SCIENCE AND ENGINEERING OF IMMUNOPROTECTED CELL TRANSPLANTS

Organizers: Michael Lysaght, Patrick Aebischer and Thomas Flanagan

April 2-8, 1995; Frisco, Colorado

Plenary Sessions *Page*

Poster Sessions

Science and Engineering of Immunoprotected Cell Transplants

Keynote Address

D1-001 ENCAPSULATION OF CNTF-RELEASING CELLS FOR THE TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS, P.Aebischer[1], S.A. Tan[1], N. Déglon[1], B. Heyd[1], A. Zurn[1], E Baetge[2], Y. Sagot[3], A Kato[3]. [1]Gene Therapy Center and Surgical Research Division, Lausanne University Medical School, Switzerland; [2]CytoTherapeutics, Inc., providence, RI, USA; [3]Division of Clinical Neurophysiology, Geneva University Medical School, Geneva, Switzerland.

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal disease characterized by the degeneration of motoneurons. Several neurotrophic factors such as ciliary neurotrophic factor (CNTF) or the neurotrophins BDNF, NT-3 and NT-4/5 have been shown to prevent the death of embryonic motoneurons in vitro and after peripheral nerve axotomy in early postnatal rats. These factors are therefore potential candidates for human application. The short half-life of CNTF and the need for local expression demand appropriate delivery techniques such as the transplantation of polymer-encapsulated cells genetically engineered to produce CNTF. Baby hamster kidney cells (BHK) cells were transfected with an expression vector containing the human CNTF gene. The CNTF released from the cells was around 1 μg/ 10^6 cells/day as determined by an Elisa assay. Both the in vitro and in vivo bioactivities of encapsulated CNTF secreting cells were analysed. CNTF-BHK cells or the parent BHK cells were loaded in microporous membranes. The in vitro bioactivity of the capsule was evidenced by an increase in ChAT activity in rat embryonic spinal motoneurons exposed to CNTF-releasing capsules. The in vivo bioactivity of the system was tested in the following two rodent models. i) The subcutaneous transplantation of CNTF releasing capsules diminished the motoneuron death occuring after facial nerve axotomy in P4 rats as compared to the BHK control capsules; ii) pmn/pmn mice, a mutant mouse model of motoneuron degeneration, implanted with the CNTF capsules, showed a significantly longer survival time and improved motor functional activity as compared to mice that received capsules loaded with the native BHK cells. The number of facial nucleus motoneurons was also significantly higher in the CNTF-treated pmn/pmn mice.

In order to proceed with a clinical trial, toxicity of CNTF was tested in rat and sheep transplanted intrathecally with encapsulated CNTF-releasing cells. No side effects such as fever or weight loss were noticed. These results suggest that immunoisolated, genetically engineered BHK cells can secrete CNTF into the CSF and that this gene therapy approach may be applied for the treatment of ALS.

Unencapsulated Cell Therapy

D1-002 CURRENT STATUS OF EXPERIMENTAL HEPATOCYTES TRANSPLANTATION, Jacek Rozga, Albert D. Moscioni, Susumu Eguchi, Achilles A. Demetriou, Department of Surgery and the Liver Support Unit, Cedars-Sinai Medical Center, 8700 Beverly Blvd., North Tower, Room 8215, Los Angeles, CA 90048

Hepatocyte transplantation is an exciting tool which has been used to treat animals with genetic liver defects and liver insufficiency. Although there is evidence that this method results in significant beneficial effects, indefinite transplanted hepatocyte survival and function at an ectopic site has not been achieved. Attempts to immuno-isolate intraperitoneally transplanted hepatocytes by using microencapsulation and other techniques, have not generally been successful. Preliminary experiments in which patients were injected with hepatocytes into the spleen, have not provided convincing evidence of transplanted cell function *in vivo*. The emerging field of gene therapy has created renewed interest in hepatocyte transplantation and investigators are actively involved in the development and refinement of cell delivery techniques. Based on current experimental evidence, it appears that the recipient liver is the optimal site for hepatocyte transplantation. It also appears that a proliferative stimulus is needed for optimal transplanted hepatocyte engraftment and proliferation. We believe that hepatocyte transplantation will be primarily useful in the treatment of genetic liver function defects, where a relatively small number of surviving functional cells could correct a specific defect. Unless cell delivery techniques are dramatically improved, it is unlikely that this technique will be useful in the treatment of acute severe liver failure where significant amounts of liver mass replacement is needed to support a failing liver.

D1-003 CHROMAFFIN CELL ALLO- AND XENOGRAFTING FOR CHRONIC PAIN, Jacqueline Sagen, Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, IL.

The transplantation of cells or tissue into the CNS is a means of delivering sustained levels of naturally-derived therapeutic agents for the alleviation of chronic disorders on a long-term or permanent basis. Our laboratory has employed this approach in the treatment of chronic pain using chromaffin cells derived from the adrenal medulla, which produce a combination of potentially useful therapeutic substances including opioid peptides, catecholamines, neurotrophic factors, and other neuropeptides. Using rodent chronic pain models for inflammatory arthritis and peripheral neuropathy, allografts of adrenal medullary tissue transplanted into the rat spinal subarachnoid space markedly reduce symptoms of chronic pain including allodynia, hyperalgesia, and spontaneous pain. The mechanism for these beneficial effects most likely involves co-activation of host spinal opioid and α-adrenergic receptors. In addition, adrenal medullary transplants can reduce the incidence of spinal neuroplastic changes associated with chronic injury such as the induction of nitric oxide synthase and immediate early gene c-*fos*, and the appearance of hyperchromatic neurons in the spinal dorsal horn.

The success of pre-clinical studies led to the initiation of limited clinical trials in cancer patients with chronic pain. Of five patients who received adrenal medullary allografts, four obtained significant pain alleviation, three for prolonged durations. However, while adrenal medullary allografts are promising, a large-scale application of this approach is limited by the availability of human donor tissue. Xenogeneic sources such as bovine chromaffin cells are a potential alternative. Findings in our laboratory indicate that bovine chromaffin cells can survive well in the rat CNS with limited short-term immunosuppression when they are substantially isolated from more potent antigenic cells such as endothelial cells. In addition, transplants of bovine chromaffin cells in the rat spinal subarachnoid space provide long-term alleviation of neuropathic pain symptoms. Together, these studies suggest that neural transplantation is a promising approach in the therapeutic management of chronic pain. Supported by NS25054.

Science and Engineering of Immunoprotected Cell Transplants

Biology of Immunoisolation

D1-004 THE ROLE OF CD4+ T CELLS IN THE REJECTION OF IMMUNOSOLATED DISCORDANT XENOGRAFTS IN NON-OBESE DIABETIC (NOD) MICE: GRAFT DESTRUCTION IS MEDIATED BY SMALL MOLECULES.

Thomas E Mandel[1], Maria Koulmanda[1], Tom Loudovaris[2] and Robert C Johnston[2]. [1]Transplantation Unit, The Walter and Eliza Hall Institute of Medical Research, Parkville, 3050 Australia, and [2]Baxter Healthcare Corporation, Applied Sciences, Baxter Technology Park. Round Lake, Illinois 60073-0490.

