detailed molecular pathology of this debilitating disease, and to utilise this knowledge in the development of a rational therapy. As the first step in the development of an antisense based therapy we have designed and constructed an antisense RNA molecule for the 5'-end of the Fib15 mRNA. The molecule has been designed as a potential Fib15 specific ribozyme and comprises 25 bases of Fib15 sequence containing a centrally located GUC ribozyme cleavage site, along with the sequence necessary for the formation of a hammerhead structure. A 75 bp oligonucleotide containing the ribozyme sequence was synthesised and cloned into the pBSIISK+ vector facilitating production of large amounts of ribozyme **in vitro** by transcription off the T3 promoter. Preliminary data suggests that the ribozyme is capable of specifically cleaving the Fib15 mRNA **in vitro**. The oligonucleotide was also cloned into the vector pRSV-RZ(cis), downstream of the Rous Sarcoma Virus promoter and upstream of a cis-acting ribozyme sequence, to allow **in vivo** production of the ribozyme. The construct is currently being transfected into normal fibroblast and smooth muscle cell lines. The **in vivo** production of ribozyme will be monitored, and its effect on both the Fib15 mRNA and the synthesis of fibrillin will be determined.

SZ 314 PRE-CLINICAL ASSESSMENT OF IN VIVO GENE THERAPY FOR METHYLMALONYL CoA MUTASE DEFICIENCY USING ASIALO-GLYCOPROTEIN/POLYLYSINE/DNA COMPLEXES. **Fred Ledley, Jozsef Stankovics, Elizabeth Andrews, Catherine Wu, George Wu. Departments of Cell Biology and Pediatrics, Baylor College of Medicine; Department of Medicine, University of Connecticut.**
Methylmalonic acidemia (MMA) is an often fatal inborn error of organic acid metabolism resulting from deficiency of methylmalonyl CoA mutase (MCM). The clinical course of MMA with dietary therapy is characterized by life threatening episodes of metabolic dyshomeostasis with organic acidosis and many secondary metabolic abnormalities. The studies presented here were undertaken in anticipation of using short term expression of MCM in the liver after asialoglycoprotein/polylysine (ASO/PL) mediated gene delivery to treat these episodes. This will require expression of MCM at therapeutic levels in the liver and repetitive administration of the ASO/PL/DNA complex. A vector containing the human MCM cDNA under transcriptional control of the CMV immediate early promotor was complexed with ASO/PL and injected into the tail vein of mice. The clearance of the complex from the blood and tissues was assayed by semi-quantitative PCR. Plasmid was cleared from the blood in 10 minutes with >95% taken up by the liver and less in lung and spleen. Plasmid was largely cleared from the liver within 24 hours. Small amounts of plasmid (<1copy/100 cells) was detectable as long as 20 days after injection and retained its bacterial pattern of methylation. mRNA was evident in the liver 6-24 hours after injection and activity increased to levels 30-50% above (normal) baseline for 12-48 hours after injection. Animals injected with this complex four times over a period of six months all had antibodies against both polylysine and asialo-orsomucoid which precipitated [^{125}I]-labeled complex at dilutions of >1:1000. There was no evidence for anti-DNA antibodies by immunoprecipitation of [^{32}P]-DNA, competition for [^{125}I]-ASO/PL/DNA precipitation, or clinical anti-nuclear antigen assay. These studies demonstrate the feasibility of achieving clinically significant levels of MCM expression which could be useful for treating acute episodes of acidosis in MMA. The generation of antibodies may limit the usefulness of this specific complex in clinical trials.

SZ 315 BIOCOMPATIBLE, RETRIEVABLE CAPSULES FOR CELL TRANSPLANTATION, M. Lysaght, S. Rudnick, CytoTherapeutics, Inc., 2 Richmond Square, Providence, RI 02906

We describe here semipermeable, biocompatible, implant vehicles which may facilitate site specific delivery and subsequent retrieval, if necessary or desirable, of recombinantly modified cells for gene therapy. The implants are small cylindrical chambers, about 1 mm in diameter and several cm long, with an internal volume of 0.5 to 5 microliters and are capable of holding from 10^4 to 10^6 cells. The wall of the cylinder is fabricated from a selective barrier whose molecular weight cutoff can be varied between 50,000 (macromolecule rejecting) and 500,000 (cell rejecting). Permeants diffuse through the wall in both directions thus providing the enclosed cells essential nutrients and oxygen and allowing therapeutically active cell secretions to reach target tissue in the host. Diffusion rate through the membrane (below the MW cutoff) is about 10-30% of that in extracellular fluid. A wide variety of hydrogel matrices can be employed inside the chamber to support graft cell growth without significantly inhibiting transport. Such implants have been extensively developed and refined as vehicles for the transplantation of primary and immortalized xenogeneic cells for the treatment of diabetes, Parkinson's disease, and chronic pain. In small and large animal models of these conditions, symptoms remain reversed and cells remain viable for periods of up to one year. Biocompatibility is excellent (often an absence of any fibrotic overcoating) in sites as diverse as subcutaneous, intraperitoneal, intrathecal, and in the brain parenchyma. Several well controlled experiments have demonstrated that appropriately designed implants can be retrieved by simple surgical techniques, that the encapsulated cells are still functioning at the time of explant, and that host tissue heals well in the period following retrieval. We believe that this technology offers a practical and advantaged approach to the therapeutic delivery of genetically modified cells.

SZ 316 **HEPATIC GENE TRANSFER FOLLOWING DIRECT IN VIVO INJECTION,** Malone R.W.[1,3] Hickman M.A.[2] Lehmann K.[3] Walzem R.[4] Bassiri M.[3] and Powell J.S.[3] Departments of Pathology[1], Biochemistry and Biophysics[2], Med:Hematology and Oncology[3], and Vet Med:Physiological Sciences[4], University of California, Davis 95616

Hepatocytes are attractive targets for gene augmentation strategies due to their critical roles in the maintenance of physiologic homeostasis and extracellular protein synthesis. In testing various gene transfer methods we have directly injected the livers of Sprague-Dawley rats and outbred cats with plasmid DNA coding for the P.pyralis Luciferase and E. coli Lac Z reporter genes under the control of CMV immediate early transcriptional elements. In our rat model, injection of 500 micrograms (mg) of pCMVL DNA resulted in expression of up to 21 picograms (pg) of luciferase protein at 48 hours, with a gradual decrease and loss of expression by10 days. Histological b-galactosidase analysis demonstrated expression within hepatocytes surrounding the injection site, as well as a prominent associated acute inflammatory infiltrate. Partial immunosupression with Dexamethasone followed by 500 mg pCMVL injection resulted in expression of up to 22690 pg of luciferase protein, and persistance of up to 4000 pg for at least 18 days following injection. Enhanced expression and significantly reduced acute inflammation were confirmed by in situ b-galactosidase staining. Similar results have been obtained following feline intrahepatic injection. Despite blanching of 1 to 2 centimeters of hepatic parenchyma during the injection, reporter gene expression was localized to within 3 millimeters of the needle tract, suggesting mechanical rather than a receptor mediated transfection mechanism. This observation was tested using cultured cells by incubating Hep G2 hepatocellular carcinoma cells in high concentrations of DNA with and without aspiration through a 16g needle. Only the cells traumatized by aspiration were successfully transfected. In sum, these results suggest that: 1) contrary to prior reports, hepatic gene transfer with hepatocytic expression following direct injection is feasible, 2) steroid suppression of the subsequent inflammation and necrosis enhances expression by at least 1000 fold, 3) transfection may occur by mechanical permeabilization.

SZ 317 **YEEGal$_3$ - A SYNTHETIC GLYCOPEPTIDE LIGAND FOR ASIALOGLYCOPROTEIN RECEPTORS (ASGPr) IN GENE THERAPY.** June Rae Merwin, Mark A. Findeis, and Steven Noell. TargeTech, Inc., Meriden, CT 06450.

In vivo gene therapy shows promise as a treatment for both genetic and acquired disorders. The hepatic ASGPr binds asialoorosomucoid (ASOR) and allows the targeted delivery of DNA to the liver. We synthesized a glycopeptide, Tyrosyl(Glutamyl) Glutamate[1-(6'-aminohexyl)-N-acetylgalactosamine)$_3$ [YEE(GalNAcAH)$_3$], referred to as YEEGal$_3$. Iodinated (^{125}I-YEEGal$_3$) and fluoresceinated (FI-YEEGal$_3$) glycopeptides were used in binding experiments to determine whether it would be feasible to replace ASOR with the glycopeptide. FI-YEEGal$_3$ was shown to bind *in vitro* to hepatocyte monolayers and was competed with ASOR. Scatchard analyses revealed high affinity binding and receptor numbers similar to the amount reported for ASGPr. ^{125}I-YEEGal$_3$ binding was competed with either YEEGal$_3$ or ASOR. The reverse was also true; ^{125}I-ASOR was competed with YEEGal$_3$. However, internalization of the receptor-glycopeptide complex required ^{125}I-YEEGal$_3$ to be coupled to human serum albumin. *In vivo* animal studies mimicked previous work done with ^{125}I-ASOR, namely: >90% recovered ligand was targeted to the liver within five minutes; CPM in the liver at 60 minutes were enhanced following partial hepatectomy; kidney, lungs, heart and spleen displayed low radioactivity. These data suggest that the YEEGal$_3$ synthetic glycopeptide can be used as a substitute for the ASOR ligand and would be an alternative ligand for targeted delivery in gene therapy.

SZ 318 **ENHANCED EFFECTIVENESS OF ANTISENSE OLIGONUCLEOTIDES IN VASCULAR SMOOTH MUSCLE CELLS (VSMC) BY HVJ MEDIATED GENE TRANSFER,** Ryuichi Morishita, Gary H. Gibbons, Kristin E. Ellison, Wendy Lee, Yasufumi Kaneda*, Toshio Ogihara*, Victor J. Dzau, Falk Cardiovascular Research Center, Stanford University, Stanford, California.

*Osaka University Medical School, Osaka, Japan.

The efficacy of antisense (AS) oligonucleotides for in vitro and in vivo studies is limited by low efficiency of uptake and instability due to degradation by nucleases. To enhance the effectiveness of AS oligonucleotides, we developed a novel gene transfer method that utilizes an inactivated Sendai virus (HVJ: Hemagglutinating Virus of Japan) and liposomes to deliver antisense (AS) oligonucleotides. We have previously shown that angiotensin (Ang) II induces VSMC proliferation in association with increased expression of autocrine basic fibroblast growth factor (FGF). This study compared the effects of AS oligonucleotides against basic FGF (AS-FGF) on Ang II induced VSMC growth using the HVJ-liposome method versus cationic liposomes method versus passive uptake of phosphothinate modified oligonucleotides alone. 20-28 hours after transfection, AS-FGF introduced by either method decreased significantly basal DNA synthesis as compared to the sense and control groups. However, 60-68 hours after transfection, only the AS-FGF transduced by the HVJ method showed a significant inhibition of DNA synthesis under basal and Ang II (10^{-6}M) stimulated conditions. The dose-dependent effect of AS-FGF utilizing all three transfection methods was assessed. The IC_{25} of the inhibitory effect of the HVJ method on thymidine incorporation was a 100-fold lower oligonucleotide concentration than the IC_{25} of the other transfer methods. The enhanced effect of the HVJ mediated transfection of AS-FGF was confirmed further by the effect on mitochondrial dehydrogenase (MD) activity, an index of cell number. At 3 days after the transfection, MD activity of VSMC stimulated with Ang II was significantly lower with the HVJ mediated AS-FGF transfection as compared to sense and control groups. In contrast, no significant inhibition of cell proliferation (as assessed by MDA) was observed with AS-FGF, sense and control treated cells using passive oligonucleotide transfer. These results demonstrate that the HVJ mediated transfer enhanced the effectiveness of AS-FGF. The HVJ method may be suitable for the use of antisense oligonucleotides to inhibit VSMC growth in vivo. Our results indicate that the HVJ-liposome transfer method is an effective and improved delivery system for antisense oligonucleotides as compared to lipofectin, or passive oligonucleotide transfer.

SZ 319 **GENE TRANSFER INTO INTESTINAL EPITHELIAL CELL LINES (IEC-6 AND RIE-1) BY AN ECOTROPIC RETROVIRAL VECTOR.** R. Adam Noel, Mônica Puppi, J. Perez-Rossello and Susan J. Henning, Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030.

Our laboratory is studying the possibility that the intestinal epithelium can be used as a site for somatic gene therapy. We believe retroviruses are the vectors of choice for these studies, as they are the most efficient means of gene transfer and the least disruptive (because they insert only a single copy of their proviral DNA into the host chromosome). The infection of intestinal stem cells is necessary for the use of the intestine as a target organ for somatic gene therapy. These stem cells are known to reside in the crypts of the intestine. There currently are two intestinal crypt cell lines, IEC-6 and RIE-1, that can be used as in vitro models to study the dynamics of infection in the intestine. Using a replication-defective retrovirus (Zen$^+$ß-gal), we delivered the reporter gene, bacterial ß-galactosidase, into IEC-6, RIE-1 and NIH3T3 cells. Successful transduction was assessed by measuring the expression of the reporter gene by X-gal histochemistry. Prior to infection we demonstrated that the mRNA for the ecotropic retroviral receptor was present in both intestinal crypt cell lines. Optimal density of all three cell lines for infection was approximately 70 cells/mm^2. Infection rate declined abruptly below a cell density of 10 cells/mm^2 or greater than 200 cells/mm^2 for retrovirus at low to moderate titers. At the appropriate density the intestinal cell lines were transduced as efficiently as NIH3T3 cells (the normal titering cell line). At a single density at plating, a linear relationship to increasing titers of retrovirus was found in all cell lines. This relationship held up to a 1:5 dilution of viral supernatant. Using undiluted viral supernatant there was a decrease in the expression of the reporter gene, which was more profound in the intestinal cell lines. The negative effect was minimized by decreasing the exposure time of the supernatant to the packaging cell line. Infection rate also was improved by using multiple application of retrovirus during the cell cycle. Because of the effect of cell density and multiple applications of retrovirus on gene transfer, we are studying the use of various growth factors to enhance retroviral transduction in these cell lines. CONCLUSIONS: Intestinal epithelial cells are highly infectable by retroviral vectors, provided that the proliferation rate, cell density and viral supernatant conditions are optimal. These studies should be useful in developing strategies for in vivo intestinal gene therapy applications.

