

gene correction at chromosomal sites in mammalian cells. We have also extended this work to demonstrate that TFOs administered systemically to mice via intraperitoneal injection can mediate gene targeting within the somatic tissues of the animals. Overall, this work raises the possibility that DNA binding ligands such as TFOs and related molecules may be useful in strategies designed to mediate genome modification and gene correction in site-specific manner.

SS404: VIRAL: VECTOR CELL INTERACTIONS

Adenovirus Cell Entry Mechanisms

Glen R. Nemerow, PhD

The mechanisms by which human adenovirus crosses the barrier of the host cell membrane remains unresolved despite the fact that Ad vectors are well known for their ability to accomplish this feat. In the studies to be presented here, we determined the relationship between Ad capsid disassembly and the generation of membrane lytic activity. Exposure to low pH or heating induced conformational changes in wild-type Ad but not in temperature-sensitive Ad (*ts1*) particles that lack the ability to escape the endosome. Wild-type Ad but not *ts1* particles permeabilized model membranes (liposomes) and facilitated the cytosolic delivery of a ribotoxin (a-sarcin). Alterations in wild-type Ad capsids were associated with the exposure of a pH-independent membrane lytic factor. Unexpectedly, this factor was identified as protein VI, a 22 kDa cement protein located beneath the peripentonal hexons in the viral capsid. Recombinant protein VI and preprotein VI, but not a deletion mutant lacking an N-terminal amphipathic α -helix, possessed membrane lytic activity similar to partially disassembled virions. These findings have prompted us to consider a revised model of Ad entry in which acidification of the endosome initiates viral capsid disassembly and subsequent exposure of a highly membranolytic capsid protein. Further biochemical and functional analyses are underway to test this model of Ad entry.

Targeting AAV Vectors

Nicholas Muzyczka, PhD

Our laboratory has focused on two approaches for changing the tropism of AAV vectors. The first is to use mutational analysis, cryo-electron microscopy and X-Ray crystallography to determine the structure and function of different regions of the AAV capsid. The second is to find regions of the AAV capsid that will accommodate the insertion of large peptide ligands. Progress in both of these areas will be discussed.

SS500: YOUNG INVESTIGATORS SYMPOSIUM

Gene Transfer into Peripheral Blood T Lymphocytes: Clinical Benefits and Safety Profile

Chiara Bonini, MD

Since the first gene therapy trials documented, in the early 90', that human peripheral blood lymphocytes can be efficiently transduced by retroviral vectors and infused to treat patients affected by severe combined immunodeficiencies, several gene transfer approaches aimed at correcting genetic defects or augmenting immune responses to microbes and tumors, were translated into clinical trials. As the field progressed, several insights on T cell biology were revealed and translated into new transduction protocols, with the purpose of increasing the overall therapeutic index of transduced T-cells. A general observation is that the transduction procedure associated to viral vectors invariably produces modifications of T-cells in terms of repertoire, maturation, cytokine secretion profile, effector functions and life-span. In an attempt of controlling transduced lymphocytes our group pioneered the retroviral mediated transfer of two genes: 1. The low affinity receptor for nerve growth factor, truncated of the intracytoplasmic domain ("LNGFR). "LNGFR is a non-immunogenic human surface marker that allows to avoid toxic and time-consuming drug selection to isolate transduced cells, resulting in preservation of T-cell function. 2. The thymidine kinase (TK) gene from Herpes Simplex virus. TK is the prototype of suicide genes, genes able to confer an inducible suicide phenotype to permit the *in vivo* selective elimination of transduced cells in case of unwanted effects. TK expression confers selective ganciclovir sensitivity to transduced cells. "LNGFR has been the first cell surface marker utilized in a gene therapy clinical trial. In a recent multicentric study, the safety of the "LNGFR cell marking molecule was supported by cumulative results on >900 animals transplanted with "LNGFR⁺ hematolymphopoietic cells.