A major problem in clinical transplantation is the dearth of suitable allogeneic donors and particularly for common diseases such as insulin dependent diabetes mellitus (IDDM) that could, potentially, be cured by islet replacement there is no chance that sufficient human donors will ever be available. However, in IDDM it is conceivable that animal donors such as pigs could be suitable and adequate numbers of such donors are readily available. Immunosuppression for xenografts remains a major problem and indeed the mode of xenograft destruction is still very poorly understood. Discordant pig islet xenografts placed into NOD mice are rapidly (but not hyperacutely) rejected and the infiltrate in the graft site differs from that seen in allografts with numerous granulocytes, mainly eosinophils, as well as many macrophages present. Treatment of recipients with anti-CD4 (GK1.5, rat IgG2b) or anti-T cell receptor (KT3, rat IgG2a) monoclonal antibodies (MAbs) allows prolonged graft survival and granulocytes are not seen at the graft site. Similarly, pig islets transplanted into either nude (athymic) or SCID mice survive indefinitely without immunosuppression despite the ability of these recipients to mount a non-lymphocytic (macrophage, NK cell and granulocytic) protective response. Thus, very heavy immunosuppression, genetic or induced, allows discordant xenografts to survive but such severe immunosuppression would be unacceptable in patients. In an attempt to reduce immunosuppression to safe levels, or perhaps even to avoid it altogether, it was decided to attempt islet transplantation in NOD mice by placing the islets inside devices that would exclude the entry of immune cells. Pig islet xenografts were compared with islet allografts and isografts to determine whether the mechanism of graft damage within the immunoisolation devices differed. Allografts generally survived well without a need for immunosuppression and recurrent autoimmune disease was also prevented in isografts, but the xenografts were destroyed despite the inability of immune cells to enter the graft. However, treatment with anti-CD4 MAb did allow the xenografts to survive for prolonged periods. Thus, it appears that xenografts, but not allografts or isografts, are damaged by molecules that can diffuse into the immunoprotective device from cells that are attracted to the vicinity of the implanted device. The identity of the cells responsible for producing the toxic molecules and the nature of such molecules is still not known but it seems that CD4+ve T cells are necessary for the response to be initiated. It is conceivable that the migration and activation of eosinophils and perhaps neutrophils as well as macrophages is required before xenograft damage is seen and that these cells are under CD4 T cell control; the mode of damage to allografts and autoimmune damage to isografts seems not to be dependent on this mechanism. It is possible that cytokines and reactive oxygen species (eg superoxide, hydrogen peroxide and NO) are involved and if this is the case immunoisolation membranes with very small pores would be required but these would also prevent the entry of nutrients and exit of insulin. Thus some form of immunosuppression may still be needed. The precise nature of the anti-xenograft response needs to be carefully analysed so that appropriate countermeasures can be developed.

Encapsulation Technologies

D1-005 POLYETHYLENE GLYCOL-BASED CONFORMAL COATINGS FOR CELL ENCAPSULATION, Jeffrey A. Hubbell[1], Gregory M. Cruise[1], and Orion D. Hegre[2], [1]Department of Chemical Engineering, University of Texas, Austin, TX, 78712 and [2]Neocrin Company, 31 Technology Drive, Suite 100, Irvine, CA, 92718.

Microencapsulation using permselective polymer membranes shows promise to achieve immunoprotection of cell and tissue transplants. Polyethylene glycol's (PEG) good biocompatibility and water solubility makes it an excellent candidate for use as a microencapsulation membrane. We are using PEG acrylates, formed by reacting the PEG hydroxyl groups with acryloyl chloride, to form membranes for immunoprotection. (1) The PEG acrylates are crosslinked using the eosin-triethanolamine initiation system. The tissue is exposed to a 0.3 mM ethyl eosin solution for 30 seconds, giving time for the ethyl eosin to adsorb to the surface of the tissue. After rinsing, the tissue is placed in a 23% w/w PEG solution with 50mM triethanolamine and 0.2% vinyl pyrroloidinone and illuminated with a light source, either an argon ion laser or a filtered xenon arc lamp. (2) The PEG acrylates are crosslinked via free radical polymerization, resulting in a conformal hydrogel membrane. The permeability of the membranes is directly proportional to the distance between crosslinks or molecular weight of the PEG since it is end activated, so coatings of various permeabilities can be generated. The coating thickness is directly proportional to the light exposure time and light flux and can easily be controlled from 10 to 100 microns. We are investigating the use of PEG conformally coated islets of Langerhans as a bioartificial pancreas for the treatment of diabetes. Using 18.5kD PEG (Polysciences) tetraacrylate (TA) coatings approximately 30 microns thick on both rat and pig islets, we have shown minimal loss of viability, using fluoroscein diacetate and ethidium bromide staining, despite the crosslinking of PEG directly on the islet surface, and function, using static glucose stimulation and perifusion. The 30 micron 18.5kD PEG TA conformal coatings prevent outgrowth by fibroblasts and passenger leukocytes. Approximately 2% of PEG coated islets exhibit cellular outgrowth when cultured in 10% matrigel, while 100% of uncoated islets have cellular outgrowth. We have shown that 30 micron 18.5kD PEG TA conformal coatings prevent complement dependent antibody mediated cytotoxicity for periods up to 2 hour. Control islets were 0% viable after 2 hour exposure to 1:4 rabbit complement (Pel Freez, Brown Deer, WI), while no loss of viability was observed for PEG coated islets. In order to test the coating's ability to protect islets from complement for longer periods of time without concerns of loss of activity, we plan to implant PEG coated islets to rabbits. Presently, we are testing 18.5kD PEG TA coated rat and pig islets in STZ diabetic immunoincompetent and immunocompentent mice.

(1) C.P. Pathak et al., *J. Am. Chem. Soc.* 114:8311 (1992); (2) A.S. Sawhney et al., *Biotechnol. Bioeng.*, 44:383 (1994).

Encapsulation As A Basic Science Tool

D1-006 ENCAPSULATED CELLULAR TRANSPLANTS: FUNCTIONAL AND IMMUNOLOGICAL PARAMETERS, Robert C. Johnson, James Brauker, Thomas Loudovaris, Laura Martinson and S. Mookie Sternberg, Baxter Healthcare Corporation, Round Lake, IL 60073.

Transplantation of encapsulated cells offers the opportunity to correct certain disease conditions by using tissues that are foreign to the recipient. Through the proper choice of the cells to be transplanted, encapsulating membrane and design of the transplantation device, it is possible to achieve allogeneic or xenogeneic transplantation without immunosuppression. The design features of the encapsulating membranes can be adjusted to allow varying degrees of passage of molecules that are involved in the immune response to the graft. The transplantation device can therefore act as a tool in dissecting the components of the immune response to the graft. We have used membranes to encapsulate allogeneic or xenogeneic tissues that were subsequently implanted into rats or mice for periods ranging from three weeks to one year. Membranes that allow the successful transplantation of allografts can have pores as large as 0.4 μm and allow passage of IgG and IgM across the membrane into the transplant device. The same membranes offer no protection for xenografts which are destroyed within two weeks. One of the hallmarks of this xenograft destruction is a severe local inflammatory response in the tissue surrounding the implanted device. Transplantation of xenografts within the same membranes but into complement deficient, CD8 T cell or B cell deficient mice still resulted in destruction within three weeks. Using the 0.4 μm pore membranes for transplantation of xenogeneic tissue into SCID mice that were reconstituted with only CD4 T cells resulted in graft destruction within three weeks. We believe that a CD4 T cell response, even in the absence of CD8 T cells and B cells, is sufficient to destroy xenografts. This occurs due to the recipient being sensitized to antigens shed by the xenograft. Indeed, reducing membrane transport properties progressively leads to a decrease in the inflammatory response to xenografts due to reduction in the amount and/or size distribution of shed xenoantigens. We believe that the key to successful encapsulated xenotransplantation in soft tissue requires restriction of the release of xenoantigens from the transplant device, thus preventing the formation of a local inflammatory response. As the permeability of the membranes are altered to restrict macromolecule passage, there is also a decrease in passage of other essential molecules that leads to lower tissue viability and/or the requirement to disperse the tissue to lower packing densities. Therefore, adjustment of membrane permeability allows one to use encapsulated cellular transplants as a method of asking basic questions about the nature of the immune response to transplants and the membrane permeability required to maintain cell viability and function.