SZ 320 ADENO-ASSOCIATED VIRUS: HIGH EFFICIENCY PRODUCTION OF RECOMBINANT TRANSDUCING VECTORS, Kevin Page, Mark Harvey, Thomas B. Okarma, and Jane Lebkowski, Applied Immune Sciences, Inc., Santa Clara, CA 95054

Adeno Associated Virus (AAV) is a single stranded DNA dependovirus which can be engineered for efficient gene transduction. In the absence of adenovirus or herpes virus helpers, AAV enters mammalian cells, uncoats in the nucleus and integrates its genome into that of the host. We have constructed a number of recombinant AAV vectors which carry one or two inserted genes and have shown that they efficiently infect both established cell lines, primary skin fibroblasts and T cells. Our current strategies in development of this vector system have focused on new, simpler strategies to produce high titer rAAV stocks without the need for repeated transfections of the producer cell line. We have constructed chimeric AAV/EBV plasmids which contain a recombinant AAV genome, the hygromycin resistance gene and the EBV oriP fragment and EBNA gene. These plasmids, when transfected into human 293 cells, maintain episomal replication, and stable cell lines carrying the plasmid can be cloned. Upon transfection of these cell clones with plasmids carrying the wt rep and cap genes of AAV and infection with adenovirus, replication of the recombinant AAV genome ensues producing recombinant viral stocks that can be used in transduction protocols. Such generated stocks have produced infection efficiencies of up to 10%. Our new methods to efficiently introduce the rep and cap genes into the AAV/EBV clones to make even higher titer stocks will be presented.

SZ 321 ADENOVIRUS-MEDIATED TRANSFER OF A HUMAN DYSTROPHIN GENE TO SKELETAL MUSCLE OF *mdx* MOUSE, Thierry Ragot[1], Leslie Stratford-Perricaudet[1], Nathalie Vincent[2], Philippe Chafey[2], Emmanuelle Vigne[1], Hélène Gilgenkrantz[2], Dominique Couton[3], Pascale Briand[3], Jean-Claude Kaplan[2], Axel Kahn[2] & Michel Perricaudet[1], 1) URA 1301 CNRS, Institut Gustave Roussy, PR2, 94805 Villejuif, FRANCE, 2) U 129 INSERM and 3) CJF 9003 INSERM, Institut Cochin de Génétique Moléculaire (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 kbp transcript. Approaches proposed to correct the dystrophin deficiency in muscle, 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. A replication-deficient adenovirus has been shown to be an efficient vector for *in vivo* expression of various foreign genes. Furthermore, it has recently been demonstrated that a recombinant adenovirus expressing the lac-Z reporter gene is able to stably infect a large variety of mouse tissues, especially muscles and heart. Then, we have tested the ability of a helper-free recombinant adenovirus, containing a 6.3 kbp Becker-like dystrophin cDNA driven by the Rous sarcoma virus promoter to direct the expression of a "minidystrophin", in infected 293 cells and C2 myoblasts, and in the *mdx* mouse. *In vivo*, we have demonstrated that it is possible to transfer a functional minidystrophin gene to a significant proportion of muscle fibres (a sarcolemmal immunostaining is obtained in up to 50 % of fibres) by intramuscular injection of the recombinant adenovirus. Further investigations will determine whether a similar result can be obtained using systemic routes of administration and whether this results in functional improvement of dystrophin-deficient muscles. We are currently constructing a helper-dependent adenovirus expressing the whole dystrophin cDNA.

SZ 322 DEVELOPMENT OF A RAT MODEL FOR ADENOVIRUS-MEDIATED GENE TRANSFER INTO THE PANCREAS, Steven E. Raper*, Yi-Ping Yang, James M. Wilson, Departments of Surgery* and Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109

Gene transfer into the pancreas would be useful for the treatment of a variety of disorders, including cystic fibrosis, diabetes, cancer, and immunomodulation during allogeneic transplantation. The purpose of these studies was to optimize delivery of an adenoviral vector, AdCMVLacZ, by a variety of surgical approaches. 175-225 gm male F344 rats were treated in one of four groups: Group 1 underwent laparotomy and injection of AdCMVLacZ into the common duct near insertion into the *duodenum* (N=2); Group 2 underwent temporary proximal duct ligation and viral injection as above (N=2); Group 3 underwent direct injection of virus into the pancreatic tissue (N=2); Group 4 underwent temporary proximal ligation of the duct, placement of a 22 Ga catheter into the duct near the duodenum, secured by a 3-0 suture. In all cases, 500 µl of vector with a titer of ~ 10^{12} pfu was injected. No mortality was seen in Groups 1-3, however, 3 of 4 died in Group 4. At day 3, pancreas and liver were harvested and fresh-frozen sections were analyzed for presence of gene transfer by Xgal staining:

Group	% Positive tissue	Islets	Ducts	Acini
1	<1%	-	+	+
2	<1%	-	+	+
3	~20%	+	-	+
4	~50%	+	+	+

The lack of selectivity of gene transfer for pancreas in groups 1&2 was demonstrated by the presence of hepatic gene transfer by Xgal staining and Southern blot analysis. Further analysis on H&E stained sections from pancreatic tissue in the surviving rat of group 4 showed severe hemorrhagic pancreatitis and necrosis. In summary, 1) groups 3&4 provided the best gene transfer in the rat, however a high mortality from severe pancreatitis was associated with group 4. 2) gene transfer was relatively non-selective in that it was not possible to infect only the duct, or acini, or islets. We conclude that adenovirus-mediated gene transfer into the pancreas is possible. Further studies are need to demonstrate persistence of the transduced gene, to attempt to selectively target individual cells types in the pancreas and to minimize the development of pancreatitis.

SZ 323 GENETIC MODIFICATION OF SYNOVIOCYTES IN VIVO USING RECOMBINANT ADENOVIRAL VECTORS, Blake J. Roessler and Beverly L. Davidson, Department of Internal Medicine, Division of Rheumatology, University of Michigan Medical Center, Ann Arbor, MI 48109-0680

Inflammatory arthropathies represent a large group of heterogeneous diseases that result in substantial morbidity. Treatment of these diseases is currently inadequate and associated with significant side effects. As an initial step in developing models for intra-articular gene therapy we examined the utility of recombinant adenoviral vectors for the genetic modification of synoviocytes *in vivo*. In these studies we used the gene for *E. coli* β-galactosidase as a reporter transgene. High titer recombinant adenoviral vectors were injected into hind knees of New Zealand White rabbits. Periodically after intra-articular injection, animals were sacrificed and the articular and periarticular tissues were examined for transgene expression using direct visualization, light microscopy, immunohistochemistry and electron microscopy. Expression of *E. coli* β-galactosidase activity was observed in synovial tissues from 3 days until at least 4 weeks post infection. Transgene expression was observed in synovial tissues overlying tendons, cancellous bone and articular cartilage. Microscopic examination of cells expressing the transgene confirmed their identity as type A and type B synoviocytes. No evidence of inflammatory arthritis or morbidity was observed in the treated animals. These data suggest that recombinant adenoviral vectors may be useful for the intra-articular delivery of therapeutically relevant proteins in the treatment of inflammatory arthropathies.

SZ 324 MUCUS ERADICATION AS A MEANS FOR OPTIMIZING INTESTINAL GENE TRANSFER, James W. Sandberg, Chantal Lau, and Susan J. Henning, Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030

The intestine is an anttractive site for somatic gene therapy because of its ease of access and large tissue mass. Previous studies in our laboratory have shown successful retroviral-mediated gene transfer into the intestinal epithelium, but at low efficiency. A potential factor causing inefficient retroviral infection of intestinal stem cells is the mucus gel layer. As a physical barrier, mucus prevents infectious agents from reaching the intestinal epithelium. As a biological barrier, its glycoproteins offer spurious attachment sites for bacteria and viruses. The goal of the current study was to develop procedures which would effect *in vivo* mucus removal without damaging the underlying epithelium. Initial experiments involved evaluation of the mucolytic agents dithiothreitol (DTT) and N-acetyl-cysteine (NAC) versus a control solution of phosphate-buffered saline (PBS). Catheters were inserted in each end of a 3-cm terminal ileal segment in a rat. Two ml of agent was instilled into the clamped segment for 2 min, removed, and repeated. The segment was taken from the animal along with an upstream control segment. Tissue was fixed in 2.5% paraformaldehyde/2% glutaldehyde and embedded in paraffin. Sections were stained for mucus with PAS. Mucus removal was assessed in two areas, between the villi and in the crypt lumen. Following blind scoring of the slides, it was found that both NAC and DTT removed significant mucus between the villi ($p < 0.05$) but failed to reach the crypt lumen ($p > 0.05$). To enhance mucus release from the crypt lumen, pilocarpine was selected due to its cholinergic properties and preferential binding to muscarinic receptors on crypt goblet cells. Follow-up studies using pilocarpine given intraperitoneally 30 min prior to the mucolytic or PBS wash revealed complete eradication of mucus down into the crypt lumen ($p < 0.05$). Further studies are under way to investigate improved retroviral transduction into the intestinal epithelium using these conditions of mucus removal. This work was supported by NIH grants HD-14094 and DK-44646, and a GI Clinical Fellowship Award from the Cystic Fibrosis Foundation.

SZ 325 DELIVERY OF DNA-LIPOSOME COMPLEXES BY SMALL-PARTICLE AEROSOL. Lindsay A. Schwarz, J. Clifford Waldrep and J. Vernon Knight. The Center for Biotechnology, Baylor College of Medicine, The Woodlands, TX 77381. The cloning of several genes responsible for maintaining normal pulmonary function such as cystic fibrosis transmembrane conductance regulator (CFTR) and α1-antitrypsin (α1-AT) has made gene therapy for individuals with these genetic defects a possibility. In addition, severe lung inflammatory diseases such as asthma, interstitial pulmonary fibrosis, sarcoid and adult respiratory distress syndrome may result in permanent lung damage or even death. Aerosol delivery of genes such as CFTR, α1-AT or anti-inflammatory oligonucleotides is appealing since the lungs can be treated topically with doses not obtainable by systemic administration. An important aspect of aerosol therapy is to generate respirable particles of a size range of 0.5-5 microns. Using a novel, patented technology to deliver drug-liposome complexes by aerosol, we have begun to test efficacy of liposome-complexed- cDNA. Plasmid cDNA (5 ug) was encapsulated in liposomes of the neutral synthetic phospholipid 1,2-dilauryol-sn-glycero-3-phosphocholine (DLPC, 1 mg/ml) and generated into an aerosol with a Puritan-Bennett 1600 nebulizer. Aerosol particles then passed through an Andersen Sampler (AS), a multistage partical size analyzer. Each stage of the AS was fitted with a nylon filter to trap the liposome-DNA complexes. Following entrapment, the filters were removed and hybridized with homologous ^{32}-P-labeled plasmid DNA to detect the presence of DNA. cDNA was detected on each stage of the AS in the range of 0.4-10 microns. These results demonstrate that DNA-liposome complexes can be delivered as respirable particles by aerosol. Efficiency of DNA delivery by neutral and cationic liposomes, quality of aerosolized DNA-liposome particles and delivery of anti-inflammatory oligonucleotides are under investigation.

Cancer, HIV, and Animal Models of Disease

SZ 400 THERAPEUTIC STRATEGIES FOR SURFACTANT PROTEIN SP-B DEFICIENCY, Robert J. Bohinski, Melanie D'Amore-Bruno, and Jeffrey A. Whitsett, Division of Pulmonary Biology, Children's Hospital Medical Center, Cincinnati, OH 45229

Surfactant protein B (SP-B) is a 79 amino acid hydrophobic protein critical to the surface tension lowering ability of pulmonary surfactant. A deficiency of SP-B gene expression in the lung causes severe pulmonary disease characterized by atelectasis, cyanosis, and respiratory failure. We have cloned and characterized the genes and cDNAs encoding both human and murine SP-B. Regions of the human SP-B gene that mediate lung epithelial cell-specific expression *in vitro* were delineated biochemically and functionally. Distinct regions flanking the SP-B promoter conferred lung epithelial cell-specific expression of chimeric genes *in vitro* and DNA footprint analysis demonstrated cell type-specific nuclear protein-DNA interactions within this region. The SP-B promoter may be useful in targeting genes to the bronchiolar and alveolar epithelium for gene therapy. Analysis of the transcriptional regulatory domains of the SP-B promoter may also provide insight for the control of SP-B gene expression for therapy of SP-B deficient pulmonary disease such as RDS, ARDS, and genetic abnormalities of the SP-B gene. The feasibility of genetic transfer of SP-B to the airway epithelium was demonstrated in transgenic animals. Transgenic mice were generated in which the full-length SP-B cDNA was expressed under the control of the surfactant protein C (SP-C) promoter-enhancer element comprising 3.7 kb of 5' flanking DNA of the human SP-C gene. Lungs from transgenic mice bearing the SP-C/SP-B chimeric gene expressed the human SP-B mRNA at readily detectable levels and immunohistochemistry demonstrated abundant human SP-B protein in respiratory epithelial cells of the transgenic mice. There was no morphological evidence of toxicity in the lungs of transgenic mice, supporting the concept that full-length SP-B proprotein can be transferred to the distal respiratory epithelial cells of the lung for genetic therapy of SP-B deficient states in the future.