T-cells transduced to express "LNGFR and TK (TK-cells) have been extensively used in the context of allogeneic hematopoietic stem cell transplantation (HSCT) performed to treat hematologic malignancies. In this context, TK-cells are utilized to promote immune-reconstitution, mediate anti-tumor activity, and selectively control graft-versus-host disease (GvHD). In 45 patients treated with donor TK-cells in the context of HSCT, we showed selective control of GvHD in 100% of cases, preservation of antiviral activity (in all patients with sustained TK-cell engraftment) and anti-tumor activity (in 65% of patients). The direct role of TK cells in mediating clinical events was documented by consistent expansion and long-term persistence of transduced cells,



that proved widely immunocompetent in terms of repertoire, phenotype, cytokine secretion profile and function. The substantial clinical benefit associated with the “TK-technology” was obtained in the absence of acute or chronic adverse or toxic effects due to the gene transfer procedure. Vector integration sites were analyzed by LM-PCR on DNA obtained from patients up to 10 years after treatment. Over 85% of proviral integrations occurred within transcription units, mostly in active genes, with a preference for first introns (27%) and regions upstream of transcription start sites (24%). Analysis of gene expression profiles showed that <1% of the 16,000 analysed genes were differentially expressed in “LNGFR⁺ vs. “LNGFR⁻ T-cells ex vivo, suggesting the substantial biological identity of transduced and untransduced cells. Administration of NGF to “LNGFR⁺ cells in culture caused no significant variation in proliferation, cytokine secretion pattern, expression of activation markers, nor in gene expression profile.

Overall, these results demonstrate the feasibility, safety, and efficacy of infusions of T-lymphocytes transduced to express TK and “LNGFR, substantially challenge the hypothesis that “LNGFR expression play a role in the leukemic transformation observed in a single murine model upon retroviral integration in the Evi1 oncogene, and show the relevance of appropriate clinical trials in which the risk/benefit ratios are carefully evaluated. A series of clinical protocols have been approved by regulatory authorities in Europe, the US, Israel, and Japan.

Development of Human Gene Therapy for Neurodegenerative Disorders

Michael G. Kaplitt, MD, PhD

Since our initial demonstration in 1994 that adeno-associated virus (AAV) can be a safe and effective vehicle for long-term gene transfer in the brain, we have focused upon developing this technology for practical use in the human clinical setting. Initially we used gene therapy to increase dopamine production in animal models of Parkinson’s disease (PD). While this was effective, the known physiology of this system and clinical experience with late-stage human PD patients suggested an alternative strategy. The loss of dopamine in PD leads to an alteration in the activity of a brain circuit which controls movement. One key malfunction is a reduction in inhibitory GABA transmission and resultant hyperactivity of the subthalamic nucleus (STN). Traditional surgery for drug-resistant PD is designed to reduce STN firing. To develop a gene therapy based upon what is already effective in human PD patients, we used AAV to deliver the gene for glutamic acid decarboxylase (GAD) into the STN. GAD is the rate-limiting step in the synthesis of GABA, so this was designed to restore inhibitory GABA transmission to key brain structures

which are abnormally hyperactive in PD. Having demonstrated the safety and efficacy of this approach in animal models of PD, we received approval to test this in human PD patients. This was the first approved human trial of direct *in vivo* gene therapy for an adult neurodegenerative disorder. This open label phase I study is designed primarily to test safety in cohorts of 4 patients at each of three escalating vector doses. To date we have treated 11 of 12 patients, with follow-up ranging from 6 weeks to 20 months, with no evidence of treatment-related adverse events. As this trial proceeds, we are also pursuing a strategy to block and possibly reverse the cellular death or dysfunction which occurs in neurodegenerative disorders. We have found that AAV expressing the human X-linked inhibitor of apoptosis (XIAP) gene could completely block cell death in a newly described rodent model of PD, believed to more accurately recapitulate features of the human disease. We are also applying this to Huntington’s disease (HD) models, and have recently observed that this vector can not only improve the life expectancy of these animals, but can also completely reverse motor deficits in one standard assay. This suggests that AAV-XIAP may not only be neuroprotective, but may actually reverse neuronal dysfunction at least in this HD model. With an increasing number of brain gene therapy protocols entering human trials, these studies will hopefully facilitate the introduction of a variety of novel gene therapeutics into the clinical setting.