D1-007 DIFFUSIBLE SIGNALS IN NEURAL COMMUNICATION, Rae Silver[1], Joseph LeSauter[1], Mike Lehman[2] and Patrick A. Tresco[3,1] Barnard College of Columbia University, New York, N.Y. 10027, [2] University of Cincinnati, Cincinnati, OH, 45267, [3]University of Utah, Salt Lake City, UT, 84012

Locomotor rhythms and sleep-wake cycles are normally synchronized to environmental cues such as light and dark, however, circadian rhythms underlying these responses are sustained, and they "freerun" in the absence of all environmental cues. In mammals, it is well established that the suprachiasmatic nucleus (SCN) of the hypothalamus is the site of an endogenous circadian pacemaker that drives many behavioral and physiological rhythms, but the coupling mechanism(s) whereby the output of the biological clock signals the brain and the rest of the body is not known. These coupling mechanisms may entail efferent fiber connections and/or diffusible signals from SCN neurons.

To examine the possible role of diffusible signals as coupling mechanisms, we have used an encapsulation technique to physically isolate the grafted fetal anterior hypothalamic neurons from the host brain. Because the donor period (about 20 hours in homozygotes and 22 hours in heterozygotes) of these *tau* period mutant hamster is easily distinguishable from the freerunning period (about 24 hours) of the wild-type hamster, restored rhythms when they occur, can be attributed unambiguously to the SCN of the donor tissue. Encapsulated grafts from wild-type hamsters fetuses implanted into the 3rd ventricle of *tau* mutant, SCN-lesioned hamsters elicit wild-type locomotor behavior in some, but not all animals owing to a poor tissue survival. Studies in progress are focusing on ways to optimize conditions for tissue survival within the capsule.

It is possible that many brain nuclei communicate via both diffusible and fiber (synaptic) mechanisms. Polymer encapsulation of transplanted tissue provides a novel tool for analysis of these distinct signaling systems. Supported by grants from NIH NS24292 and AFOSR F49620-94-2-0294.

Tailoring of Cells

D1-008 DEVELOPMENT OF CORRECTLY-REGULATED PANCREATIC ß-CELL LINES, Shimon Efrat[1], David Fusco-DeMane[1], Manju Surana[2], Margarita Leiser[2], Obaidullah Al Emran[1], and Norman Fleischer[2], [1]Departments of Molecular Pharmacology and [2]Medicine, Albert Einstein College of Medicine, Bronx, NY 10461.

The development of cell encapsulation approaches opens the way for utilizing pancreatic ß cells for treatment of type I and insulin-requiring type II diabetes. However, this approach is limited by the supply of donor material and the difficulty of isolating large numbers of islets from the pancreas. Insulin-producing ß-cell lines could provide a readily available and virtually unlimited source of donor cells with well-defined and reproducible properties. We have employed transgenic mouse approaches to develop murine ß-cell lines with desired properties as a model for transplantation therapy of diabetes. Since normal ß cells do not grow well in culture, the SV40 T antigen (Tag) oncoprotein was used to transform ß cells of transgenic mice. Cell lines derived from the mice, denoted ß tumor cells (ßTC), maintain many of the properties of normal ß cells. ßTC cells synthesize insulin in amounts comparable to normal islets, and release it in response to all the major insulin secretagogues, most notably glucose, in a manner similar to that of normal ß cells. Isolation of correctly-regulated single-cell clones in early passages of the lines allows maintenance of a stable phenotype during prolonged propagation in culture. The clonal cells maintain the pattern of expression of glucose transporters and glucose-phosphorylating enzymes characteristic of normal ß cells, which is required for correct glucose sensing and secretion of accurate amounts of insulin. By placing the Tag oncogene under an inducible gene expression system, it has become possible to regulate the proliferation of ßTC cells as needed, both in culture and in vivo. This ability is crucial to prevent uncontrolled cell expansion in vivo and may also enhance the differentiated properties of the cells. In addition, an approach is being developed to manipulate the immunogenicity of ß cells to facilitate their transplantation.

D1-009 ENGINEERING A GLUCOSE RESPONSIVE INSULIN SECRETING CELL LINE, Christopher B. Newgard and Hector BeltrandelRio, University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75235

Our laboratory focuses on application of techniques of molecular biology for engineering of glucose-stimulated insulin secretion in insulin-producing cell lines. Our studies have established that stable transfection of glucose-unresponsive insulin secreting cell lines such as ArT-20ins or RIN 1046-38 with the GLUT-2 glucose transporter (but not GLUT-1) confers glucose-stimulated insulin secretion. In RIN cells, GLUT-2 gene transfer causes a four-fold increase in glucokinase enzymatic activity which appears to be necessary but not sufficient for the glucose response. GLUT-2 expressing cell lines exhibit a maximal response to glucose at subphysiological concentrations of the sugar (10-50 μM) but can be adjusted towards the physiologic range by inhibition of hexokinase enzymatic activity. Inhibition of hexokinase activity by 50-70% has been achieved in our laboratory both by a chemical approach, involving preincubation of the cells with 2-deoxyglucose or 5-thioglucose, and by the molecular approach of expressing hexokinase in antisense orientation. Expression of antisense hexokinase was achieved by transducing RIN1046-38 cells engineered for stable expression of GLUT-2 with a recombinant adenovirus (AdCMV-HKIrev) containing the full-length hexokinase I cDNA in antisense configuration. Reduction of hexokinase activity resulted in cell lines with a maximal insulin secretory response to insulin at 5 mM glucose. Current studies are focused upon producing lines with a stable reduction in hexokinase activity and testing of their insulin delivery function when transplanted into rodent models of insulin-dependent diabetes mellitus.

D1-010 GENE THERAPY FOR NEUROMUSCULAR DISORDERS. Jon A. Wolff, University of Wisconsin, Waisman Center, Departments of Pediatrics and Medical Genetics, Madison, WI 53705.

Neuromuscular disorders are excellent candidates for early attempts at gene therapy because most are not adequately treatable. In my laboratory, we discovered that when naked plasmid DNA is injected intramuscularly, the muscle cells have the remarkable ability to take up and express the plasmid DNA. Contrary to the current dogma, the plasmid DNA was maintained in an extracellular, open-circular state and had not replicated even though it did not contain any known origins of replication. We have used the mdx model for Duchenne muscular dystrophy to explore gene therapy treatments. When dystrophin expression vectors were intramuscularly injected into the quadriceps of the mdx mouse, approximately 1-2% of the myofibers were dystrophin-positive. The human dystrophin expression caused the re-expression of the dystrophin-associated glycoprotein complex and prevented the muscle fibers from dying. These results suggest that post-natal human dystrophin gene transfer would be beneficial to patients with Duchennes if one would get gene transfer into a sufficient number of myofibers.

The efficiency of gene transfer and expression in muscle must be increased before muscle can be used as a target tissue for the treatment of DMD. A variety of *in vivo* and *in vitro* systems have been used to understand naked DNA uptake into muscle. A preliminary accounting of the mechanism by which muscle cells take up the pDNA will be described.

Encapsulation for Diabetes: Approaching the Clinic

D1-011 ENCAPSULATION STRATEGIES FOR CELLULAR MEDICINE, Robert P. Lanza, Willem M. Kuhtreiber, and William L. Chick, BioHybrid Technologies Inc., Shrewsbury, MA 01545.