SZ 401 CONSTRUCTION AND CHARACTERIZATION OF REPLICATION-DEFECTIVE HIV-1 PACKAGING CELL LINES. R. Carroll[1], J.T. Lin[1], J. Kim[2], J. Mosca[1], M. Vahey[2], D. Burke[3], and D. St. Louis[1]. [1]The Henry M. Jackson Foundation, 1500 E. Gude Dr., Rockville, MD, 20850, [2]Division of Retroviral Research, [3]Division of Retrovirology, Walter Reed Army Institute of Research and the Military Consortium for Applied Retroviral Research, 13 Taft Ct., Rockville, MD, 20850.

We have generated HIV-1 packaging cell lines as a component of an HIV-1-based retroviral vector system. Packaging cell lines were constructed by transfecting human and monkey cells with an HXB2-derived expression vector capable of synthesizing HIV-1 structural and regulatory proteins as well as the hygromycin phosphotransferase gene product. Both HIV-1 LTRs and the viral packaging signal were deleted from this vector in order to prevent packaging of the HIV-1 genome. Hygromycin-resistant cell clones expressing the highest level of p24 antigen were characterized further. Radioimmunoprecipitation analysis of these packaging lines demonstrated that HIV-1 structural protein precursors were synthesized at levels almost equivalent to those synthesized by wild type HIV-1. Additionally, electron microscopy revealed the presence of virus-like particles with both mature and immature morphologies. These particles were not infectious when assayed on SupT1 cells. The cultures were monitored for infectious particles over a 28 day period by p24 antigen capture and reverse transcriptase assays. The ability of the packaging cell lines to generate transducing particles was measured by transfecting them with a retroviral vector containing the neomycin phosphotransferase gene and the HIV-1 packaging signal, flanked by HIV-1 LTRs. Supernatant from transfected packaging cell lines was used to infect SupT1 cells, and transducing particle titre was determined by limiting dilution of the culture supernatant.

SZ 402 OVEREXPRESSION OF DYSTROPHIN IN TRANSGENIC *MDX* MICE ELIMINATES DYSTROPHIC SYMPTOMS WITHOUT TOXICITY, Gregory A. Cox[1], Neil M. Cole[2], Stephanie A. Phelps[1], John A. Faulkner[2], and Jeffrey S. Chamberlain[1,3], [1]Department of Human Genetics, [2]Department of Physiology, [3]Human Genome Center, University of Michigan Medical School, Ann Arbor, MI 48109-0618.
Mutations in the dystrophin gene lead to Duchenne and Becker muscular dystrophies (DMD/BMD). We are exploring the feasibility of gene therapy for DMD by introducing dystrophin expression vectors into myogenic cultures and *mdx* mice, an animal model for muscular dystrophy. A variety of dystrophin vectors have been created that are expressed at different levels in mice. Most of these vectors include a full-length, 14kb, murine dystrophin cDNA under the control of muscle creatine kinase (CK) gene regulatory regions. Transgenic *mdx* mice carrying these contructs express dytrophin in skeletal and cardiac muscle at levels up to 10 times greater than in wild-type mice. A variety of assays indicate that these mice display a complete correction of dystrophic symptoms in muscle. These include: elimination of elevated serum CK levels, restoration of normal muscle morphology, and proper sarcolemmal localization of the expressed dystrophin. A functional correction of the dystrophy was demonstrated by measurement of mechanical properties of the diaphragm. The progressive degeneration observed in *mdx* mouse diaphragm muscles is particularly severe, and closely resembles that observed in DMD patients. Diaphragm specific force and normalized power in transgenic *mdx* animals were indistinguishable from those of wild-type mice, and significantly greater than in *mdx* mice. These results indicate that delivery of exogenous dystrophin to muscle can completely eliminate dystrophic symptoms. In addition, mice overexpressing dystrophin display no evidence of pathological abnormalities, suggesting that the excess dystrophin is not toxic. Delivery of dystrophin clones to muscle of DMD/BMD patients may therefore be an effective method of treatment for muscular dystrophy.

SZ 403 REGULATED GENE TRANSFER BY CO-DELIVERY OF A CIS- ACTING DNA ELEMENT AND A TRANS- ACTING PROTEIN FACTOR TO MAMMALIAN CELLS WITH CATIONIC LIPOSOMES, Hassan Farhood[1], Xiang Gao[1], James Barsoum[2], and Leaf Huang[1], [1]Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, and [2]BIOGEN, Fourteen Cambridge Center, Cambridge, MA 02142.
Gene expression is highly regulated in mammalian cells through transcriptional controls. HIV-1-Tat protein controls the specific activation of HIV transcription by stabalizing the mRNA transcripts initiated from the HIV-LTR promoter sequence. We have used cationic liposomes to co-deliver the specific trans-acting protein factor Tat and a cis-acting DNA element coding for a reporter gene under the control of HIV-LTR sequence to human epidermoid carcinoma (A431) cells in culture. Expression of the reporter gene was used as an assay for the highly efficient co-delivery of protein and DNA. The delivery system had negligible toxicity on the cells since low concentrations of liposomes, DNA and protein were used for a high level of gene expression. Activation of the reporter gene expression was controled by the dose of Tat Protein, DNA or liposomes incubated with the cells. This co-delivery system is presented as a model for regulated gene therapy. Ultimatly, gene therapy is most effective if gene expression is controlled transcriptionally. **Supported by NIH grants AI 29893, HL 50256 and CA 59327.**

SZ 404 INJECTION OF EXPRESSION VECTORS CONTAINING ANTIGEN GENES INDUCE CELLULAR, HUMORAL AND PROTECTIVE IMMUNITY, PL Felgner[1], Gary H Rhodes[1], VJ Dwarki[1], J Felgner[1], LH Hawe[2], JJ Donnelly[2], JB Ulmer[2], MA Liu[2], AM Abai[1] SH Gromkowski[1] and SE Parker[1],; [1]Vical Inc., San Diego CA 92111 and [2]Merck Research Laboratory, West Point, PA 19486
Intramuscular injection of plasmid DNA results in expression of the genes encoded by the plasmid. We have investigated the immunological consequences of injection of an expression vector containing the genes for two well characterized antigens: the gp120 gene from HIV-1 and the nucleoprotein (NP) gene from influenza A virus. A single injection of DNA encoding for either gene induces IgG antibodies which appear 2 to 3 weeks after injection and persist for more than 12 months. The antibody response is dose dependent with 10 μg of DNA required for a consistent antibody response. Maximum antibody titers for both antigens were in the range of 1/10,000 to 1/30,000. A single injection of DNA is also sufficient to produce cytotoxic T lymphocytes (CTL) which recognize target cells coated with synthetic peptides representing the major T cell epitopes. Priming of CTL may occur independently of antibody since some animals injected with low amounts of DNA develop CTL activity without measurable IgG antibodies. Once induced, the CTL activity persists for more that 6 months. The induced immunity appears to be functional in vivo as animals injected with the gene for influenza NP from strain A/PR/8/34 (H1N1) are protected from a subsequent lethal viral challenge of a homologous (H1N1) and heterologous (H3N2) strains of influenza virus. The ability to directly prime CTL may allow the development of subunit vaccines which are more efficacious than killed virus or recombinant protein derived vaccines. Similarly, the induction of CTL could be useful for a therapeutic vaccine against chronic viral infections.

SZ 405 **EXPRESSION OF HUMAN LIPOPROTEIN LIPASE IN TRANSGENIC MICE.** Michael R Hayden[+], Ming-Sun Liu[+], Frank Jirik[*], Yuahong Ma[+], René LeBouef[#], and John D Brunzell[#], [+]Department of Medical Genetics, [*]Biomedical Research Center, University of British Columbia, Canada; [#]Department of Medicine, University of Washington, WA.
The aim of over-expressing human lipoprotein lipase (LPL) in transgenic mice is to further investigate the pivotal role of this enzyme in lipid metabolism as well as its potential role in prevention of atherosclerosis. To achieve high level expression of human LPL in transgenic mice, an expression vector, consisting of the human LPL cDNA under the control of the human cytomegalovirus (CMV) promoter, was microinjected into embryos of (C57BL/6J X CBA/J) F1 mice. After screening 23 offspring by Southern blotting, one of the 3 founder lines was found to express over 350 hemizygous animals, which is approximately 2x higher than that observed in normal human plasma. Our observations of mice fed regular mouse chow, showed that the growth and development of transgenic mice expressing human LPL is normal. Transgenic LPL mice fed a high carbohydrate diet had HDL cholesterol levels of 55.1±11.1 mg/dl compared to levels in nontransgenic littermates of 36.3±6.1 mg/dl ($p<0.001$). Levels of human LPL activity in transgenic mice showed a positive correlation ($R=0.80$) with HDL cholesterol. A decrease in HDL is a most potent predictive risk factor for presence of atherosclerosis. Our data suggests that upregulation of LPL activity might represent a new target for increasing HDL cholesterol levels. Comparison of the susceptibility of the transgenic LPL mice to atherosclerosis versus their nontransgenic siblings will determine whether the increased LPL and HDL levels confer resistance to development of atherosclerosis.

SZ 406 **LYSIS OF OVARIAN CANCER CELLS BY HUMAN LYMPHOCYTES REDIRECTED WITH A CHIMERIC GENE COMPOSED OF AN ANTIBODY VARIABLE REGION AND THE Fc RECEPTOR γ CHAIN**, **P. Hwu, G. Shafer, J. Treisman, R. Cowherd, Z. Eshhar, and S.A. Rosenberg, National Cancer Institute, Bethesda, MD 20892**

A limitation in the use of adoptive cellular immunotherapy for cancer lies in the difficulty in obtaining specific tumor infiltrating lymphocytes (TIL) for many histologic types of cancer. In contrast, many monoclonal antibodies have been described which bind human tumor-associated antigens shared by tumors of the same histology. In order to combine the effector function of T cells with the anti-tumor specificity of antibodies, we have constructed chimeric receptor genes containing the variable region domains from monoclonal antibodies (mAb) linked to the Fc receptor-associated γ chain, which has been shown to be capable of mediating signal transduction in T cells. Chimeric receptor genes were made using single-chain V_L/V_H domains from an anti-trinitrophenyl mAb as well as from MOv18, a mAb which binds the 38 kD folate binding protein highly expressed on most ovarian adenocarcinomas. Human TIL retrovirally transduced with the anti-TNP chimeric receptor gene (TNP-TIL) could lyse TNP-labeled Daudi cells (60% specific lysis at Effector:Target of 90:1) and TNP-labeled EBV-B cells, but did not lyse unlabeled cells. Moreover, TIL retrovirally transduced with the MOv18 construct (MOv-TIL) could lyse the ovarian carcinoma cell line IGROV (57% specific lysis at E:T of 90:1), but did not lyse non-ovarian cell lines. In addition, TNP-TIL specifically secreted GM-CSF when cocultured with TNP-labeled Daudi and EBV-B cells (> 512 pg/ml/10^6 TIL/24 hours), while MOv-TIL specifically secreted GM-CSF when cocultured with IGROV cells (269 pg/ml/10^6 TIL/24 hours). These studies indicate that TIL can be stably modified genetically to be redirected against new antigens, defined by monoclonal antibodies. This approach is potentially applicable to a number of neoplastic and infectious diseases, and may allow adoptive immunotherapy against histologies not previously amenable to this treatment modality.

SZ 407 ANALYSIS OF *p53*-DEPENDENT AND -INDEPENDENT PATHWAYS IN DEVELOPMENT OF MURINE BREAST TUMORS, D. Joseph Jerry[1], Daniel Medina[2], and Janet S. Butel[1], Division of Molecular Virology[1] and Department of Cell Biology[2], Baylor College of Medicine, Houston, TX 77030

Effective treatment of breast cancer relies on early detection. Preneoplastic lesions can be detected as focal regions of hyperplasia, but the genetic changes associated with transition of mammary epithelial cells to hyperplastic cells remain undefined. To study the molecular basis for mammary preneoplasia, a series of transplantable murine mammary cell lines has been established. These cell lines form hyperplastic alveolar nodules (HAN) when transplanted into mammary fat pads of syngeneic hosts. Overexpression of *p53* protein was accompanied by mutations in the *p53* nucleotide sequence in three cell lines (COMMA-D, TM2H, TM3). The mutations included aberrant splicing, premature stop signal, amino acid substitution, and insertion of 3 nucleotides. Overexpression of *p53* was also evident in TM9 outgrowths and tumors, but the nucleotide sequence was wild-type throughout the entire coding region. Expression of *mdm2* was elevated in 3/5 TM9 tumors compared to 1/5 tumors from outgrowth lines that express low levels of wild-type *p53*. Somatic cell hybrids were constructed between two cell lines (COMMA-D and TM3) to test complementation and dominance of the outgrowth phenotypes. Both cell lines are EGF-dependent in vitro and form HAN in vivo, but vary in tumor incidence. Fusion of parental HAN cell lines resulted in retention of EGF-dependence for proliferation in vitro. However, hybrids between TM3 and COMMA-D lines resulted in EGF-independence, indicating complementary mutations. Outgrowth phenotypes are presently under investigation. These experiments demonstrate 1) roles for mutation of *p53* and overexpression of *mdm2* in murine mammary tumorigenesis; and 2) that acquisition of EGF-independent growth and mutation of *p53* are not necessarily linked. The data are consistent with alterations in *p53* or downstream targets being important to development of HAN. Therefore, wild-type *p53* is being introduced via a retroviral vector to test whether cell phenotypes can be altered.

