S03-17

CD69 EXPRESSION BY MURINE MACROPHAGES AND NITRIC OXIDE PRODUCTION UPON CD69 STIMULATION.
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Nitric oxide (NO), produced by activated murine macrophages (mø), plays an important role in the regulation of immune function. CD69 is an early activation antigen of lymphocytes and and serves as a signal transducing molecule in many cell types. Induction of NO production by human monocytes after CD69 crosslinking was recently reported. We examined CD69 expression and function in murine mø. CD69 staining, evaluated by flow cytometry and immunohistochemistry, was negative in resting mø, but was induced by overnight LPS stimulation. CD69 upregulation was unaffected by an inhibitor of NO production or infection with *Leishmania* and was blocked by cycloheximide. Stimulation of gamma-interferon primed mø with anti-CD69 mAb in the presence of polymyxin B induced the release of high amounts of nitrite. Mø triggering was inhibited by EGTA, as shown for other CD69-dependent functions. Taken together, these results suggest that CD69 may serve as signaling receptor in murine mø.

Somatic Gene Therapy

S04-02

CYTOKINES IN SOMATIC CELL AND GENE THERAPY: PERSPECTIVES IN ONCOLOGY
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The discovery and clinical evaluation of cytokines is having a rapidly increasing impact on cancer therapy by providing unprecedented opportunities for therapeutic modulation of hematopoiesis and the immune system. To facilitate chemotherapy dose intensification, colony stimulating factors (CSFs) together with peripheral blood progenitor cells (PBPCs) have been shown to shorten the period of profound pancytopenia following high-dose chemotherapy.
The second area of cancer therapy moving rapidly ahead following the discovery of cytokines and cytokine-mediated effector mechanisms is cancer immunotherapy. Given the fact that induction of anti-tumor immunity by cytokine gene transfer into tumor or bystander cells has been demonstrated by different groups in mouse tumor models, we have initiated a phase I clinical protocol for patients with refractory neoplasias. Hematopoietic and immunostimulating cytokines are beginning to have an impact on cancer treatment outcome. Not only cytokines themselves, but also cells mobilized by and grown ex vivo in the presence of cytokines as well as cell transfected with therapeutic genes of interest are being developed as novel therapeutic modalities.

S04-04

GENE THERAPY FOR NEUROLOGICAL DISEASES: FIRST CLINICAL RESULTS IN AMYOTROPHIC LATERAL SCLEROSIS

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Neuronal growth factors hold promise for providing therapeutic benefits in various neurological disorders. As a means of ensuring adequate CNS delivery of growth factors and minimizing significant adverse side effects associated with systemic delivery methods, we have developed an *ex-vivo* gene therapy approach to protein delivery using encapsulated genetically modified xenogeneic cells. One neurotrophic factor in particular, ciliary neurotrophic factor (CNTF), has been shown in various rodent models to reduce the motor neuron cell death similar to that seen in amyotrophic lateral sclerosis (ALS). The initial clinical trial focusing on the systemic administration of CNTF resulted in severe side-effects mandating the interruption of the trials, therefore preventing determination of the efficacy of the molecule. In order to deliver CNTF directly to the nervous system, we conducted a phase I study in which six patients with amyotrophic lateral sclerosis (ALS) were implanted with capsules containing genetically-engineered baby hamster kidney (BHK) cells releasing around 1.0 µg of CNTF per day *in vitro*. The CNTF-releasing implants were surgically placed within the lumbar intrathecal space. Serial CSF sampling showed CNTF ranging from 170 to 6,282 pg/ml for at least 17 weeks post-implantation whereas no CNTF was detected prior to implantation. On explant, all devices showed good BHK cell viability and CNTF output when measured *in vitro*. The patients showed no weight loss, severe coughing or response of acute phase reactants over the course of implantation which were the limiting side effects observed with systemic CNTF administration. These results demonstrate that measurable levels of human CNTF can be continuously delivered within the CSF of humans by an *ex vivo* gene therapy approach opening new avenues for the treatment of neurological diseases.

S04-05

AN OPTIMIZED PROTOCOL FOR ADENOVIRUS-MEDIATED GENE TRANSFER: EXPRESSION OF THE CHICKEN NEURONAL CELL ADHESION MOLECULE NgCAM IN RODENT NEURONS

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Neuronal cell adhesion molecules of neurites and growth cones play an important role for neurite outgrowth and pathfinding in the development of the nervous system. The ability to manipulate the expression of these glycoproteins in neurons would provide a powerful tool to study their function in vitro and in vivo. Here we report the ectopic expression of chicken NgCAM in rodent neurons by adenovirus-mediated gene transfer. We constructed the replication-defective recombinant adenovirus Ad.CMVNgCAM containing the full-length cDNA of chicken neuronal glial cell adhesion molecule NgCAM under the transcriptional control of the cytomegalovirus (CMV) promoter in the early region 1 (E1) of the adenoviral genome. The transcription unit of the Ad.CMVNgCAM virus consists of more than 5 kb and, thus, is at the reported upper limit of the cloning capacity for E1-deleted recombinant adenoviruses. The size of the adenoviral genome of Ad.CMVNgCAM was 106% of the size of wild-type adenoviral genome. An optimized protocol for the construction of a replication-defective recombinant adenovirus is presented and the ectopically expressed NgCAM is structurally and functionally characterized.