Encapsulation systems have been developed in which transplanted cells are separated from the immune system of the host by permselective barriers. These systems do not require a life-long regimen of high dose immunosuppressive drugs to prevent immune rejection. Furthermore, they offer a solution to the problem of human cell procurement by permitting use of cells and tissues isolated from animal sources. Three basic types of encapsulation systems have been studied in our laboratory. These include devices anastomosed to the vascular system as AV shunts, diffusion chambers (both hollow fibers and wider-bore tubular membranes), and spherical micro- and macroreactors. This technology is applicable to treating a number of diseases by transplantation of cells which produce specific bioactive substances. In essence, this approach constitutes a living drug delivery and detoxification system. Our work has focused mainly on developing a new treatment for diabetes using encapsulated pancreatic islets and, to a lesser extent, the use of encapsulated gene-modified cells for the treatment of hemophilia B. In the first type of encapsulation system, canine and porcine islets were distributed in a chamber surrounding a permselective acrylic membrane (nominal M, exclusion of 80kD), and the devices implanted into diabetic, totally pancreatectomized dogs without use of immunosuppression. Fourteen of the recipients (11 canine and 3 porcine islets) maintained graft function (>10 Units/day) for periods of time ranging from two months to more than a year. In the second type of encapsulation system, the islets were sealed within the acrylic membranes and the chambers implanted into the peritoneum of diabetic, pancreatectomized dogs (canine islets), STZ-induced diabetic rats (canine, bovine and porcine islets), and spontaneously diabetic BB/Wor rats (canine islets) without use of immunosuppression. All but one of the dogs maintained graft function for at least 1 month, and 2 animals for at least 7 and 8 months, respectively. In the rats, these chambers restored normoglycemia $\geq$1 month in all of the animals, and for >6 months in 10 of the 17 STZ recipients (canine, 3/8 [38%]; bovine, 4/5 [80%]; porcine, 3/4 [75%]). In the third type of encapsulation system (spherical reactors), the islets were implanted into the peritoneum of spontaneously diabetic dogs (canine islets) with low dose immunosuppression, into STZ-induced diabetic mice without use of immunosuppression and into STZ-induced diabetic rats (bovine and porcine islets) both with and without use of low dose immunosuppression. Implantation of the spheres completely supplanted exogenous insulin therapy in the dogs for 60 to >120 days (4 of the 5 implants continue to maintain function). The fasting blood glucose (FBG) concentrations averaged 122$\pm$4 mg/dl (mean $\pm$SEM) for these animals during the first 2 months. The glycosylated hemoglobin (Hb_{A1C}) levels during this period dropped from 6.7$\pm$0.5% to 4.2$\pm$0.2% ($P<0.001$). IVGTT K-values (decline in glucose levels, % min) at 1 and 2 months postimplantation were 1.6$\pm$0.1 and 1.9$\pm$0.1, respectively compared with 0.71$\pm$0.3 before implantation. In the mice, without immunosuppression, nonfasting plasma glucose concentrations promptly dropped from a preimplantation value of 498$\pm$47 to 142$\pm$6 (bovine) and 178$\pm$7 mg/dl (porcine) during the first week. All the animals sustained these levels for at least 1 month. Two mice implanted with bovine islets subsequently reverted to diabetes at 43 days postimplantation. The remaining grafts maintained function for >10 weeks. In contrast, nonencapsulated islets failed to function, or sustained euglycemia for <4 days. In the rats, with use of low dose immunosuppression, the spheres reversed the hyperglycemic state of all 8 of the recipients. Nonfasting plasma glucose levels dropped from a preimplantation level of 530$\pm$8 to 149$\pm$17 (bovine) and 156$\pm$12 mg/dl (porcine) during the first week. All of the the animals maintained these levels for >100 days. In rats, without use of immunosuppression, newer types of reactors have reversed hyperglycemia for >10 weeks (still ongoing). Histologic evaluation of all three types of pancreas devices (1-20 months postimplantation) revealed viable islets with granulated β-cells. These results represent an important step in determining the use of these different encapsulation strategies in the treatment of human disease.

D1-012 ENCAPSULATED ISLET TRANSPLANTATION IN TYPE I DIABETES: PRELIMINARY RESULTS OF PHASE I/II CLINICAL TRIALS, Patrick Soon-Shiong, Islet Transplant Center, Santa Monica, CA 90404.

The findings of the Diabetes Complication and Control Trial have provided encouragement to both the diabetic patient and the scientific community that maintenance of tight glycemic control can prevent or delay the devastating secondary complications of diabetes. Whole organ pancreas transplantation is the only form of therapy currently available that can consistently achieve euglycemia in insulin-dependent diabetic patients. However, this is accomplished at a severe cost, both in terms of morbidity and health care dollars. Intensive insulin therapy as demonstrated by the DCCT trial, is another alternative, but again, at a cost of increased incidence of episodes of severe hypoglycemia. Islet transplantation, even with high dose immunosuppression, has been disappointing and has failed to live up to its promise of providing a safe, simple cure for insulin-dependent diabetic patients.

The challenge that scientists in the field of diabetes care now face is to identify and develop a therapy which will at least achieve the results of the Diabetes Complication Control Trial, without the dangers of severe hypoglycemia, without the surgical risk, morbidity and cost of whole organ pancreas transplantation, without the life-long need for high dose immunosuppression, and finally, without the disappointing and inconsistent results of intraportal islet transplantation. Transplantation of islets immunoprotected by a semi-permeable porous membrane (encapsulated islets) may prove to be a possible therapeutic approach which could meet this challenge.

Intraperitoneally encapsulated islet cell therapy may be such an approach, whereby patients may receive an injection of immunoprotected, insulin-secreting cells without the need for life-long immunosuppression and achieve tight glycemic control, and even insulin-independence, with ultimate prevention or reversal of secondary diabetic complications. The preliminary results of our clinical trials, offers a glimpse into the possibilities that indeed this may be achievable. We have demonstrated in our first patients that insulin-independence is achievable following encapsulated islet therapy. Eighteen months after the initial procedure (to date) the patient demonstrates ongoing islet function, tight glycemic control, ongoing improvement of peripheral nerve conduction with no evidence of symptomatic hypoglycemic events.

Encapsulation for CNS: Clinical & Preclinical Trials

D1-013 **ENCAPSULATED BOVINE CHROMAFFIN CELLS XENOTRANSPLANTATION FOR THE TREATMENT OF INTRACTABLE PAIN: A PHASE I CLINICAL STUDY.** J.M. JOSEPH[1], B. HEYD[1], J. FAVRE[2], E. BUCHSER[3], F. MOSIMANN[1], M. GODDARD[4], P. AEBISCHER[1], [1]Surgical Research Division, [2]Neurosurgical Department, Lausanne University Medical School, Switzerland; [3]Pain Clinic, Morges, Switzerland; [4]CytoTherapeutics Inc, Providence, RI, USA.

Chromaffin cells are known to release a cocktail of antinociceptive substances such as catecholamines and opioids. Intrathecal transplantation of allogeneic chromaffin cells has been shown to alleviate pain in rodent models and in human terminal cancer patients. To circumvent the problem related to the shortage of human cadaver donors, we are investigating the possibility of transplanting encapsulated xenogeneic cells. Chromaffin cells were isolated from surgically harvested calf adrenal glands and enclosed within a tubular (5 cm long, 900 µm diameter) semipermeable acrylic derived membrane capsules which allow diffusion of nutrients and neurotransmitters but prevent rejection of the graft by the host immune system. The loaded capsules were then mounted on a silicone tether and kept in vitro for a week. Bacteriological analysis and catecholamine release were performed before implantation. The nicotine evoked norepinephrine release from the capsule ranged from 0.7 to 5nM/2ml/30min prior to implantation.