SZ 408 HAEMOPHILIC MICE: USE OF HOMOLOGOUS RECOMBINATION TO PRODUCE AND STUDY TRANSGENIC MOUSE MODELS OF HUMAN COAGULATION DISORDERS, Louise K. Jones, Edward G.D. Tuddenham and John H. McVey, Haemostasis Research Group, Clinical Research Centre, Harrow, Middx. HA1 3UJ, U.K.

Haemophilia A is an X-linked recessive disorder which results in inappropriate bleeding into joints and muscles. Haemophilia is caused by a deficiency in factor VIII and affects 1 in 5000 males. Identification of carriers of haemophilia A and prenatal diagnosis is essential in order to offer genetic counselling and the prevention of the disease. 30% of haemophilia cases however arise due to spontaneous new mutations. It will therefore never be possible to eliminate this disease from the population by carrier diagnosis and genetic counselling.

Current treatment of haemophilia A involves the injection of factor VIII concentrates prepared from pooled plasma donations and therefore carry a high risk of serious viral infection. Recombinant factor VIII has entered clinical trials but may prove too expensive for widespread prophylactic use.

The ultimate therapeutic goal is the correction of the defect by somatic gene therapy. The haemophilias are ideal candidates for treatment by replacement gene therapy, since a low level of unregulated expression would transform a severe disease with frequent life threatening haemorrhages to a mild disorder requiring only occasional treatment.

In order to devise and test new therapies, it is necessary to have a whole animal model. Spontaneous haemophilia A is found in cats and dogs, however, these animals are difficult and expensive to maintain: a mouse model of haemophilia would have the advantages of cost, convenience, lifespan and generation time.

It is now possible to induce alterations in an endogenous gene of cultured embryonic stem cells through homologous recombination with cloned copies modified *in vitro*. Bacterio phage clones containing portions of the murine factor VIII gene have been isolated and partly characterised. We have prepared two targeting constructs, which carry the HSV-tk gene in a region of non homology and contain 1.2kb and 6kb of homology in which a neomycin resistance gene disrupts exon 19 of the factor VIII gene.

Low passage 129 derived ES cells known to contribute to the germline are currently being transfected with the constructs and subjected to double selection with G418 and gancyclovir. Double positive clones isolated are being screened using a combination of PCR and southern blotting, the results of which will be presented.

Recent work has indicated that ES cells electroporated using isogenic DNA have a greater likelihood of undergoing homologous recombination.

We are presently using constructs derived from CBA genomic DNA, however, we have also isolated factor VIII clones from 129 mouse DNA, and are currently in the process of making further constructs with this DNA for transfection.

SZ 409 **HIV-1 EXPRESSION IN HUMAN STROMAL CELLS DOES NOT EFFECT CYTOKINES PRODUCTION,**

Kaushal, S*, Gartner, S*, La Russa, S^, Liu, Y*, Yu, Z*, Ritchey, D*, Xu, J*, Perera, P§, Kim, J^, St. Louis, D*, Vahey, M^, Redfield, R^, Burke, D^, Mosca, J*, *Henry M. Jackson Foundation, Rockville, MD 20850, ^Walter Reed Army Institute of Research, Rockville, MD 20850, §NIH, Bethesda, MD 20894

Numerous investigators agree that HIV infected individuals have an overall decrease in hematopoietic potential. However, little agreement exist on the mechanism to account for the observed hematopoietic suppression. We initiated studies to evaluate the ability of HIV infection human stromal cells to express cytokines and support growth of progenitors.

A series of human immortalized stromal cell lines were established from human bone marrow by transfection of a plasmid containing the SV40 large T antigen (pSV3gpt), and isolating foci exhibiting a transformed phenotype. Attempts to infect one line, Lof11-10, with HIV-1 was unsuccessful, however, the HIV-1 genome was introduced into the Lof11-10 cell line by calium phosphate mediated gene transfer. Two HIV-1 expressing Lof11-10 lines were established using HIV-1 provirus constructions expressing either the neomycin gene, Lof-proNEO, or the hygromycin gene, Lof-proHYGRO. All cells in these lines expressed HIV-1 as detected by in-situ hybridization, RNA PCR, and p24 antigen production (1-10ng/ml).

Cytokine production was measured by RNA PCR in the Lof11-10 (uninfected) and the Lof-proNEO (HIV infected). Out of 24 cytokines tested, expression of 8 cytokines were detected. The RNA expression level for IL1beta, IL8 and M-CSF were equal to that of beta-actin, whereas the expression of other cytokines, IL1alpha, GM-CSF, G-CSF, TGFalpha and IL6 were superinducible upon cycloheximide treatment. Treatment of Lof11-10 cells with cycloheximide resulted in a 9-, 33-, 14-, 4- and 5-fold increase in IL1alpha, GM-CSF, G-CSF, TGFalpha, and IL6 respectively, representing RNA levels 3, 19, 21, 100, 33 percent of that observed for beta-actin RNA. The data suggest that the cycloheximide effect is at the level of RNA stabilization, since removal of the protein synthesis block results in a rapid decrease, within 2 hrs., in the accumulated RNA. The specificity for the cycloheximide response in the Lof11-10 cell lines was shown by the lack of accumulation of cytokine RNA in another human cell line. Protein production of IL1alpha, IL1beta, IL8, and GM-CSF were shown by ELISA. Throughout all the RNA PCR and ELISA analysis, the Lof-proNEO cell line (HIV-infected) showed only 2- to 3-fold difference in cytokine production when compared to the uninfected Lof11-10 cell line.

SZ 410 A HIGH COPY NUMBER VECTOR EXPRESSING ANTISENSE REVERSE TRANSCRIPTASE RNA EFFICIENTLY INHIBITS HIV-1 REPLICATION IN JURKAT CELLS. Hans J.Lipps[a], Jobst Meyer[a], Friedrich Grummt[b], Sigrid Nick[c], Thomas Stamminger[c], Gerhard Jahn[c]. a: Medizinisch-Naturwissenschaftliches Forschungszentrum, University of Tübingen, Tübingen, FRG; b: Institut für Biochemie, University of Würzburg, FRG; c: Institut für Klinische und Molekulare Virologie, University of Erlangen-Nürnberg, Erlangen, FRG.

We describe the construction of a high copy number expression vector for human T-lymphocytes which contains a portion of the HIV-1 reverse transcriptase gene in antisense orientation. The most important property of this vector is the presence of an amplification promoting sequence derived from the nontranscribed spacer region of the murine rDNA. This sequence promotes the spontaneous amplification of plasmid DNA to a copy number of up to 100 copies per cell. The amount of transcript is proportional to this high copy number. Compared to untransfected control cells or cells containing the corresponding single copy expression vector, HIV-1 multiplication is dramatically reduced (over 98 %) in cells harbouring the high copy number construct. Therefore this kind of vector represents an important improvement not only for genetic engineering of eucaryotic cells but also for gene therapy of some human diseases.

SZ 411 *A COMBINED GENETIC AND BIOCHEMICAL MOUSE MODEL FOR LESCH-NYHAN SYNDROME,* *Jim McWhir, Chao-Liang Wu and David Melton, Institute of Cell and Molecular Biology, Edinburgh University, Edinburgh EH9 3JR, Scotland.*

The inherited disease Lesch-Nyhan Syndrome (LNS), is characterised by self injurious behaviour (SIB) and mental retardation. LNS is associated with a deficiency of the purine salvage enzyme hypoxanthine phosphoribosyltransferase (HPRT). Until recently no spontaneous behavioural abnormalities had been reported in HPRT-deficient mice which had been generated using the embryonic stem cell (ES) system. In preliminary experiments, we observed that HPRT-deficient ES cells, which normally die in hypoxanthine-aminopterin-thymidine (HAT) medium, were rescued following supplementation with 10^{-4} M adenine. We then set out to test the hypothesis that mice were more tolerant of HPRT deficiency than man because of their greater reliance on adenine phosphoribosyltransferase (APRT) than on HPRT for purine salvage. Administration of the APRT inhibitor 9-ethyladenine to ES cells had a reversible cytostatic effect as determined by [^{3}H] thymidine incorporation. Growth inhibition was more pronounced on HPRT-deficient than wild type cells, indicating that the effect was not a general toxicity and that 9-ethyladenine was suitable for administration to HPRT-deficient mice. Primary cultures of brain tissue from mice sacrificed following 4 injections (ip) with 9-ethyladenine showed a significant reduction in APRT activity for both HPRT-deficient and wild type mice. A further 5 HPRT-deficient animals were administered with 9-ethyladenine between 5 and 20 times. All 5 animals developed self inflicted trauma in from 48 to 130 days. SIB did not occur in an age matched, uninjected control group. In a further experiment both wild type and deficient animals were injected with saline, 9-ethyladenine or caffeine. 9-ethyladenine treatment, and to a lesser extent, caffiene treatment, led to significant increases in the frequency of SIB in HPRT-deficient mice. This combined genetic/biochemical model of LNS will permit the evaluation of novel therapies involving the introduction of functional HPRT genes to affected animals.

SZ 412 PHARMACOLOGIC ANALYSIS OF GM-CSF PRODUCED BY A GM-CSF GENE-TRANSDUCED MURINE B16 MELANOMA CELL VACCINE, Janet A. Meurer, Audrey J. Lazenby, Hyam I. Levitsky, Liz M. Jaffee, Glen Dranoff, Richard C. Mulligan, and Drew M. Pardoll, Department of Medicine, Johns Hopkins University Medical School, Baltimore, MD 21287

The long range goal of cancer immunotherapy is to generate an anti-tumor immune response by the host immune system. Recent studies using retrovirally transduced tumor cells secreting various lymphokines have indicated that these cells can act as effective vaccines in vivo. Murine vaccination studies from this laboratory, using lethally irradiated B16 melanoma cells transduced to secrete GM-CSF, have demonstrated that mice receiving the vaccine are capable of rejecting a subsequent challenge of non-transduced tumor cells. It is thought that the local production of GM-CSF by the tumor vaccine enhances the immune response against tumor antigens, while systemic GM-CSF toxicity is minimal. In order to address the pharmacologic characteristics of GM-CSF produced by our lethally irradiated GM-CSF-producing melanoma cell vaccine, we have carried out in vitro and in vivo time course analyses to determine the amount of GM-CSF produced at various cell doses. In vitro analysis demonstrated a low-level production of GM-CSF by $1x10^5$ cells up to 36 hours post-irradiation, followed by a 3-fold increase in GM-CSF from 48-120 hours. Thereafter, levels dropped off and cells began to die. Subcutaneous injections of doses of $1x10^6$ and $1x10^7$ cells into C57BL/6 mice demonstrated detectable systemic levels of GM-CSF in the serum that increased steadily after 1 hour post-injection, with peak levels occuring at 42 hours. Serum GM-CSF levels began to decline thereafter and were barely detectable after 120 hours. Compared to in vivo pharmacokinetic analyses of glycosylated recombinant GM-CSF administered subcutaneously, the peak levels of GM-CSF attained in the mouse serum at the highest vaccine dose were 10-fold lower than levels defined as toxic (20-100 ng/ml). This analysis indicated, furthermore, that the decline in systemic GM-CSF after 42 hours post-injection was due to an in vivo effect on the cell vaccine itself, given that these cells in vitro had not yet reached the maximum secretion of GM-CSF (48 hours). A histologic examination of the injected site of the tumor cell vaccine demonstrated an influx of monocytes and macrophages by 3 days post-injection, with no viable tumor present by day 5. Therefore, this study would suggest that the decline of serum GM-CSF levels correlated with tumor cell vaccine destruction by the host immune system, and that peak levels of GM-CSF produced by the vaccine in vivo are an order of magnitude below those levels achieved from subcutaneous administration of the mean tolerated dose of glycosylated recombinant GM-CSF.

SZ 413 TOWARDS TARGETED INACTIVATION OF THE ADENOSINE DEAMINASE GENE, Alexandra A. J. Migchielsen, Marco Breuer, Anton Berns[1] and Dinko Valerio, Dept. of Gene Therapy, TNO-Institute of Applied Radiobiology and Immunology, 2280 Rijswijk, [1]Div. of Molecular Genetics, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

The absence of adenosine deaminase (ADA) in humans causes severe combined immunodeficiency disease (SCID). The disorder is caused by a single gene defect and serves as the paradigm in the initial attempts at human gene therapy. To study therapeutic approaches for ADA SCID we plan to generate an ADA deficient mouse. This model will be useful for the testing of existing gene therapy protocols, but is also invaluable for the development of new strategies such as gene correction therapy.

To disrupt one of the endogenous ADA alleles via homologous recombination in mouse embryonic stem (ES) cells, different targeting vectors were constructed using mouse ADA sequences isogenic with the employed ES cell line (E14). All three constructs are Ω type replacement vectors. In two constructs the incomplete ADA gene is disrupted by a deletion as well as an insertion of a selectable marker gene with promoter. The third construct incorporates an incomplete (promoterless) selectable marker gene. In this latter construct 5' *cis*-acting regulatory sequences are necessary for expression of the marker gene.

Southern analysis of transfected, resistant colonies is in progress. The results will be presented at the meeting.

SZ 414 DERIVATION AND CHARACTERIZATION OF EMBRYONIC STEM CELL LINES FROM THE RABBIT. Randall W. Moreadith and Kathy H. Graves, Molecular Cardiology Laboratories, University of Texas Southwestern Medical Center, Dallas, TX 75235.