S04-06

RETROVIRUS-MEDIATED GENE TRANSFER OF 6-PYRUVOYL-TETRAHYDROPTERIN SYNTHASE CORRECTS H_4-BIOPTERIN DEFICIENCY IN FIBROBLASTS FROM HYPERPHENYLALANINEMIC PATIENTS

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H_4-biopterin (BH_4) deficiency is primarily caused by mutations in the gene encoding the 6-pyruvoyl-tetrahydropterin synthase (PTPS), and is associated with hyperphenylalaniemia and lack of dopamine and serotonin. We have previously identified and characterized several mutations in patients with PTPS deficiency. This study reports the *in vitro* correction of BH_4 deficiency by using retro-viral-mediated transfer of the PTPS cDNA into primary fibroblast cultures from patients. Following PTPS-cDNA transfer, BH_4 biosynthesis could be restored in originally defective cells, providing a direct proof that the mutations in PTPS were causative for the mutant phenotype. Moreover, sustained complementation of the metabolic defect was observed over a period of several months. This is the first step towards gene therapy as a potential approach to treat BH_4 deficiency.

S04-07

CNS DELIVERY OF CNTF BY POLYMER ENCAPSULATED GENETICALLY ENGINEERED C_2C_{12} CELLS

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Demonstration of the neuroprotective effects of trophic factors in the central nervous system and in various animal models of neurodegeneration has lead to the development of strategies for the treatment of motoneuron diseases. Using the immunoisolation technology into pmn/pmn mice, an animal model displaying a neuronopathy resembling ALS, we were able to show that transplantation of polymer capsules containing ciliary neurotrophic factor (CNTF) secreting BHK (Baby hamster kidney) cells delay the progression of the disease. However, BHK cells divide until they fill the capsule and an accumulation of debris is observed after several months. In order to overcome this problem, we have tested the mouse C_2C_{12} cell line which can be differentiated into a post-mitotic state upon exposure to low serum containing medium. The mouse C_2C_{12} myoblast cells have the advantage of rapidly dividing cells, they can be grown in large quantity in vitro, transfected to express proteins and selected clones can be isolated.
Mouse C_2C_{12} myoblasts have been transfected with the pNUT expression vector containing the human CNTF gene. The level of expression of the hCNTF gene and the bioactivity of the factor were analyzed by Northern blot, Elisa assay, and ChAT activity on embryonic spinal cord motoneuron cultures. One C_2C_{12} clone has been found to secrete approximately 0.2 µg CNTF/10^6 cells/day. The rate of secretion of hCNTF is not altered upon differentiation of C_2C_{12} myoblasts. Furthermore C_2C_{12}-hCNTF can rescue neonatal facial motoneurons from axotomy-induced cell death. Long-term intrathecal implantation of encapsulated hCNTF-C_2C_{12} cells or control C_2C_{12} cells into rats confirmed the excellent viability of these mouse myoblasts and the potential use as a source of neurotrophic factors for the teatment of neurodegenerative diseases.

S04-08

A HELPER VIRUS-FREE PACKAGING SYSTEM FOR HSV-1 PLASMID VECTORS

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Vectors based on herpes simplex virus type 1 (HSV-1) are attractive for genetic intervention in the brain because HSV-1 can efficiently infect neural cells and can persist indefinitely in neurons. HSV-1 plasmid vectors (or amplicons) contain only ~1% of the 150 kb HSV-1 genome and have been packaged into HSV-1 particles using a helper virus. Although these vectors have been used to alter neuronal physiology, their effectiveness has been limited by several problems: (i) acute cytopathic effects and an immune response, largely due to gene expression from the helper virus, (ii) potential interactions between helper virus and endogenous viruses, (iii) instability of gene expression, (iv) potential helper virus-mediated oncogenesis, and (v) reversion of the helper virus to wt HSV-1. To essentially eliminate many of these problems we developed a helper virus-free packaging system. The DNA cleavage/packaging signals were deleted from a set of cosmids that represents the HSV-1 genome. Cotransfection of cells with this modified cosmid set and vector DNA resulted in the packaging of vectors into HSV-1 particles; however, no wt HSV-1 was produced. Vectors which contained the *Escherichia coli lacZ* reporter gene efficiently transduced neural cells with minimal cytopathic effects. Following injection into the rat brain, ß-galactosidase positive cells were observed for at least 1 month, and vector DNA persisted for this period.