Ten patients suffering from terminal cancer pain (n=8) or denervation pain (n=2) were transplanted with encapsulated bovine chromaffin cells. Eight patients received lumbar implants under local anesthesia through a cannula guiding system. In two patients suffering from upper body pain, the devices were implanted in the lateral ventricle by a stereotaxic approach. No complication was observed except for post lumbar puncture headaches in 3 patients with the lumbar intrathecal implants. In six patients the devices were retrieved at the time of death 11 to 165 days post-transplantation. In three other patients, the devices were retrieved 41 to 84 days post-transplantation. One patient is still alive, 11 month after transplantation. Excellent biocompatibility was observed in all 9 retrieved devices. The external surface of the explanted capsules were characterized by the absence of host cell reaction. Intracapsular populations of healthy chromaffin cells were observed in 8 out of 9 explanted devices. Three out of 3 explanted capsules tested were releasing catecholamines at the time of explantation. Cells positive for tyrosine hydroxylase and methionine enkephalin immunostaining were observed throughout the explanted capsules. Diminution of analgesic drug intake in 4 out of 6 opioid responder patients, while maintaining adequate pain scores, and improvement of pain scores in 3 out of 3 non opioid responder patients were observed.

These preliminary results illustrate the potential of the encapsulation technology for xenogeneic cell transplantation and speak for the need of a double blind randomized phase II study.

D1-014 GRAFTS OF POLYMER ENCAPSULATED CELLS GENETICALLY MODIFIED TO SECRETE HUMAN NERVE GROWTH FACTOR PREVENT THE DEGENERATION OF CHOLINERGIC NEURONS IN YOUNG AND AGED NONHUMAN PRIMATES: IMPLICATION FOR THE TREATMENT OF ALZHEIMER'S DISEASE. Jeffrey H. Kordower[1], Yue-Ting Liu[1], Elliott J. Mufson[1], Shelly R. Winn[2], Joseph P. Hammang[2], E. Edward Baetge[2], and Dwaine F. Emerich[2]. Department of Neurological Sciences, Rush Presbyterian Medical Center, Chicago Illinois 60612 and [2] Cyto Therapeutics, Providence, Rhode Island 02906.

Nerve growth factor (NGF) supports the viability of cholinergic basal forebrain neurons, a population of cells which consistently degenerate in Alzheimer's disease (AD). Based upon these data, NGF treatment has been proposed as a novel treatment strategy for this disorder. Prior to initiating widespread clinical trials, however, studies demonstrating the efficacy of NGF in nonhuman primates, especially aged nonhuman primates have been recommended. Towards this end, two series of studies have been carried out.

In the first study, baby hamster kidney (BHK) cells were genetically modified to secrete high levels of human NGF (hNGF). Following polymer encapsulation, these cells were grafted (BHK-hNGF) into the lateral ventricle of four young adult cynomologus monkeys immediately following a unilateral transection/aspiration of the fornix. Three control monkeys received identical grafts, with the exception that the BHK cells were not genetically modified to secrete NGF (BHK-Con)). One monkey received a fornix transection only. Control monkeys displayed extensive losses of choline acetyltransferase (ChAT) and p75 nerve growth factor receptor (NGFr)-ir neurons within the medial septum (53% and 54) ipsilateral to the lesion. In contrast, monkeys receivingBHK-hNGF implants exhibited only a modest loss of cholinergic septal neurons (19% and 20). Only monkeys receiving BHK-hNGF grafts displayed a dense sprouting of cholinergic fibers within the septum which ramified against the ependymal lining of the ventricle adjacent to the transplant site. Examination of capsules retrieved from monkeys prior to sacrifice revealed numerous healthy-appearingNissl stained cells which produced ELISA detectable levels of hNGF in a sufficient concentration to differentiate PC12 cells in culture.

In the second study, six Rhesus monkeys between 24 and 29 years of age received unilateral transections of the fornix. Three monkeys then received intraventricular BHK-hNGF grafts and three received BHK-Con grafts. Monkeys receiving BHK-Con grafts displayed extensive reductions in the number of ChAT (57-75%) and p75 NGFr-ir (53%) medial septal neurons ipsilateral to the lesion/implant. In contrast, monkeys receiving BHK-hNGF transplants display only a modest loss of ChAT-(0-36%) and p75 NGFr-(7-22.4%)-ir septal neurons. Only monkeys receiving hNGF-secreting implants displayed robust sprouting of cholinergic fibers within the septum ipsilateral to the transplant. Just prior to sacrifice, the capsules were retrieved and determined to contain viable BHK cells releasing biologically relevant levels of hNGF. These data demonstrate that hNGF can provide trophic and tropic influences to primates including aged primate basal forebrain neurons undergoing lesion-induced degeneration supporting the contention that hNGF may prevent degeneration of cholinergic basal forebrain neurons in Alzheimer's disease.

Emerging Applications

D1-015 **IMMUNOISOLATION IN SOMATIC CELL GENE THERAPY** J. Brauker, V. Carr-Brendel, S. Neuenfeldt, R. Clarke, D. Hodgett, C. Vergoth, W. Stone,*V. Dwarki, *R. Chen, *T. Nijjar, T. Loudovaris, L.Martinson, S. Young, S. Jacobs, R. Geller, D. Maryanov, S. Levon, W. Johnston and R.C. Johnson. Gene Therapy Unit, Baxter Healthcare, Round Lake IL 60073 and *Somatix Therapy Corp. Alameda CA, 94501.

Immunoisolation is the encapsulation of therapeutic tissue within a membrane that protects the tissue from the immune response. We developed an immunoisolation device that becomes heavily vascularized at the membrane-tissue interface. We have shown survival of allogeneic rat embryonic lung tissue (Sprague Dawley into Lewis rats) in devices for one year with excellent maintenance of differentiated phenotype (ciliated epithelia). These cells were rejected within two weeks when holes were poked in the membrane. The pore size of the protective membranes was 0.45 µm, which is sufficient to allow high rate permeability for molecules larger than factor VIII. The use of an immunoisolation device allows for (i) segregation of the implanted cells from the host, (ii) the ability to remove the cells quantitatively, (iii) the use of a single cell clone for all patients, resulting in improved reproducibility and quality control, and (iv) the use of allogeneic cells that will be destroyed if they escape from the device. We have demonstrated utility of the device in three areas: (i) Rat pancreatic cells were implanted into NOD mice. These animals were corrected of diabetes as assessed by blood glucose levels and glucose tolerance tests. (ii) Murine tumor cells were implanted within devices into C57 black mice and shown to prevent subsequent tumor formation. (iii) Human fibroblasts expressing human Factor IX were implanted in nude mice and rats resulting in plasma levels of 200-500 ng/ml for more than 80 days without any apparent diminishment. These results show the utility of the Baxter immunoisolation device for implantation of large amounts of viable, functional tissues for Gene Therapy.

D1-016 APPLICATION OF IMMUNOISOLATION TO SOMATIC GENE THERAPY, Patricia L. Chang, Hong-wen Liu, Mary Hughes, Ayman Al-Hendy and Gonzalo Hortelano, McMaster University, Hamilton, Ontario, Canada.