The derivation of pluripotent embryonic stem (ES) cells has ushered in a new era in mammalian molecular genetics. These cells enable investigators to design precise mutations, propagate animals which carry these mutations, and study the consequences in an intact animal throughout development. However, studies are currently limited to the mouse. We report here the derivation and characterization of pluripotent ES cells from preimplantation rabbit embryos. Four - five day rabbit blastocysts were harvested from naturally mated 6-7 mo. old does. Embryos were washed and zona pellucidae enzymatically or mechanically removed before being placed individually on fibroblast feeder layers. Six to seven days later the embryos were trypsinized and replated onto feeder layers. Two cell types emerged as independent clonal cell lines. The first has morphology identical to primary trophectodermal outgrowth and is capable of spontaneous vesicle formation. The second is morphologically similar to ES cell lines derived from the mouse. These ES cells grow as undifferentiated colonies in culture and are feeder dependent. They are capable of "embryoid body" (EB) formation and differentiate into spontaneously contracting muscle, as well as neuronal, epithelial and pigment producing cells.

Characterization of these cells by karyotype, growth properties and electron microscopy of terminally differentiated EBs will be presented. The availability of a rabbit embryonic stem cell should facilitate a variety of genetic experiments in an animal large enough to provide detailed physiologic measurements. Rabbit models of human genetic diseases should prove to be extremely useful in testing novel approaches to genetic therapy and its consequences.

SZ 415 ENDOTHELIAL CELL-DIRECTED CANCER IMMUNOTHERAPY: EXPRESSION OF HUMAN RECOMBINANT CYTOKINE GENES BY ENDOTHELIAL CELLS IN VITRO. John Ojeifo, Alex MacPherson, Ning Su, Una Ryan[1], Udit Verma, Amitaba Mazumder, and James Zwiebel. Department of Medicine, Divisions of Hematology and Oncology, and the Lombardi Cancer Research Center Georgetown University, Washington, DC 20007, and [1]The Monsanto Company, St. Louis, MO.

Recent studies have demonstrated the feasibilty of cytokine gene transfer to enhance the anti-tumor activities of immune cells. We have previously shown that we can deliver genetically-modified endothelial cells to sites of tumor angiogenesis. Thus, endothelial cells are attractive cellular vehicles for the delivery of cytokine molecules in order to effect tumor immunotherapy. We constructed two retroviral vectors containing a cDNA encoding either interleukin-1α (IL-1α) or interleukin-2 (IL-2), called LNCIL-1α & LNCIL-2, respectively, and studied the expression of the two cytokines *in vitro* in primary endothelial cells. Human umbilical vein endothelial cells (HUVEC) transduced with LNCIL-1α or LNCIL-2 secreted 1.8 ng/10^6 cells/24h and 40 ng/10^6 cells/24h of biologically active IL-1α and IL-2, respectively. Mouse microvascular endothelial cells (MMEC) transduced with LNCIL-1α and LNCIL-2 secreted 1.5 ng/10^6 cells/24h and 6.8 ng/10^6 cells/24h of biologically active IL-1α and IL-2 proteins, respectively. Cocultivation of HUVEC-IL-2 and MMEC-IL-2 with normal human bone marrow cells generated cytotoxic activity against K562 and Daudi cell targets in a ^{51}Chromium release assay. Cytokine-secreting HUVEC and MMEC retained their normal cobblestone morphology, with the exception of MMEC-IL-1α cells, which became spindle-shaped in culture. However, the cytokine-secreting endothelial cells still retained other endothelial cell features, including uptake of acetylated-low density lipoprotein (Ac-LDL) and expression of von Willebrand factor. All transduced endothelial cells proliferated normally and were euploid as shown by flow cytometry. These results demonstrate that endothelial cells can maintain the expression of biologically active IL-1α and IL-2 *in vitro*.

SZ 416 TRANSCRIPTIONAL REGULATION OF HIV Matija Peterlin, Alicia Alonso, Andreas Baur, Subir Ghosh, Xiaobin Lu and Ying Luo, Howard Hughes Medical Institute, Departments of Medicine, Microbiology, and Immunology, University of California, San Francisco, San Francisco, CA 94143-0724.

Long terminal repeats (LTRs) of HIV-1 and 2 are transcriptionally activated following T cell or macrophage activation, growth, and proliferation. Cellular transacting factors that participate in this response include nuclear factors κB (NF-κB) and of activated T cells (NF-AT) in HIV-1 and NF-κB and AP-3 in HIV-2. Serine/threonine and tyrosine phosphatases modulate cytoplasmic to nuclear translocation and activities of these regulatory proteins. Following activation, HIV LTRs are transactivated by their respective transactivators (Tats). Tat interacts with an RNA stem-loop called TAR, which is located 3' to the site of initiation of viral transcription. For optimal interactions between Tat and the bulge in TAR RNA, cellular TAR RNA-binding proteins are required. The defect in rodent cells that leads to low levels of transactivation by Tat and that is complemented by human chromosome 12 maps to these TAR RNA-binding proteins. Tat modifies an unstable to a stable (i.e. elongation competent, processive) transcription complex. Orther DNA-binding proteins, i.e. NF-κB, SP-1, and LBP-1, are responsible for efficient loading of RNA polymerase II at the HIV TATA box. By fusing Tat to the MS2 coat protein, a prokaryotic RNA binding protein, and replacing TAR with the MS2 operator, activation and RNA binding domains of Tat were mapped. Besides determining differences in modes of action of Tat presented by RNA and DNA, we also constructed a minimal functional lentiviral Tat of only 25 amino acids. Here, 15 and 10 aa consitute activation and RNA-binding domains, respectively. This minimal Tat was subjected to structural studies. Mixing and matching different strains of the HIV LTR and Tat, i.e. constructing chimeric targets and effectors, revealed structural features of these LTRs and Tats that might explain distinct clinical courses of HIV-1 and HIV-2 infections. Based upon these observations, we developed a very sensitive test of viral burden and replication in infected individuals.

SZ 417 GROWTH OF SENSITIVE AND DRUG RESISTANT HUMAN MYELOID LEUKEMIA CELLS IN SCID MICE. Pavel Pisa, Milan Beran, Anna Porwit, and Magnus Björkholm, Department of Medicine, Division of Hematology, Department of Pathology, Karolinska Hospital and Institute, Stockholm, Sweden, and Department of Hematology, Division of Medicine, University of Texas MD Anderson Cancer Center, Houston, TX

Multidrug resistance is a critical problem in therapy of hematological malignancies. Recent advances in the transplantation of human normal and transformed hematopoietic cells into severe combined immunodeficient (SCID) mice provide an unprecedented opportunity to study the biological and molecular events mediating resistance against chemotherapy. We studied the engraftment of several myelogenous leukemia cell lines sensitive and resistant to mAMSA, Vincristine, Hycamptamine, Methotrexate or Doxorubicine. The distribution and growth potential of these cells was evaluated using histological and molecular technologies. Inoculation of 1 x 10^7 leukemic cells lead to manifestation of disease and subsequent tissue analysis showed evidence of leukemia. The survival of mice varied from 21 to 135 days, and it appeared to be longer after i.v. inoculation, compared to i.p. or s.c. routes. Terminally the animals showed either symptoms of wasting, development of local tumor or both. Massive leukemic involvement with infiltration of bone marrow and various organs including lungs, spleen, liver, ovaries and the brain was detected. No apparent differences in the tissue distribution of sensitive compared to resistant leukemia cells was observed. These findings demonstrate that human leukemic cells retain in SCID mice the clinico-pathological picture reminiscent of the original disease behaviour in humans. The combination of this *in vivo* model with high efficiency gene transfer methods will provide an important system to test the molecular alterations involved in drug resistance and leukemic progression.

SZ 418 TARGETING GENE EXPRESSION TO THE EPIDERMIS: POTENTIAL APPLICATIONS, Dennis R. Roop, Joseph A. Rothnagel, Xiao-Jing Wang, and David A. Greenhalgh, Departments of Cell Biology and Dermatology, Baylor College of Medicine, Houston, TX 77030

The ability to stably introduce genes into the germline of mice has greatly enhanced prospects for the generation of animal models of human diseases. The need for such animal models is becoming increasingly apparent as novel pharmaceuticals are developed which are specifically designed to inhibit expression of viruses which have been implicated in the etiology of certain diseases, or counteract the effect of mutated proteins which have been identified as the cause of specific diseases. Current efficacy assessments of these new therapeutic agents are restricted to in vitro models which do not allow evaluation of delivery routes nor assessment of other factors known to affect disease processes in vivo. In addition, the prospects for utilizing gene therapy to treat HUMAN DISEASES are coming closer to reality. Therefore, animal models of human diseases would be useful to assess the therapeutic potential of these approaches. Using a vector which specifically targets gene expression to the epidermis, we have generated transgenic mouse models for epidermolytic hyperkeratosis (a hereditary skin disorder), psoriasis and skin cancer. In anticipation of the eventual use of gene therapy in the treatment of these and other important diseases, we have developed several epidermal vector systems. Current research is directed toward examining the applicability of these vectors for in vivo gene therapy.

SZ 419 p53-DEFICIENT EMBRYONIC FIBROBLASTS EXHIBIT DECREASED SENSITIVITY TO DNA DAMAGE AND ALTERED CELL CYCLE CONTROL

Arthur T. Sands, Lawrence A. Donehower and Allan Bradley, Department of Molecular Genetics, Baylor College of Medicine, Houston, TX, 77030.

Embryonic fibroblasts were derived from a line of mice that contains a p53 null mutation which was generated by homologous recombination in mouse embryonic stem cells. It has been previously shown that approximately 74% of mice homozygous for the p53 mutation have developed tumors in a wide range of tissues by six months of age. Using embryos derived from this line of mice as a source of cells for in-vitro experiments, wild-type embryonic fibroblasts were compared to cells heterozygous (p53 +/-) and homozygous (p53-/-) for the mutation. Preliminary experiments indicated that these cells exhibited marked growth rate differences and altered cell cycle control by flow cytometry. In addition, early studies of cells homozygous for the p53 deficiency have demonstrated a decreased sensitivity to DNA damaging agents in a colony survival assay as compared to wild-type controls. Flow cytometric studies have also indicated different responses to DNA damaging agents between wild-type and p53 -/- cells. Assays for differences in rate of mutation are currently being explored.

SZ 420 RETROVIRUS-MEDIATED GENE DELIVERY AND SPECIFIC CYTOTOXICITY TO MOUSE GLIOMA CELLS BY GLIOMA-SPECIFIC PROMOTERS, K Shimizu, Y Miyao, M Yamada, K Ikenaka*, K Nakahira*, N Morita*, J Nakao*, K Mikoshiba**, T Hayakawa, Dept of Neurosurg, Osaka Univ Med Sch, Osaka 553, Japan, *Okazaki Natl Res Inst, Okazaki 444, Japan, **Dept of Mol Neurobiol, Inst of Med Sci, Tokyo Univ, 108, Japan

We demonstrated that the retrovirus-mediated genes were transferred to mouse glioma cells in meningeal gliomatosis models(Jpn J Cancer Res 83, 1992[in press]). This retroviral vector contains the *E. coli* β-galactosidase (β-gal) gene as a marker for integration of the *lacZ* gene which was controlled by the SV40 (non-specific) early promoter. Now, we investigate whether or not the *lacZ* genes are able to be specifically controlled in mouse glioma (RSV-M glioma or 203 glioma) cells by the glioma-specific promoters. As candidates for glioma-specific promoters, we chose glia-specific promoters, the 2.5-kb 5'-flanking region of the mouse glial fibrillary acidic protein (GFAP) gene, the 1.3-kb 5'-flanking region of the mouse myelin basic protein (MBP) gene and the 1.5-kb 5'flanking region of the mouse myelin proteolipid protein (PLP) gene. Supernatant of the psi-2 packaging cells which were transfected each retroviral vectors (GFAP promoter-, MBP promoter-, and PLP promoter-*lacZ*) containing 8 μg/ml polybrene was incubated with mouse glioma cells, mouse fibroblast cells (NIH-3T3) and mouse myeloma cells (A20-2J) for three to four hours. The blue β-gal stain was detected in all cell lines infected retrovirus BAG vector which the *lacZ* gene was controlled by the Moloney leukemia virus 5'-LTR. On the other hands, the blue stain was only detected in these two glioma cell lines which were infected glia-specific promoter-*lacZ* retroviruses. The retrovirus containing MBP promoter directing herpes simplex virus thymidine kinase (HTK) gene could be infected to various cells. However, mouse glioma cells, which were transfected MBP promoter-HTK retroviral vectors, were sensitive to the antiviral drugs, aciclovir (ACV) or ganciclovir (GCV), but no other cells (fibroblast and lymphoma cells) were insensitive to the antiviral drugs, even if these cells were transferred MBP promoter-HTK retroviral vectors.

SZ 421 PACKAGING OF RECOMBINANT HIV VECTORS IN COS CELLS, Eiji Shinya and Takashi Shimada, Dept. of Biochemistry and Molecular Biology, Nippon Medical School, Tokyo, JAPAN

We recently developed a packaging system for the human immunodeficiency virus (HIV) based retrovirus vectors. This packaging system is based on transient expression of virus proteins from the helper plasmid and of packageable RNA molecules from the HIV vector plasmid in Cos cells and therefore, enable us to prepare recombinant vectors within only two to three days. There was no detectable wild-type HIV. Since the recombinant HIV vectors can introduce genes into only CD4(+) cells with very high efficiency, this system should be important for developing gene therapy of AIDS. In this work, we have studied the mechanism of packaging of HIV using this system. By analogy to Moloney murine leukemic virus, the sequence between the splicing donor site and the gag initiation codon has been thought to be essential for packaging of viral genome into particles. Accordingly, we mutated 34 out of 46 nucleotides of this putative packaging signal sequence (psi) in the helper plasmid and the HIV neo plasmid, yielding the psi(-)helper and the psi(-)neo, respectively. Hybridization analysis of RNA extracted from the recombinant HIV showed that the psi(-)helper RNA was efficiently packaged along with the neo RNA into virus particles. The transduction efficiency of CD4(+) HeLa cells with HIV vectors containing the psi(-)neo RNA was 20% of that containing the psi(+)neo. PCR analysis of DNA from G418 resistant cells transduced with the psi(-)neo vector showed that only the psi(-)neo sequence was integrated into chromosomal DNA, but the helper sequence was not detectable. These results suggest that this particular sequence is not essential for packaging of the recombinant HIV. Further modification of the helper plasmid may be required to avoid transfer of the packaging function.