S04-09

TRANSFECTION OF KERATINOCYTES FROM LAMELLAR ICHTHYOSIS PATIENTS WITH TRANSGLUTAMINASE K (TGK) cDNA CONSTRUCTS.

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Mutations in the keratinocyte transglutaminase gene are causing lamellar ichthyosis, a disfigurating skin disease. As a first step towards gene therapy, cultured TGK-deficient keratinocytes were cotransfected with TGK and ß-galactosidase cDNA constructs driven either by the CMV promoter (CMVP) or the involucrin promoter (INVP). TGK activity was increased by 6-fold (CMVP) respectively 2-fold (INVP) in the membrane fractions. Cytosolic ß-gal activity was increased 30-fold (CMVP) respectively 5-fold (INVP). The higher increases of the ß-gal activity suggests low transcription-translation efficiency of the TGK gene or instability of the TGK protein. The level of TGK activity in transfected cells was only about 4 % of the one in unaffected individuals, indicating the need for stronger promoters, TGK constructs with increased stability and/or better delivery systems.

S04-10

A MOUSE MODEL FOR THE LIDDLE'S SYNDROME

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Liddle's syndrome is a heritable form of salt-sensitive hypertension. Recent studies demonstrated that a mutation in the β subunit of the epithelial Na^+ channel causes this disorder. This mutation (a premature stop codon) results in a truncation of the cytoplasmic C-terminus of the β subunit, thereby generating a hyperactive channel.
We are generating a mouse model for the Liddle's syndrome by a gene targeting strategy, including the positive/negative selection in a first step and the CRE/lox-mediated gene replacement system in a second step. We have now identified several targeted ES cell clones which will be used for the second selection step by transient CRE-recombinase expression.
In the future, this animal model should help us to understand the physiopathology of this disease and to design rational therapies.

S04-11

DESTRUCTION OF THE TUMOR VASCULATURE AS A THERAPY AGAINST SOLID TUMORS

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The growth of primary solid tumors and metastases depends strictly on the rapid ingrowth of newly formed blood vessels. Tumor endothelial cells are easily accessible for therapeutics and the destruction of a few vessels might result in the elimination of a broad tumor area. Therefore, inhibition of angiogenesis and the selective destruction of the tumor vasculature is an attractive strategy for future tumor therapies.
Using a magnetic cell sorter, endothelial cells were isolated from experimentally induced tumors or from normal mouse tissue such as lung and liver. Purified endothelial cells are used to create monoclonal antibodies specific for the tumor endothelium. Different approaches such as phage display library screening are also under evaluation. To establish anti-angiogenic tumor therapies in immuno-competent mouse models we investigate previously described tumor endothelial surface markers such as receptor type tyrosine kinases (e.g. Flk-1). Ligand molecules recognizing such endothelial markers are used to generate cytotoxic T lymphocytes (CTL) which express a chimeric T cell receptor subunit, consisting of the intracellular signalling domain of the ζ chain and an extracellular ligand domain. The CTLs are tested for recognition and lysis of the tumor endothelium.

S04-12

Ectopic Adenoviral Vector Directed Expression of Semaphorin III/Collapsin-1 in Neuronal Explants Repels Growing Axons of Primary Sensory Neurons.

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Semaphorin III/collapsin-1 (sema/coll), the first cloned chemorepellent, is a 100 kDa glycoprotein which has been shown to selectively induce growth cone collapse and paralysis of NGF-responsive dorsal root ganglion neurons in vitro. The expression pattern of sema/coll mRNA in the spinal cord suggests a role of this chemorepellent in patterning ingrowing axons of primary sensory neurons. By homologous recombination we constructed a first generation adenovirus based vector AdCMVcol, containing an expression cassette of rat sema/coll in the E1-region of the viral genome. The functionality of this viral vector was confirmed by Northern blot analysis and in situ hybridization of AdCMVcol infected Vero cells. To test the biological activity of virus vector derived sema/coll we infected dorsal root ganglia explants of E15 rat embryos with AdCMVcol and compared the axonal growth pattern to uninfected and AdlacZ infected cultures. In control cultures neurites did grow in a regular radial orientation and formed only small fascicles. In contrast, in AdCMVcol infected cultures, axonal growth was slower and the regular radial pattern was clearly disturbed. Neurites exhibited a tendency to bend away from non-neuronal cells and formed fascicles probably in attempt to avoid sema/coll expressing cells. Defasciculation occurs apparently when axon bundles have passed sema/coll expressing cells. These studies show that recombinant adenoviral vectors are powerful tools to manipulate gene expression in primary cells in neural explants and may be applied in vivo thus, representing an attractive alternative to transgenic mice.