Current gene therapy protocols mostly depend on genetic modification of the patients' own cells. We propose an alternate approach of "**non-autologous somatic gene therapeutics**" in which a *universal* cell line is genetically modified to secrete a desired therapeutic gene product. By enclosing such non-autologous recombinant cells in immunoisolation devices, the same cell line can be implanted into different patients with the same product requirements. To demonstrate the feasibility of this idea *in vitro*, we showed that genetically-modified allogeneic fibroblasts can remain viable within immuno-protective alginate-poly-L-lysine-alginate microcapsules and continued to secrete recombinant gene products such as human growth hormone, factor IX and lysosomal enzymes. The versatility of this strategy was further enhanced when the recombinant gene product can be engineered to acquire novel properties as required. Human adenosine deaminase, the first gene product used in human gene therapy, is not normally secreted. However, fusing a signal sequence to the 5' terminus of its cDNA directed this enzyme into a secretory pathway, thus allowing its secretion from encapsulated cells and demonstrating the potential of treating metabolic diseases caused by cytosolic enzyme deficiencies. To test this strategy *in vivo*, we implanted normal mice with microcapsules enclosing allogeneic mouse fibroblasts transfected with the *human growth hormone* gene. The implanted animals acquired human growth hormone in the circulation within the first two weeks. By ~3 weeks, antibodies against human growth hormone developed in the microcapsule-implanted mice. The antibody titer continued to escalate for more than three months, thus demonstrating indirectly the continued delivery of the growth hormone. The persistent expression of the transgene and survival of the transfected cells were verified when the microcapsules were retrieved to show that the encapsulated cells remained viable, proliferative, and productive of human growth hormone even by 78-111 days. We have since improved the delivery to more than six months with another recombinant product, human factor IX for the eventual treatment of hemophilia B. Since growth hormone is species-specific, the clinical efficacy was proven when we engineered a *mouse* growth hormone-producing myoblast cell line and implanted them in microcapsules into dwarf mice which were genetically deficient in growth hormone. Four weeks after implantation, significant increases in body weight and length, internal organs and tibial growth plates were obtained above the controls, in some cases to >25%. Thus, encapsulated recombinant cells can be engineered to provide therapeutic products which may be engineered to acquire novel properties. *In vivo* experiments demonstrated the clinical efficacy of such non-autologous implants to correct a mutant phenotype. Therefore, application of immunoisolation technologies to somatic gene therapy should have potential clinical and economic benefits.

D1-017 MICROENCAPSULATED HEPATOCYTES, Vivek Dixit, Department of Medicine, University of California, Los Angeles, CA.

Recent advances in tissue culture and cell immobilization techniques have significantly enhanced the therapeutic potential of isolated hepatocytes for treating a wide array of liver disorders. Microencapsulation of hepatocytes is one such advance that has demonstrated efficacy in providing long-term liver-specific function in experimental animal models of acute and chronic metabolic liver diseases. Microencapsulation facilitates the immunoisolation of transplanted cells in a foreign environment. The microcapsule's semipermeable membrane functions as a physical barrier that prevents the body's immune system from detecting encapsulated cells as foreign. This innovative technique provides a unique method for transplanting living cells without the need for immunosuppression. We have shown that unlike free hepatocytes that evoke strong immunogenic response following xenotransplantation, microencapsulated hepatocytes remain non-antigenic even after repeated exposure to the host's immune system. We showed that intraperitoneally transplanted microencapsulated hepatocytes can provide sufficient metabolic support to significantly improve the survival of animals with fulminant hepatic failure. In other studies we demonstrated the effectiveness of microencapsulated hepatocytes in reducing congenital hyperbilirubinemia in Gunn rats for periods of 4-6 weeks. Beyond this period, the transplanted microencapsulated hepatocytes underwent considerable degeneration. We showed that cell-surface interaction properties play an important role in these degenerative changes. This can be delayed if an improved cell attachment substrate *(Matrigel™)* is used to prepare the microcapsules. Sustained correction of hyperbilirubinemia for at least 6 months was also demonstrated in the Gunn rat by repeated monthly transplantations of microencapsulated hepatocytes. In other studies we showed that microencapsulated hepatocytes can be successfully cryopreserved for several weeks with little or no loss in viability and function. This observation is important in developing liver support systems that can be easily stored and be accessible on demand. We recently proposed a novel extracorporeal hepatocyte bioreactor involving the direct hemoperfusion of microencapsulated hepatocytes. Since microcapsules have a very high surface area to volume ratio, this system exceeds other bioreactor designs in providing both an augmented surface area for solute mass-transfer and a high capacity for hepatocyte mass. Our recent efforts have also focused on microencapsulated fetal porcine hepatocytes for use in transplantation and in extracorporeal liver support systems. Porcine hepatocytes are advantageous in that they are hardy and can be readily obtained in large quantities. Our preliminary results suggest that these cells are ideal for use in humans where large volumes of hepatocytes would be required to treat severe liver disease.

D1-018 **EXTRACORPOREAL CELL BASED LIVER PROSTHESIS,** Claudy J-P. Mullon[1], Hugo O. Jauregui[2], Achilles Demetriou[3], Barry A. Solomon[1], [1]W. R. Grace & Co.-Conn, Lexington, MA, [2]Rhode Island Hospital, Providence,RI, [3]Cedars Sinai Medical Center, Los Angeles, CA.

Liver transplantation is the only clinically effective method of treatment for fulminant hepatic failure. However, liver transplantation is limited by the number of organ donors, high cost, and the need for life-long immunotherapy. Several cell based extracorporeal liver support systems (LSS) are currently being developed to support patients awaiting liver transplantation or to prevent transplantation by allowing the patient's liver to regenerate.

A liver assist device (LAD) consisting of a hollow fiber (microporous polysulphone fibers) and hepatocytes (from rabbits and pigs) was developed by W. R. Grace. The efficacy of the LAD was successfully demonstrated in an animal model of acute liver failure. The Grace LAD technology was later merged with the Bioartificial Liver (BAL) technology used at Cedars Sinai Medical Center. The BAL based extracorporeal liver support system (LSS) consists of a plasma separator, a hollow fiber cartridge containing pig hepatocytes and a charcoal unit. Thirteen patients were treated with the BAL. An IND was filed and phase I clinical trials were initiated at Cedars Sinai Medical Center in September 1994.

Key steps in the development of the LSS will be reviewed in this presentation.

Late Abstract

IMMUNE RESPONSES TO THE CELL TRANSPLANT TO BE OVERCOME BY ENCAPSULATION. Peter J. Morris. Nuffield Department of Surgery, University of Oxford, Oxford Radcliffe Hospital, Oxford OX3 9DU, UK.

The immune response to an allogeneic or xenogeneic cell transplant comprises both a specific humoral and cellular response. In addition there may be a significant non-immune inflammatory response mediated by macrophages, NK cells and polymorphonuclear leukocytes. There is also an important difference between the allogeneic and xenogeneic response in that in the latter situation there may be pre-existing natural cytotoxic antibodies against xenoantigens on the transplanted cells.

The humoral side of the immune response is mediated by antibody and complement (C) which is the major effector mechanism of cell lysis of the humoral response. The complement cascade is triggered by the interaction of antibody and antigen (classical pathway) but can also be triggered non-specifically by the interaction of the C3 component with a foreign surface such as exhibited by bacteria (alternative pathway). It should be noted that sensitisation of the host against encapsulated allogeneic cells can occur by passage of soluble MHC molecules out of the capsule and indirect presentation of these molecules by host antigen presenting cells to host T cells.

A major difference between the alloresponse and the xenoresponse is that in the latter there may be pre-existing natural cytotoxic antibody directed against xenoantigens on the cellular transplant, depending on the species combination. Man possesses natural cytotoxic antibody against most species except the higher order primates, the target epitope being a Gal-α 1,3-Gal carbohydrate moiety expressed by most tissues in species carrying the antigen. These natural antibodies are mostly IgM, but there can be a significant IgG component.

The size of the molecules involved in the humoral arm of the response ranges from 25 to 900,000KD (e.g. IgG-150,000, IgM-900,000, Clq-410,000, C3-190,000). Access to encapsulated cells, be they allogeneic or xenogeneic, depends on the pore size of the capsule, which will range around the average. Whether cell damage will be produced depends on the number and the class of antibody molecules gaining access, the affinity of the antibody, and the access of the various complement components. It is also possible that the alternative C pathway might be triggered by the membrane itself resulting in C mediated damage if access is possible for the C components involved in this pathway.