SZ 422 A SEARCH FOR THE HUMAN XLA (*X-LINKED AGAMMAGLOBULINEMIA*) GENE, A CANDIDATE DISEASE FOR GENE THERAPY.

C.I. Edvard Smith, Igor Vorechovsky, Paschalis Sideras, Lennart Hammarström, Jill Holland, David R. Bentley and David Vetrie.
Center for BioTechnology, Karolinska Institute, NOVUM, S-14157 Sweden and Division of Medical and Molecular Genetics, Guy's Hospital, Guy's Tower, London SE1 9RT, UK.

X-linked agammaglobulinemia (XLA) results in a B-lymphocyte differentiation defect causing agammaglobulinemia and increased susceptibility to infections in affected males. The disease is a candidate for gene therapy. The gene was previously mapped using genetic linkage analysis to Xq22. No recombinants have to date been reported between the XLA locus and the marker DXS178 in over 30 informative meioses. Two overlapping yeast artificial chromosomes (YACs), which cover a region of approximately 1000 kb around DXS 178, and form part of a larger YAC contig, were hybridised to an ordered cosmid library constructed from a human fibroblast cell line with the karyotype 49,XXXXX. Positive cosmid clones were gridded in high density arrays onto nylon filters and rescreened with a series of YACs, YAC end probes and additional markers from the region. A 640 kb YAC hybridising to DXS178 (p212) was used for direct selection of PCR-amplified cDNA from two cDNA libraries. Enriched clones were hybridised back to cosmids and positive clones were hybridised to the subcloned cDNA libraries. Candidate cDNA clones have been isolated, sequenced and are currently being used for the analysis of the corresponding genes in patients with XLA.

SZ 423 TUMOR GROWTH ALTERATION WITH LOCAL INTERLEUKIN-12 SECRETION ACHIEVED BY GENE TRANSFER.

Tahara, H.[1,2], Zeh, H. III[1], Pappo, I.[1], Nastala C.[1], Robbins, P.D.[2], Lotze, M.T.[1,2] Departments of [1]Surgery, and [2]Molecular Genetics and Biochemistry, University of Pittsburgh, Pittsburgh, PA 15261

Interleukin-12 (IL-12), a heterodimeric cytokine composed of p35 and p40 subunits, activates NK cells, facilitates the generation of specific cytolytic T lymphocytes, promotes the expansion of tumor infiltrating lymphocytes, and induces the secretion of IFN-γ from both T and NK cells *in vitro*. The effect of local secretion of IL-12 on growth of tumor cells in a murine model was investigated using fibroblasts (NIH3T3) transfected to express both genes of murine IL-12. Graded doses of BL6 cells, a poorly immunogenic murine melanoma, were admixed with IL-12 expressing fibroblasts (IL-12 group), and inoculated into C57/B6 mice intradermally. Tumor emergence was measured and compared with those of controls (tumor only or tumor with non-transfected 3T3). The date of tumor emergence in the animals of IL-12 group was delayed significantly compared with either control group at multiple tumor dose challenges. However, all of the animals eventually developed tumors. These results show that locally secreted IL-12 can alter tumor growth, but may not induce complete rejection of the BL6 tumor. We have constructed retroviral vectors that can express either of the two chains of IL-12 (MFG-p35 and MFG-p40). To obtain higher efficiency of expression of both subunits, we also constructed a retroviral vector containing both genes and an internal ribosome entry site sequence of the encephalomyocarditis virus (DFG-mIL-12). We have recently demonstrated high level of expression of IL-12 in NIH3T3 cells transfected with the DFG-IL-12 provirus (3100 U/24hr./10^6 cells). The application of these retroviral vectors expressing the subunits of IL-12, either separately or coordinately, for cytokine gene therapy will be discussed.

SZ 424 ENHANCEMENT OF IL-2 INDEPENDENT KILLING ACTIVITY BY INTRODUCTION OF MUTANT *lck* GENE INTO CYTOTOXIC T CELLS.
-POSSIBLE APPLICATION FOR GENE THERAPY OF CANCER-

Toshihiko Torigoe, Russell S. Taichman+, Juan A. Millan and John C. Reed
La Jolla Cancer Research Foundation, La Jolla, CA 92037, +University of Michigan, Ann Arbor, MI 48109

Interleukin-2 (IL-2) has been used extensively in attempts to bolster immune responses to tumors. Recently the activity of the protein tyrosine kinase encoded by the *lck* proto-oncogene has been found to be regulated by IL-2, and appears to be essential for cytolytic function of at least some T-cells. Here we describe initial attempts to utilize a gene transfer strategy involving *lck* for augmenting the killing activity of T-cells against tumor target cells.

For these experiments, we stably introduced into the murine IL-2-dependent T-cell line CTLL-2 an expression plasmid encoding a mutant version of p56-LCK that contains a Tyr ->Phe substitution at position 505 (F505-LCK) that renders the kinase constitutively active. When tested for killing of the NK-sensitive target cell line YAC-1, F505-LCK-containing CTLL-2 cells displayed 6-fold higher cytolytic activity relative to parental CTLL-2 cells. Furthermore, killing by F505-LCK-producing CTLL-2 cells occurred in an IL-2-independent manner, in contrast to CTLL-2 cells transfected with an expression plasmid encoding normal p56-LCK where cytolytic activity was augmented by IL-2 in a concentration-dependent fashion. Despite the high levels of protein tyrosine kinase activity in F505-LCK-containing CTLL-2 cells, growth of these cells remained completely dependent on exogenously supplied IL-2. Thus, production of the F505-LCK kinase in these cytolytic T-cells resulted in markedly enhanced killing of tumor target cells without necessity of IL-2. This approach therefore could potentially be extended to tumor infiltrating lymphocytes as a gene therapy for cancer.

SZ 425 CONSTRUCTION OF RETROVIRAL VECTORS CONTAINING CHIMERIC IMMUNOGLOBULIN / T CELL RECEPTOR GENES SPECIFIC FOR RENAL CELL CARCINOMA.

M.E.M.Weijtens*, E.Braakman*, D.Valerio†, R.A.Willemsen*, R.L.H.Bolhuis*. *Dept. of Medical and Tumor Immunology, Dr. Daniel den Hoed Cancer Centre, Rotterdam, The Netherlands. †Dept. of Gene Therapy, ITRI/TNO, Rijswijk, The Netherlands.

In order to combine the specificity of tumor-selective mAb with the efficacy of T lymphocytes to destroy tumor cells, we have constructed chimeric immunoglobulin / T cell receptor (Ig/TCR) genes composed of the TCR constant (C) domains coupled to the antibody variable (V) domains. cDNA sequences encoding V-regions of the heavy (V_H) and light (V_K) chain of the renal cell carcinoma specific antibody G250 were isolated and cloned from G250 secreting hybridoma cells. The V-region gene segments were fused in frame to the C-region gene segment of either the α or β TCR chain by recombinant polymerase chain reaction (PCR). These chimeric genes were cloned into the pLXSN or pLXSH retroviral vector containing the neomycine or hygromycine resistance genes, respectively. Recombinant virus producing cell lines were generated by transfection of the pL($G250V_K$-$C_β$)SN vector into GP+E86 cells. Ecotropic recombinant virus, produced by these cells, was used to infect the amphotropic packaging cell line PA317. After selection in G418 containing medium, clones were obtained that produced helpervirus free amphotropic recombinant retroviral vectors (titer $4x10^3$). Mutant Jurkat cell lines, containing a defect in the transcription of either TCR α or β genes, and consequently lacking CD3/TCRαβ expression at the cellsurface, were used to analyse the functional expression of the chimeric Ig/TCR genes. Restoration of CD3/TCRαβ surface expression was analysed in these cells after transfection or infection with the various chimeric vectors.

SZ 426 INHIBITION OF HIV REPLICATION IN HUMAN MONOCYTES BY DEFECTIVE HERPESVIRUS VECTOR DELIVERY OF AN INTERFERON α GENE, Jerry P. Weir and Karen L. Elkins, Henry M. Jackson Foundation, Department of Cellular Immunology, Walter Reed Army Institute of Research, Rockville, Maryland 20850.

Human monocytes and macrophages are non-dividing cells that serve as a major reservoir for human immunodeficiency virus (HIV) at all stages of infection. To investigate viral mediated gene delivery as a means of inhibiting HIV replication in human monocytes, a defective herpes simplex virus (HSV) vector was developed that expressed human interferon α (IFNα). Monocytes infected with this defective HSV vector expressed IFNα and, when challenged with HIV, showed dramatically reduced cytopathic effects and HIV replication compared to control vector treated or mock treated monocytes. Similar effects on HIV replication were observed if monocytes were first infected with HIV and then treated with the recombinant vectors. These results demonstrate that defective HSV gene delivery of IFNα directly to human monocytes can greatly decrease HIV replication, and suggests that such a vector, or possibly vector-treated cells, might deliver therapeutically important genes directly to sites of HIV infection.

SZ 427 A CHIMERIC SUICIDE GENE FORMED BY FUSING RAT GROWTH HORMONE SIGNAL PEPTIDE SEQUENCE TO THE CYTOSINE DEAMINASE GENE MARKEDLY ENHANCES SECRETION OF ACTIVE ENZYME, L. Keoki Williams,* Craig A. Mullen,§ and R. Michael Blaese,§ *Howard Hughes Medical Institute and §Cellular Immunology Section, Metabolism Branch, National Cancer Institute, National Institute of Health, Building 10, Room 4B47, Bethesda, MD 20892

One use of gene therapy in the treatment of cancer is to selectively destroy tumor cells through the introduction of "suicide" genes. Most "suicide" genes are non-mammalian genes that work by converting otherwise innocuous prodrugs to toxic metabolites at the tumor site. The genes cytosine deaminase (CD) and thymidine kinase (TK) are two commonly used negative selection systems. CD works by converting 5-fluorocytosine (5-FC) to the toxic metabolite 5-fluorouracil (5-FU); and TK works by phosphorylating the nucleoside analogs, acyclovir and ganciclovir, to active forms. Mixed cellular assays and *in vivo* studies have shown that in the presence of acyclovir or ganciclovir TK transduced cells have the ability to cause "bystander" killing of untransduced cells. This property is important in the complete eradication of tumor cells *in vivo*. CD, on the other hand, does not possess similar "bystander" killing in mixed cellular assays. In order to increase "bystander" killing in the CD system a chimeric "suicide" gene was created by joining the signal peptide region from the rat growth hormone gene to a modified *E. coli* CD gene. The resulting chimera was named pRGHCD. A retroviral vector producer line was then created by transfecting the packaging line, PA317, with pRGHCD. Gene expression was measured by the ability of the CD to convert ^{3}H-cytosine to ^{3}H-uracil. Expression studies using the pRGHCD transfected producer line showed enzyme activity in the culture medium but not in cell lysates, confirming both synthesis and secretion of the gene product. Further expression studies were performed using NIH-3T3 and murine adenocarcinoma 38-2 cell lines transduced with vector containing either RGHCD or CD2. The latter contains the CD gene without the secretion signal. Cell lysates from those cells transduced with pCD2 producer line retrovirus showed higher enzyme activity than pRGHCD transduced cell lines. However, cell lines transduced with virus from the pRGHCD producer line showed higher levels of CD activity in the culture medium compared to those cells transduced from the pCD2 producer line. Cell lines transduced with control pLXSN retrovirus, which contains only a neomycin resitance gene, did not show any activity in either the culture medium or the cell lysate. These results suggest that the addition of rat growth hormone signal peptide to the proximal end of the CD causes it to be secreted in its active form. The ability of pRGHCD transduced cell lines to secrete CD in its active form may provide a more effective negative selection system for eradicating localized tumors than the CD2 gene alone. By increasing the extracellular conversion of 5-FC to 5-FU, secreted CD may enhance bystander killing of untransduced tumor cells.

SZ 428 RESTORATION OF AUDITORY EVOKED POTENTIAL BY MYELIN BASIC PROTEIN (MBP) GENE THERAPY IN SHIVERER MICE. T.J. Yoo[1], T. Fujiyoshi[1], Leroy Hood[2], C. Readhead[2], Univ. of TN, Memphis, TN 38163[1], Cal Tech, Pasadena, CA 91125[2]

One of the major challenge of modern medicine is developing a proper gene therapy. We have used the shiverer mouse as a model for the correction of hearing impairment due to gene defect. The mouse has an autosomal recessive mutation of the myelin basic protein (MBP) structural gene located at the distal end of chromosome 18, and this mutation results in the absence of MBP message in the brain. The gene encoding MBP is largely deleted with only the first two exons remaining. The deletion extends from a region between second and third exon to 2 kb 3' to the last exon. MBP is a major protein component of central nervous sytem (CNS) myelin.

