environment in the pancreas at diabetes onset, a higher interferonγ:interleukin-4 (IL4) concentration ratio is evident in the pancreata of diabetic female NOD mice in comparison to non-diabetic NOD mice. Adminstration of recombinant cytokines has previously been demonstrated to prevent disease onset in the nonobese diabetic (NOD) mouse model of T1D.

Here we investigate the utility of biolistic-mediated gene transfer as a non-invasive therapy to prevent disease onset through expression the murine IL4 cDNA. Following epidermal delivery of only 2μg of DNA, it was possible to produce transient levels of mIL4 in the serum using a conventional cDNA expression vector. However, a cDNA expression vector stabilized by the incorporation of the EBNA1/*oriP* episomal maintenance replicon of the Epstein-Barr virus was able to provide for the production of higher levels of serum mIL4 which also persisted for 12 days following gene transfer. Although three biolistic inoculations were sufficient to reduce insulitis and prevent disease onset for both expression vectors, the protracted mIL4 expression profile afforded by the EBNA1/*oriP*-based vector was significantly more effective and resulted in a shift to Th2-responses in the periphery and pancreas. We conclude that our approach of using biolistic gene delivery in combination with stabilized cytokine gene expression is a clinically viable therapeutic approach to prevent the onset of type I diabetes.

816. Expression Level-dependent Human Proinsulin Processing by Furin in Primary Hepatocytes

Peter Thule'*†, Jing Ming Liu*
*Atlanta VA Medical Center
†Emory University School of Medicine

The success of insulin gene therapy requires that metabolic need regulate the magnitude of insulin action. By utilizing a glucose and insulin responsive promoter to drive an insulin expression sequence, we have established an in vivo model of metabolically responsive hepatic insulin production. In this model insulin production is sufficiently robust to dramatically reduce hyperglycemia, and sufficiently responsive to avoid lethal hypoglycemia. Insulin secretion increases in response to glucose loading, and declines upon glucose withdrawal. However, occasional glucose levels of <50mg/dl indicate temporal disregulation of insulin action. Incomplete proinsulin processing is a potential cause of hypoglycemia in our system, since it would produce insulin-intermediates that exert glucose-lowering effects, and possess prolonged serum half-lives. Our insulin expression sequence (2xfur) is designed to permit furin dependent proinsulin processing in hepatocytes. Others, using similar furin dependent constructs, report proinsulin-processing efficiencies of 70-100% in non-B-cells, including hepatocytes. However, prior studies have utilized constitutive promoters, and have not examined the effect of variable proinsulin expression on processing efficiencies. In contrast to published studies, we show that proinsulin processing in hepatocytes is indeed less efficient at high levels of proinsulin expression. Immunoprecipitates of [^{32}S]-radiolabeled insulin from medium conditioned by hepatocytes transfected with pCMV 2xfur, a construct encoding a furin-susceptible transgenic insulin product, were subjected to SDS-PAGE under highly denaturing conditions. Densitometric analysis of autoradiographs obtained from multiple experiments revealed that the proportion of insulin relative to proinsulin decreased as the sum of insulin and proinsulin increased, suggesting that the processing capacity of the hepatocytes was exceeded. To test this hypothesis, we utilized adenovirus/polyethylenimine/DNA particles to co-transfect hepatocytes with an adenovirus containing a glucose-regulated insulin transgene, and either pCMVBgal, or pCMVfurin. Thus, exposing transfected cells to high glucose maximized proinsulin expression, with, or without furin overexpression. Measurement of mature insulin in conditioned media with a double-monoclonal antibody ELISA indicated that cells co-transfected with pCMVfurin secrete greater amounts of mature insulin than cells co-transfected with pCMVBgal.

Thus, furin-mediated proinsulin processing efficiency in hepatocytes may be saturable, and vary with the level of proinsulin expression. This suggests that co-transfection of furin expression sequences with 2xfur constructs in vivo may improve proinsulin processing efficiencies, particularly when proinsulin production is high. Future studies will confirm whether over-expression of furin reduces the frequency of hypoglycemia in our model of insulin gene therapy.

817. Regulated Insulin Gene Delivery for Treatment of Type I Diabetes

A Auricchio*, GP Gao*, P Zoltick*, A Shifrin†, S Raper†, V Rivera‡, JM Wilson*
*Institute for Human Gene Therapy, The Wistar Institute, Depts. of Cellular and Molecular Engineering and
†Dept. of Surgery, University of Pennsylvania, Philadelphia, PA
‡Ariad Pharmaceuticals, Cambridge, MA

Ectopic and regulated insulin production are two major and achievable goals of gene therapy for treatment of type I diabetes. For this purpose we produced adeno- associated (AAV) and adenoviral vectors encoding the human and mouse proinsulin as well as the two b cell-specific proteases (PC2 and PC3) required for the efficient conversion to mature insulin. In addition, we genetically engineered the pro-insulin sequence to achieve cleavage by the ubiquitous protease furin. Using Western blot, RIA/Elisa, NH2-sequence and a functional assay based on the insulin receptor phosphorylation, we show that both the furin-modified and the PC2/PC3-processed insulin produced by our vectors in cell culture are mature and active.Adenoviral transduction of liver, skeletal muscle and pancreas in a chemically-induced (streptozotocin) mouse model of type I diabetes mellitus results in rapid correction of the hyperglycemia with detection of the vector-encoded insulin in the target tissue and in the serum. The most robust systemic release of insulin follows liver transduction by the adenoviral vector encoding the furin-modified pro-hormone. To regulate insulin expression we are using transcriptional activators pharmacologically induced upon administration of small, non-toxic molecules such as rapamycin. We introduced the components of these systems as well as the various proinsulin sequences into our DNA vectors and are currently characterizing the kinetics of the regulated insulin expression in cell culture and in the diabetic mice. Since the adenoviral-mediated gene expression is transient due to a vigorous immune response towards the viral proteins, we are currently examining features to enhance the potential of AAV as a vector for insulin gene delivery to liver, muscle and pancreas.

818. Gene Delivery to the Liver *In Vivo* Using rSV40 Vectors

David Strayer*†, Mark A. Zern‡§, J. Roy Chowdhury‡¶
*Dept. of Pathology
†Thomas Jefferson University
‡Dept. of Medicine
§University of California, Davis
¶Albert Einstein College of Medicine

A wide variety of genetic and acquired diseases have been considered to be reasonable candidates for therapeutic manipulation by gene delivery to the liver. However, this goal has been elusive: limitations in available vector systems and delivery techniques have constrained progress in this direction. Recombinant SV40 vectors (rSV40) are derived from T antigen-deleted SV40 genomes, and packaged by COS-7 cells, to yield very high concentrations of infectious gene delivery vehicles (up to 10(12) infectious units (IU)/ml). We have applied recombinant SV40-derived (rSV40) vectors to deliver potentially therapeutic transgenes to rodent models of two different genetic diseases of the liver: deficiency of bilirubin UDP-glucuronosyl transferase (BUGT, as in Crigler-Najjar Syndrome); and deficiency of alpha-1-antitrypsin (a1AT). In both cases, rSV40s carrying the therapeutic transgenes were delivered between 1 and 7 times via a catheter in the