Specific cell lysis of encapsulated cells would depend on cell to cell contact and hence should not be a significant factor in damaging encapsulated cells. However, non-specific activation of cells such as neutrophils or macrophages by the capsule might result in the production of cytokines and free radicals, for example, which could then gain access to the encapsulated cells causing damage.

Science and Engineering of Immunoprotected Cell Transplants

D1-100 THE IMMUNE RESPONSE AGAINST THE H-2-DEFECTIVE CELL LINE LR.4 IN C57BL/6J MICE MAY LEAD TO REJECTION OR TO THE DEVELOPMENT OF CANCER DORMANCY. Alfaro, G., R. del Río and E. Verástegui. Depto. de Inmunología, Instituto de Investigaciones Biomédicas, UNAM. Apartado Postal 70228, Ciudad Universitaria, 04510 México, D.F. Div. de Inv. Clínica, Instituto Nacional de Cancerología, Av. San Fernando Nº 22, Tlalpan 14000, México, D.F.

Identification, confinement and eventual destruction of neoplastic cells through immune mechanisms are mediated by cytotoxic T lymphocytes (CTL). Other cell types, such NK and macrophages are less specific but can be involved in the control of malignant cell populations.

This investigation demonstrates that the H-2-deficient cell line, LR.4, is lethal in different strains of mice expressing a given haplotype (H-2a, H-2b and H-2k). However, C57BL/6J mice and (C57BL/6J X BALB/c)F1 hybrids developed an specific and protective immune response leading to the rejection of the tumor after an initial phase of growth. In vitro, neither CTLs nor NK cells could lyse LR.4 and therefore it was concluded that they were not the effector mechanisms used by the immune system against the cell line under investigation. On the other hand, the combined participation of specific antibodies and NK cells from the spleen of C57BL/6J mice were shown to mediate lytic activity against the tumor. It is noteworthy that a small percentage of mice carried live cells 150 days after macroscopic signs of tumor activity have ceased. Under our experimental conditions, this emergent population was undistinguishable from that used for the graft; therefore, if is conceivable that some cells escaped immune detection or were not lysed by immune effector mechanisms and were driven to an state of latency which in a few animals was broken and followed by accelerated growth and dead of the animals.

Although the evidence presented here suggest that the immune system was involved, the participation of other factors, not necessarily related to the immune response must be considerated since aged females failed to reject the tumor.

D1-101 TISSUE ENGINEERING OF A BIOHYBRID KIDNEY FILTER AND KIDNEY TUBULE, H. D. Humes, Nephros Therapeutics and University of Michigan, Ann Arbor, MI

Long-term ex vivo therapy for kidney failure has been achieved, so that the kidney may be the first solid organ in which tissue engineering constructs can produce an implantable device for long-term in vivo replacement. To replace the kidney's excretory function, an implantable biohybrid kidney requires both a device to replace blood ultrafiltration performed by renal glomeruli and a device to replace transport regulatory function of the renal tube. Early prototypic constructs have been formulated to reproduce these functions. Using a single hollow fiber bioreactor, polysulphone hollow fibers were prelined with various extracellular matrix components (collagen IV, Pronectin F, and fibronectin) and seeded with mammalian renal tubule cells or endothelial cells. After 5-7 days in the bioreactor, confluent growth of a monolayer of cells was accomplished along the inner aspect of the hollow fiber as demonstrated with atomic force microscopy and scanning electron microscopy. Permselective convective fluid transfer under physiologic pressure and flow conditions was demonstrated in the biohybrid filter and active transport of salt and water was achieved in the biohybrid tubule. Thus, early prototypic biohybrid constructs have been developed to replicate the necessary excretory functions of the kidney.

D1-102 PROLONGED *IN VITRO* CULTURE OF FISH (TILAPIA) ISLETS: MAINTENANCE OF DIFFERENTIATED ISLET CELL MORPHOLOGY

Laura Timares Lebow, Eugenio Morsiani, Jacek Rozga and Achilles A. Demetriou, *Liver Support Unit, Department of Surgery, Cedars-Sinai Medical Center. Los Angeles, CA 90048*

Currently, islet cells for transplantation into diabetic patients are harvested from either human cadaveric or mammalian tissues. Unlike mammalian systems, islet cells in some teleost (bony) fish are macroscopic and anatomically separate from exocrine tissue representing a practical source of islets. The tropical fish Tilapia (*O. nilotica*) survives at mammalian body temperatures. These studies investigate the ability of Tilapia islet tissue to maintain differentiation under mammalian physiologic conditions in vitro.

Cultures were set-up in parallel on either trans-well inserts or in suspension. Either trypsinized islets yielding single cells or whole islets were cultured in CMRL-1066 medium containing 10% FBS. After 1 to 2 weeks in culture, adherent cell monolayer outgrowths, from whole islet explants, preceded refractile islet tissue outgrowth. In single cell cultures adherent cells underlayed refractile islet cell clusters suggesting a feeder cell requirement. After 1 month at 37°C, 5% CO_2 in air, cultures contained Dithizone (islet-cell specific) positive cell clusters within whole islets and single cells stained positive in intracellular vesicles. Single-cell cultures rapidly declined after one month, while whole islets remained viable and spread out radially with central necrosis. Ultrastructural examination (TEM) of whole islets maintained for almost 5 months in suspension or on transwell filters reveal well granulated α-, δ-, and β-cells. Dithizone positive islets are still detected after 7 months in culture.

These results demonstrate prolonged Tilapia islet survival and maintenance of normal differentiated morphology under mammalian physiological conditions. This remarkable longevity in culture suggests that Tilapia may serve as a potential islet cell source for bioartificial pancreas development. Culture and examination of islet function and morphology within immunoprotective chambers is currently in progress.

D1-103 **CANCER IMMUNOTHERAPY UTILIZING AN IMMUNOISOLATION DEVICE, S. A. Levon, D. A. Maryanov, S. K. Neuenfeldt, T. J. Thomas, J. H. Brauker. and R. L. Geller, Gene Therapy Unit, Baxter Healthcare Corp. Round Lake IL 60073 USA.**

Baxter Gene Therapy has developed a membrane-based immunoisolation device to protect implanted cells from direct contact with immune cells of the host. Use of an immunoisolation device for cancer immunotherapy has several advantages: (i) it will permit introduction and maintenance of live cells that can stimulate the immune system for extended periods of time, (ii) there is a reduced risk of tumor development as tumor cells are sequestered from the host and (iii) following treatment, the cells can be quantitatively removed. Using the mouse colon carcinoma cell line, MCA-38 as a model system, we implanted syngeneic C57/B6 mice with two devices each containing 10^6 MCA-38 cells. After three weeks the animals were challenged by intramuscular injection of 10^6 MCA-38 cells. All of the control animals (lacking implants or with empty devices) developed tumor at the challenge site while animals that received devices containing tumor cells remained tumor free. Five of the animals received a second challenge with 10^6 MCA-38 cells two months after the first challenge. After the second challenge 4/5 animals were still tumor free at >90 days. The one animal that developed a tumor after the second challenge was implanted with only one device. These data suggest that a potent anti-tumor immune response can be generated in the absence of direct cell contact. It is our hypothesis that antigens shed from tumor cells within the device are taken up by antigen presenting cells of the host and thus indirectly initiate the activation of the immune system.

D1-104 **CORRECTION OF DIABETIC NOD MICE WITH PANCREATIC ISLET TISSUE IMPLANTED WITHIN BAXTER IMMUNOISOLATION DEVICES.**

T. Loudovaris, S. Young, S. Jacobs, D. Maryanov, L. Martinson, S. Neuenfeldt, J. Brauker, R. Johnson. Gene Therapy Unit, Baxter Healthcare Corp. Baxter Technology Park, Round Lake, IL, 60073-0490.