In order to test the hypothesis, that the complex shiverer phenotype of hypomyelination of CNS, including the hearing impairment, was due to a deletion in the MBP gene, we introduced the wild-type MBP gene as well as 4 kb of 5' and 3 kb of 3' flanking sequence; and DNA was microinjected into the pronucleous of fertilized eggs. The transgenic mice had MBP message levels of 25% of normal, the MBP transgene was expressed in the normal pattern, and neither tremors nor seizures were observed.

The auditory evoked potential study shows interpeak latency in shiverer mice from peaks I to V was prolonged significantly, while those transgenic mice improved significantly. Under electron-microscopic observation of the proximal portion of the cochlear nerve, a greater number of axons in MBP-transgenic mice were myelinated than in shiverer mice, but the myelin sheath was not as thick as in controls.

This experiment is the first successful gene transfer to restore hearing in mammals.

Late Abstracts

ADENOVIRAL MEDIATED TRANSFER OF MUSCLE GLYCOGEN PHOSPHORYLASE INTO C2C12 MYOTUBES. Susanna Baqué[1], Joan J. Guinovart[1], Robert D. Gerard[2], , Christopher B. Newgard[2] and Anna M. Gómez-Foix[1],[1]Departament de Bioquímica i Fisiologia, Universitat de Barcelona, 08028-Barcelona, Spain and [2]Southwestern Medical Center, University of Texas, Dallas, TX 75235.

Mouse skeletal muscle cell line C2C12, which differentiates into myotubes, was used as an *in vitro* model for studying the feasibility of adenoviral mediated gene transfer into muscle nondividing mature cells.
C2C12 myotubes were obtained, as assessed by morphological evidence and creatine kinase activity, by 7-day-incubation of myoblasts in high-glucose DMEM medium containing 10% horse serum. Myotubes were infected with an adenoviral vector containing rabbit muscle glycogen phosphorylase cDNA under the transcriptional control of the CMV promoter-enhancer (AdCMV-MGP). The effectiveness of the muscle phosphorylase delivery was evaluated by a quantitative radiometric assay of phosphorylase activity. A non-denaturing acrylamide gel analysis was performed to detect the presence of different isoforms of glycogen phosphorylase. AdCMV-MGP infected cells showed a 2-fold increase in glycogen phosphorylase activity, measured both in the presence and in the absence of 5 mM AMP, compared to uninfected or wild-type adenovirus infected cells. In native gel assays, an increase in an activity band, the mobility of which corresponds to that of the muscle isoform of phosphorylase, was observed in AdCMV-MGP infected cells with respect to C2C12 control cells. Further studies are being performed to measure the efficiency of the gene transfer process. Our experiments demonstrate the ability of the adenovirus vector system to deliver and express foreign genes in C2C12 myotubes, providing support for its usefulness in muscle gene therapy.

SPECIFIC ABLATION OF HIV-tat EXPRESSING CELLS BY CONDITIONALLY TOXIC RETROVIRUSES, Elaine A. Dzierzak, Colin Miles, Daniel Pennington and Hugh J.M. BradY, National Institute for Medical Research, London U.K. NW7 1AA.

Until recently, therapeutic strategies to combat HIV infection have focused on traditional methods such as drug or vaccine treatment of AIDS patients. Current approaches utilise molecular decoys or inhibitors to prevent viral infection. We have developed another strategy, whereby a recombinant DNA sequence encoding a conditional toxin gene can be delivered to haematopoietic cells via retroviruses for the specific killing of HIV-tat expressing cells. To achieve this we generated molecular constructs using portions of the HIV genome (HIV2 LTR) and a toxin-encoding gene (HSV1 thymidine kinase (TK), in the context of retroviral vectors for subsequent delivery to the blood system. Since this system of intracellular molecular ablation requires that the HIV promoter is active only in the presence of Tat, we developed constructs which could be *trans*-activated efficiently but provide no basal transcriptional activity. We have made minimal HIV2 promoter constructs incorporating substitutions and deletions in the NFKB and Sp1 sites upstream of the TATA box and TAR region of the HIV2 LTR. We have found that certain promoter modifications reduce basal transcription while maintaining high *trans*-activated levels of expression when transfected into several different cell lines. Furthermore we have constructed retroviruses containing HIV-TK and have found intact passage of these sequences after infection. Infected 3T3, HeLa and CEM cells can be Tat *trans*-activated to express the TK gene. This expression results in the differential ablation of infected cells in the presence of Ganciclovir. The retroviruses produced in these studies may be applicable to HIV ablative therapy.

AN ANTI-CANCER DRUG DELIVERY APPROACH USING GENE-MODIFIED TUMOR CELLS. S.M. Freeman, K.A. Whartenby, C.N. Abboud, F.L. Moolten, David S. Koeplin, and G.N. Abraham. Tulane University Medical Center, University of Rochester Medical Center, and Edith Nourse Veterans Hospital. We have developed a new tumoricidal agent by genetically altering tumor cells to express the herpes simplex thymidine kinase gene (HSV-TK). These cells demonstrated a potent anti-cancer effect on unmodified "bystander" tumor cells after exposure to ganciclovir. Irradiation of the HSV-TK cells did not diminish their tumoricidal effect since they remained toxic to tumor cells. This toxic effect was not dependent on tumor type or species of either the gene-modified cells or bystander cells. Two types of experiments were performed to elucidate the mechanism of the cytotoxicity. First, HSV-TK positive and negative cells were mixed and exposed to ganciclovir. The dying HSV-TK positive cells formed apoptotic vesicles which were phagocytized by nearby unmodified tumor cells as evidenced by studies using electron microscopy and fluorescent activated cell sorting. Second, we demonstrated an immunological component to the bystander effect using immunodeficient mice. The ability of HSV-TK positive tumor cells to kill unmodified tumor cells was dependent on the mice having an intact immune system. These findings were extended to an intraperitoneal tumor model. We demonstrated the ability to kill a pre-existing intraperitoneal tumor which directly relates to the treatment of ovarian cancer. This approach is applicable for the development of new therapeutic modalities for the treatment of localized and metastatic cancer.

Large Scale Gene Transfer in Early Human Hematopoietic Progenitors. Antoinette Hatzfeld[1], Angelo Cardoso[1], Pascal Batard[1], Jean Pierre Levesque[1], Ma Lin Li[1], Roland Levinsky[2] and Jacques Hatzfeld[1]. 1) CNRS UPR 272 Rue Guy Mocquet 34802 Villejuif France, 2) Institute for Child Health, London, UK

The human hematopoietic stem cell compartment represents about 0.01% of the mononuclear bone marrow cells. Most of these cells are quiescent which prevents an efficient gene transfer with retroviral vectors. Using the nls LAC Z vector (see reference) we have studied various ways to improve gene transfer in SBA^- $CD34^+$ $CD38^-$ cells. These early progenitors can develop in vitro into colonies containing granulocytes, monocytes, megakaryocytes, and erythroid cells when optimal concentrations of various cytokines are added. We have previously shown that antisense Transforming Growth Factor - ß1 or Retinoblastoma oligonucleotides can release early CFU-GEMM from quiescence (Hatzfeld et. al. J. Exp. Med. 174, 925-929:1991). These CFU-GEMM are earlier progenitors which provide up to $1.5x10^5$ cells containing the various hematopoietic lineages. We will compare various conditions which release these cells from the Go phase and determine how this stimulation will promote an efficient and stable transfer of the ß galactosidase gene.

N. Ferry, O. Duplessis, D. Houssin, O. Danos, and J.M. Heard. PNAS 88 8377-8381:1991

EXPRESSION OF A HETEROLOGOUS GENE FROM A RECOMBINANT RETROVIRUS IS DOWN REGULATED BY DNA METHYLATION OF PROMOTER IN A MURINE MODEL OF MELANOMA. Christie King*, Brigitte Schott, Preet Chaudhary and Igor Roninson, *Wellcome Research Labs, Research Triangle Park, NC, 27709 and University of Illinois Medical Center, Chicago, Illinois, 60012. Recombinant retroviruses provide a system having a high efficiency of integration for use as gene transfer vehicles, but few studies have compared the stability of expression of heterologous genes in vitro and in animal models. To generate a murine model for multi-drug resistance (MDR), B16/F10 melanoma cells were infected with a replication-defective amphotropic retrovirus, LMDR1L6, containing the human MDR-1 gene transcribed from MoMLV-LTR promoter. Cells were selected in vinblastine to generate lines having varying levels of expression and amplification of hMDR-1. In culture, all the lines retained expression of the heterologous gene for at least four months in the absence of drug. However, in vivo growth of the same cells following subcutaneous or intraperitoneal injection into nude mice resulted in a dramatic reduction in gene expression. Several cell lines having an amplified hMDR-1 gene were recovered from animals. Southern analysis showed that the transfected gene was still amplified, but it had undergone methylation during growth in vivo. To try to overcome cellular mechanisms that inactivate expression of genes from retroviral vectors in murine systems, we generated B16 cell lines expressing hMDR-1 from the constitutively expressed beta-actin promoter. Comparison of the stability of hMDR-1 expression in vitro and in vivo will be presented.

HIV-1 based retroviral vectors - necessity for *cis*-acting signals outside the 5′ leader region and utility for gene transfer to CD4+ cells

Andrew M.L.Lever, Jennifer H.Richardson and Lisa A.Child
University of Cambridge School of Medicine, Cambridge, CB2 2QQ, U.K.

We have designed a series of retroviral vectors based on HIV to investigate the *cis*-acting signals required for packaging of HIV-1 RNA into virions and explore the usage of HIV based vectors for gene therapy in AIDS. Using a helper virus system, which rigorously excludes the possibility of plasmid DNA mediated transfer, we have determined that the two minimal signals required for RNA packaging are the 5′ leader sequence and the 3′ end of the envelope gene. Absence of either of these completely abrogates encapsidation as judged by analysis of virion encapsidated RNA and vector mediated transfer of antibiotic resistance. Enhancement of packaging can be achieved by inclusion of *gag* gene sequences in *cis*. Vectors with the appropriate signals can be transferred helper free in a cotransfection system and we are currently using an HIV based packaging cell line to enhance titres of these vectors. We are able to demonstrate transfer of vectors containing antiviral genes to CD4 cells and significant inhibition of HIV replication in some vector transduced cell lines.

IMPLANTATION AND SURVIVAL OF A HUMAN FETAL ASTROCYTE CELL LINE IN THE BASAL GANGLIA OF THE NON-HUMAN PRIMATE, RHESUS MONKEY. Carlo S. Tornatore[1], Kris Bankiewicz [2], Daniel Lieberman[2], and Eugene O. Major[1]. [1]Laboratory of Viral and Molecular Pathogenesis and [2]Surgical Neurology Branch, NINDS, NIH, Bethesda, MD,20892.

The grafting of fetal tissue in the treatment of neurodegenerative diseases would be greatly facilitated if a viable human fetal cell line could be substituted for primary fetal tissue. A permanent, immortalized human fetal astrocyte cell line (SVG) has been established (Major E. et al. PNAS 1985) which is anchorage dependent for growth, does not form tumors in nude mice and maintains characteristics of fetal astrocytes such as GFAP expression. To determine the viability of the SVGs as a neural xenograft, the cells were implanted bilaterally into the basal ganglia of six rhesus monkeys. One month post-operatively two of the monkeys were sacrificed and in both cases the SVG cells could be easily identified in the CNS parenchyma both morphologically and by identification of an SVG marker protein, T antigen. The cells remained confined to the site of implantation without evidence of inflammation, graft rejection or tumor formation. The remaining monkeys have been followed using Magnetic Resonance Imaging of the CNS and in no case has a tumor forming mass been identified. This study demonstrates that an immortalized human fetal astrocyte cell line can be successfully grafted and survive in the CNS of the primate. The potential of these cells in the treatment of human neurodegenerative diseases is discussed.

DIRECT IN VIVO GENE TRANSFER TO THE CENTRAL NERVOUS SYSTEM USING REPLICATION DEFICIENT RECOMBINANT ADENOVIRUS VECTORS, Andrea Mastrangeli, Gianluigi Bajocchi, Sanford H. Feldman and Ronald G. Crystal, NHLBI, Bethesda, MD 20892

The ependymal cells of the central nervous system (CNS), the epithelial-type cells lining the cerebral ventricles, have direct access to both the cerebral spinal fluid (CSF) and the underlying brain parenchyma, and thus are potentially ideal cells targets for CNS gene transfer. One appealing strategy to deliver genes to ependymal cells is with replication deficient recombinant adenovirus vectors, with natural tropism for cells of epithelial origin and with the demonstrated ability to efficiently transfer genes to differentiated cells of a variety of organs of adult animals in vivo. To evaluate this concept, the vectors Ad.RSVβgal [containing the intracellular marker gene β-galactosidase (βgal)] and Ad-αlAT [containing the human αl-antitrypsin (αlAT) cDNA, a model secreted protein] were administered directly to the CNS of rats via a catheter in a lateral ventricle. Whereas control animals showed no histologic evidence of βgal activity in the CNS, rats evaluated 4 d after administration of Ad.RSVβgal had striking βgal expression in ependymal cells of the lateral, third and fourth ventricles, as well as in the leptomeningeal layer on the surface of the brain. Following administration of Ad-αlAT into a lateral ventricle, ELISA analysis of CSF sampled from the fourth ventricle demonstrated dose-dependent, easily detectable levels of human αlAT 2 to 4 d later. Unexpectedly, stereotactic administration of Ad.RSVβgal to the region of the globus pallidus and striatum showed localized βgal activity at the site of administration as well as in the substantia nigra, suggesting gene transfer to the CNS parenchymal cells in the local region. Thus, adenovirus vectors can be used to directly deliver genes in the CNS in vivo, with expression for at least several days, suggesting a variety of possibilities for future gene therapy applications.