The use of xenogeneic tissues may be the solution to the shortage of pancreas or islet allografts for the treatment of Insulin Dependent Diabetes Mellitus (IDDM). But before we can use xenogeneic islets two major problems have to be overcome, recurrence of autoimmunity and xenogeneic rejection. Our approach to treat diabetes is with islets inside immunoisolation device implants. The devices used are comprised of a cell impermeable membrane, a 0.45μm pore polytetrafluoroethylene (PTFE) membrane laminated to a 5μm pore PTFE vascularising membrane. The device allows for high density of islet packing in a relatively small area and has been found to be successful in preventing allograft rejection and autoimmune disease recurrence. However, a cell impermeable membrane alone may be insufficient for the protectiion of densely packed xenogeneic tissues, because they are destroyed within three weeks. In this study we examined the use of immunosuppression to overcome this problem. Diabetic NOD (Non-Obese-Diabetic) mice were implanted with devices containing xenogeneic islets and treated with either anti-CD4 (0.2mg weekly) or Cyclosporin A (CsA, initially, 50mg/kg daily for three weeks and then 25mg/kg daily) or with allogeneic or isogeneic islet tissue. Controls included immunosuppressed diabetic NOD mice implanted with islets under the kidney capsule or mice receiving devices with islets but no immunosuppression. The device together with the anti-CD4 treatment allowed for the survival of xenogeneic islets with correction of diabetes for more than 14 weeks, as determined by non-fasting blood glucose levels and intraperitoneal glucose tolerance tests. The device with CsA treatment allowed for the survival of xenogeneic islets with correction of diabetes for more than 10 weeks. The device with no immunosuppression allowed for the survival of allogeneic or isogeneic islet tissues with correction of diabetes for more than 20 weeks. The use of immunosuppression with immunoisolation devices to correct diabetes in human IDDM patients is very possible, due to the existence of IDDM patients who are presently on immunosuppressive therapy for organ transplants.

D1-105 IMPLANTATION OF MACROENCAPSULATED FISH (*TILAPIA*) ISLET TISSUE PROVIDES NORMOGLYCEMIA IN STREPTOZOTOCIN-DIABETIC MICE, Morsiani E., Lebow L.T., Rozga J., Demetriou A.A., Department of Surgery, Cedars-Sinai Medical Center, Los Angeles, CA 90048

The ultimate goal of pancreatic islet transplantation is the treatment of insulin-dependent diabetic patients early enough during the course of the disease to prevent the long-term complications of insulin therapy. This goal could be achieved if a large source of xenogeneic insulin-producing tissue became available, and if the problem of immunologic rejection of the xenograft is solved. The insular tissue in some teleost fish is anatomically separate from the exocrine one and therefore represents a practically useful source of islet tissue. Tropical fish, such as Tilapia *(Oreochromis nilotica)* has been demonstrated to survive at mammalian body temperature (Wright JR, *et al., Diabetes*, 41:1528, 1992) and was used in this study. Athymic (nude) mice and C57BL/6J (B6) mice were rendered diabetic by streptozotocin (non-fasting blood glucose >400mg/dl) and then implanted with encapsulated islets i.p. The permselective chamber consisted of variable amount of islet tissue (150-200 μl), embedded in 1% alginate in serum-free CMRL-1066 medium and gelled in 2% $CaCl_2$ within regenerated cellulose dialysis tubing (Spectra/Por^R RC; MWCO: 25,000 D). Blood glucose decreased to <100 mg/dl in nude and B6 the day after implant and remained normal for 7 days. Then, slightly increased to 250-300 mg/dl during the second week. Subsequent removal of the implants raised the blood glucose to pre-transplant levels. Histological examination of the membranes showed viable insulin-positive cells and minimal inflammatory reaction around the devices. Islet cell integrity was important in maintaining normoglycemia, since implantation of encapsulated control killed-islets induced hypoglycemic coma and death in all the recipients within 12-24 hr. Our experience suggests that the dose of islet load is important in maintaining normoglycemia with time. Studies are in progress to study the feasibility of fish tissue in immunoisolation devices to achieve long-term normoglycemia in diabetic recipients.

D1-106 DENSITY ADJUSTED CELL SORTING (DACS), A NOVEL METHOD TO PURIFY RARE CELL POPULATIONS SUCH AS CD34 HEMATOPOIETIC STEM CELLS, Peter Van Vlasselaer, George Strang, Jeff Zeitung and Peggy Hung, Department of Applied Immunology and Hematology, Activated Cell Therapy, Inc., Mountain View, CA 94043

Therapies using living cells require the purification of relevant target cells prior to their *in vitro* manipulation and re-transfusion *in vivo*. We have developed a novel method to purify rare cell populations from heterogeneous cell mixtures by binding irrelevant bystander cells to dense carrier beads and subsequently removing them by centrifugation on a specific density solution. The target cells are thereby enriched and remain at the interface on top of the density solution. The use of SimpleSep tubes allows the rapid and efficient collection of target cells by simply decanting the tube rather than by pipetting which generally results in significant cell loss, especially when the target cells represent a rare population. This method, referred to as Density Adjusted Cell Sorting (DACS) was screened for its efficacy in purifying CD34 hematopoietic stem cells from the blood of G-CSF treated Non-Hodgkin's Lymphoma transplant patients. Peripheral blood samples were first incubated with anti-CD16 and anti-CD20 coated beads which bind to granulocytes and B and lymphoma bystander cells and then centrifuged on a Percoll solution. The cell population decanted from the SimpleSep tube represented approximately 5% of the initial cell number and contained the majority of CD34 cells. In contrast, no detectable CD16 or CD20 positive cells were observed as defined by FACS analysis. Hence, this indicates the feasibility for the use of DACS technology for simultaneous CD34 stem cell enrichment and tumor purging in bone marrow and peripheral blood stem cell transplantation in cancer patients. In conclusion, DACS offers attractive features for the purification of rare cell populations for living cell therapy such as; 1) its ability to process large quantities of a heterogeneous cell mixture, 2) its high yield of target cells, 3) the fact that target cells are not in sustained contact with animal products, 4) no sophisticated equipment is required and 5) the fact that it can be performed in a closed system, the SimpleSep Syringe. We are currently evaluating this technique in a clinical setting in peripheral blood stem cell transplantation in NHL patients.

Late Abstract

CELL ENCAPSULATION IN ALGINATE: ELIMINATION OF FOREIGN BODY REACTION AND MICROCAPSULE VOLUME MINIMISATION. Jangaard N.O., Wilkinson C. K., Kelco International, San Diego. Zimmermann U., Klöck G., Groehn P., Institute for Biotechnology, Würzburg.

Alginate is commonly used as a matrix for immobilization of cells for biotechnological processes and for immunoisolation of cells and tissues in vivo. We have demonstrated previously that commercial alginates contain mitogenic impurities which are responsible for adverse side effects such as cytokine release and inflammatory reactions, therefore we have developed a protocol for the chemical purification of alginates on a large scale. Beads made from alginates purified by this procedure did not induce a foreign body reaction when implanted for three weeks either intra-peritoneally or beneath the kidney capsule of Lewis or BB/OK rats. These purified alginates can now be manufactured from weed sources with various mannuronic:guluronic acid ratios, according to Good Pharmaceutical Manufacturing Practice, in newly built clean room facilities.

The increase of the total volume of microcapsules which have to be transplanted compared to the volume of free cells is another major hindrance to the clinical application of microencapsulation. We present a new method in which small barium alginate capsules were produced for encapsulation of rat and porcine islets. The islet-containing capsules were purified from the empty capsules by discontinuous density gradient centrifugation. The recovery of encapsulated rat and porcine islets was about 70%. Using this technique, the total volume of the resulting microcapsules increased by only a factor of 2 compared to the volume of free islets.