GENE TRANSFER INTO PRIMARY CULTURES OF HUMAN HEPATOCYTES.

J.C. Pagès 1, A. Weber 1, M. Andréoletti 1, M. Bennoun 1, D. Houssin 1, D. Franco 2 P. Briand 1, J. Chapman 3, R. Benarous 1, P. Lehn 1
1. ICGM and Laboratoire de chirurgie de l'hopital Cochin, Paris.
2. Hopital A. Béclère, Clamart. 3 Hopital de la Pitié, Paris

Homozygous familial Hypercholesterolemia (FH) is a genetic disorder featuring a functional deficiency in cellular Low Density Lipoprotein Receptor (R-LDL) leading to early occurence of life threatening coronary heart problems. As no curative clinical traitment is available, gene transfer is an attractive approach. Thus we chose to investigate the transfer of the human R-LDL cDNA into primary culture of human hepatocytes in vitro via a retroviral vector. In view of their low spontaneous rate of mitosis hepatocytes represent an intractable target for retroviral mediated gene transfer. To overcome this problem we have used different infection conditions with various growth factors, namely the Hepatocyte Growth Factor (HGF). Transduction efficiency was first evaluated by using a retrovirus containing the E. Coli ß-galactosidase gene. We consistently obtained an infection rate of 30% while preserving the differentiated status of the hepatocytes. In order to obtain a high level of gene expression over a long period , we designed a retroviral vector in which the R-LDL cDNA is under the transcriptional control of strong, liver specific regulatory sequence, e.g. pyruvate kinase control region (with or without the CMV enhancer). These vectors can be evaluated under our infection conditions and compared with vectors based on ubiquitous promoters such as the ß-actine promoter.

RETROVIRUS-MEDIATED GENE TRANSFER AND EXPRESSION OF HUMAN ORNITHINE DELTA-AMINOTRANSFERASE INTO EMBRYONIC FIBROBLASTS: AN ALTERNATIVE APPROACH TO SOMATIC CELL GENE THERAPY

Luis J. Rivero, Alexander Kozhich, Robert B. Nussenblatt and Moncef Jendoubi*, Laboratory of Immunology, National Eye Institute, National Institutes of Health and Human Services, Bethesda, MD 20892

Ornithine delta-aminotransferase (OAT) is a nuclear-encoded mitochondrial matrix enzyme which catalyzes the reversible transamination of ornithine to glutamate semialdehyde. In man, genetic deficiency of OAT results in gyrate atrophy of the choroid and retina, a blinding chorioretinal degeneration usually beginning in late childhood. This disorder has been shown to be autosomal recessive, and often caused by missense, nonsense and frameshift mutations in the OAT gene. With the view of applying gene therapy, Moloney murine leukemia virus based recombinant retrovirus vectors have been constructed. The human OAT cDNA was placed under the control of the enhancer-promoter regulatory elements derived from the Moloney murine leukemia virus long terminal repeat. The construct was transfected into a safe packaging cell line GP+E-86 to produce provirus particles.

We made ectopic virus OAT producer cell lines and transduced mouse C57B1/6 embryonal fibroblasts and embryonic stem cells. We showed that the produced virus transfers the OAT gene to the recipient cells which produce an immunoreactive OAT. Northern blot analysis confirmed the presence of an OAT transcript in the transfected cell lines even after a long period of time.

E1⁻ E3⁻ REPLICATION DEFICIENT RECOMBINANT ADENOVIRUS VECTOR CONTAINING THE HUMAN CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR (CFTR) cDNA DOES NOT REPLICATE IN HUMAN RESPIRATORY EPITHELIAL CELLS

Melissa Rosenfeld, Chin-Shyan Chu, Prem Seth, H. Ari Jaffe, Tyrone C. Banks and Ronald G. Crystal, NHLBI, Bethesda, MD 20892

Cystic fibrosis (CF), a common genetic disorder caused by mutations of the CFTR gene, is characterized by defective regulation of cAMP-mediated chloride permeability across the apical membrane of epithelial cells. Given that transfer of the normal CFTR cDNA to CF epithelial cells in vitro corrects this defect, the fatal respiratory manifestations of CF should be amenable to gene therapy with the normal CFTR cDNA. In this regard, AdCFTR, a replication deficient recombinant adenovirus (Ad) vector lacking Ela, most of Elb, and most of E3, efficiently transfers the human CFTR cDNA to the respiratory epithelium of cotton rats in vivo. For human gene therapy, a critical safety issue is whether this vector can replicate in respiratory epithelial cells (REC) in vivo as a consequence of Ela or Ela-like proteins endogenous to REC. To evaluate this, REC were obtained from normal individuals using a cytologic brush and evaluated for adenoviral DNA replication and structural protein synthesis. REC were infected (48, 72 hr) with either Ad5 or AdCFTR [10-1000 plaque forming units (pfu)/ cell] and labelled in phosphate free medium containing $^{32}PO_4^{=}$ (20 hr). The cells were lysed and viral DNA extracted, cleaved with EcoRI and analyzed by agarose gel electrophoresis and autoradiography, allowing specific identification of de novo synthesized Ad5 and AdCFTR DNA. Importantly, REC supported the replication of Ad5 DNA but not AdCFTR DNA as a function of time and dose. To further evaluate the potential of AdCFTR to replicate, REC were infected (24 hr) with either Ad5 or AdCFTR (100 pfu/cell) followed by ^{35}S-methionine labelling (12 hr) and cell lysates were analyzed for Ad structural proteins by SDS gel electrophoresis and autoradiography. Ad5 infected REC produced hexon protein while AdCFTR infected REC did not. These data demonstrate that AdCFTR DNA will not replicate or produce hexon in REC, the target cells for gene therapy of the respiratory manifestations of CF, suggesting the concept that AdCFTR can be safely used in human trials.

REPLICATION DEFICIENT ADENOVIRUS-MEDIATED DELIVERY OF UNLINKED PLASMID DNAs TO CELLS: AN EFFICIENT AND SIMPLE METHOD OF DNA TRANSFECTION

Prem Seth, Melisa A. Rosenfeld, James N. Higginbotham and Ronald G. Crystal. NHLBI, Bethesda, MD 20892

As viruses enter cells via specific receptors, they carry along other molecules in a process called "cointernalization". Based on this concept, we recently demonstrated that replication deficient adenoviruses (Ad) can mediate the delivery and expression of unlinked plasmid DNA into target cells (Yoshimura, K. et al ., J Biol Chem, in press). To evaluate the ability of other transfection enhancing processes to enhance Ad-mediated plasmid cointernalization, AdCFTR (an E1⁻ E3⁻ recombinant Ad containing the human cystic fibrosis transmembrane conductance regulator cDNA) and pRSVL (a plasmid containing the Rous Sarcoma Virus long terminal repeat and a luciferase reporter gene) were added to Cos-7 cells in the presence of lipofectin, polybrene or transfectam. Whereas AdCFTR enhanced pRSVL expression 10^3-fold, expression was enhanced further by at least 10-fold (by lipofectin) or 10^2 to 10^3 -fold (by polybrene or transfectam). Using a beta-galactosidase reporter gene, qualitative assessment of the proportions of cells transfected showed that with AdCFTR and either polybrene or transfectam, 90-100% of Cos-7 expressed beta-galactosidase. The same methodologies could efficiently transfect a variety of cell lines including HeLa, CV-1, KB, and several human muscle cell lines (A-673, RD, A204), C_2C_{12} (in the form of unfused muscle cells and fused myotubes). To evaluate the mechanism of the cointernalization process, AdCFTR and pRSVL were added to Cos-7 cells, in the presence of free fiber, an Ad capsid protein responsible for binding of Ad to its receptor. Free fiber effectively blocked the ability of AdCFTR to enhance pRSVL expression as did conditions which block the escape of Ad from the endocytic vesicles to cytosol such as heating AdCFTR (45°,15 min), addition of chloroquine (100 µM) or treating AdCFTR with low concentration of antibody against AdCFTR, supporting the role of receptor-mediated endocytosis (RME) of AdCFTR enhancement of pRSVL expression. Moreover, AdCFTR enhancing activity did not require Ad genome as exposure of AdCFTR to UV (2 min) completely destroyed the ability of AdCFTR to grow in 293 cells, whereas the enhancing activity of AdCFTR was reduced by less than 20%. Consistent with this, empty capsids of AdCFTR devoid of genomic DNA also effectively increased the delivery of pRSVL plasmid DNA to cells. Together, these observations demonstrate that the ability of AdCFTR to enter cells by RME can be exploited alone, or in combination with other transfecting agents, to deliver unlinked plasmid DNA to a variety of cell types.

MOLECULAR ANALYSIS OF SKIN CELL LINEAGE IN TRANSGENIC MICE, Douglas J.C. Strathdee and Allan Balmain, Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow, Scotland, G72 8PU.

Cells are constantly shed from the surface of the epidermis as it performs its function, protecting the organism from the environment. These cells must be replaced by division of the underlying epithelial cells. It seems likely that the replacement of cells occurs via a proliferative hierarchy populated by the division of stem cells. The exact nature of this hierarchy and the location of stem cells in skin is yet to be determined. Existing evidence implicates both the basal layer of the epidermis and the outer root sheath of the hair follicle as possible locations for the stem cells. In order to analyse cell repopulation in the epidermis a cell lineage marker, based on the *E. coli lac Z* gene, has been designed for lineage analysis of single cells in transgenic mice. The marker system has been tested *in vitro* where it is able to give distinct colonies of expressing cells. Transgenic mice have been generated containing a number of test and control constructs, and work is currently underway testing the strategy in the mice.

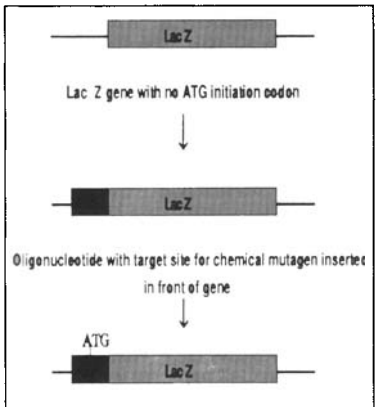

Figure 1. Diagram of the lac Z fusion gene strategy. The construct is generated by cloning specific oligo sequences into a lac Z fusion vector with no ATG initiation codon.

GENE TRANSFER IN REGENERATING MUSCLE

Maurizio Vitadello, Andrea Novelli, Anne Picard, Maria Vittoria Schiaffino*, Maurizio Scarpa*, Stefano Schiaffino, CNR Unit for Muscle Biology and Physiopathology, Department of Biomedical Sciences, *CRIBI and *Department of Pediatrics, University of Padova, Via Trieste 75, Padova, Italy

We have analyzed the level and duration of expression of genes injected in normal and regenerating rat muscles. We used three constructs, containing the chloroamphenicol acetyl transferase (CAT), ß-galactosidase (ß-gal) and cardiac troponin I (cTnI) genes under the control of the HSV, RSV and CMV viral promoters, respectively. Plasmid DNA was injected in the soleus muscle of adult rats. Muscle regeneration was induced by a myotoxic drug under conditions which cause the almost complete destruction of preexisting fibers and their substitution with regenerating fibers. The efficiency of gene transfer was evaluated 7 days after DNA injection in normal muscles and in muscles pretreated with drug at 1, 3 and 7 days before DNA injection. Muscles injured 3 days before DNA injection showed numerous fibers reactive for ß-gal or cTnI and much higher (>100 fold) CAT activity compared to uninjured control muscles. In contrast, only a small increase in CAT activity was found in muscles injected 7 days after the lesion and no significant increase in muscles injected 1 day after the lesion. Transgene expression persisted in regenerated muscles at significantly higher levels than in control muscles, however CAT activity decreased about 5-fold after 1 month and about 15-fold after 2 months.

APPROACHES TO DIABETES GENE THERAPY USING INSULIN GENE DRIVEN BY P-ENOLPYRUVATE CARBOXIKINASE PROMOTER, Alfons Valera, Cristina Fillat, Cristina Costa, Joana Visa, Jordi Sabater, Anna Pujol, Joan Enric Rodriguez-Gil and Fatima Bosch, Department of Biochemistry and Molecular Biology, Autonomous University of Barcelona, 08193-Bellaterra, Barcelona, Spain

Future protocols for the insulin-dependent diabetes mellitus gene therapy have to be centered in the introduction of the insulin gene i) in a tissue different from pancreas, and ii) under the control of a regulated promoter to achieve blood physiological levels of the hormone. The expression of the P-enolpyruvate carboxykinase (PEPCK) gene is increased in diabetic animals as the result of the raise in glucagon and the decrease in insulin blood levels. The PEPCK promoter contains regulatory elements responsive positively to cAMP and glucocorticoids, and negatively to insulin. A PEPCK/insulin chimeric gene has been obtained by linking the PEPCK gene promoter to the human insulin gene. This chimeric gene could be regulated in a physiological manner when introduced into an animal. To test this hypothesis, we have obtained transgenic animals containing the PEPCK/insulin chimeric gene. These mice express insulin in the tissues where endogenous PEPCK is mainly expressed (liver, kidney, adipose tissue and jejunum), and the animals are healthy and normoglycemic. Transgenic animals did not show the metabolic effects of the diabetic process after intravenous streptozotocin injection, when compared with diabetic control mice. This indicates that the PEPCK/insulin chimeric gene could be a good candidate to be used in diabetes gene therapy protocols. In this regard, the chimeric gene has been introduced in a retroviral vector and different cell lines (mioblasts, hepatocytes, etc) have already been infected. Studies on trasplantation of these cells to diabetic animals will be presented.

Supported by a grant from the FISss 90/0302.