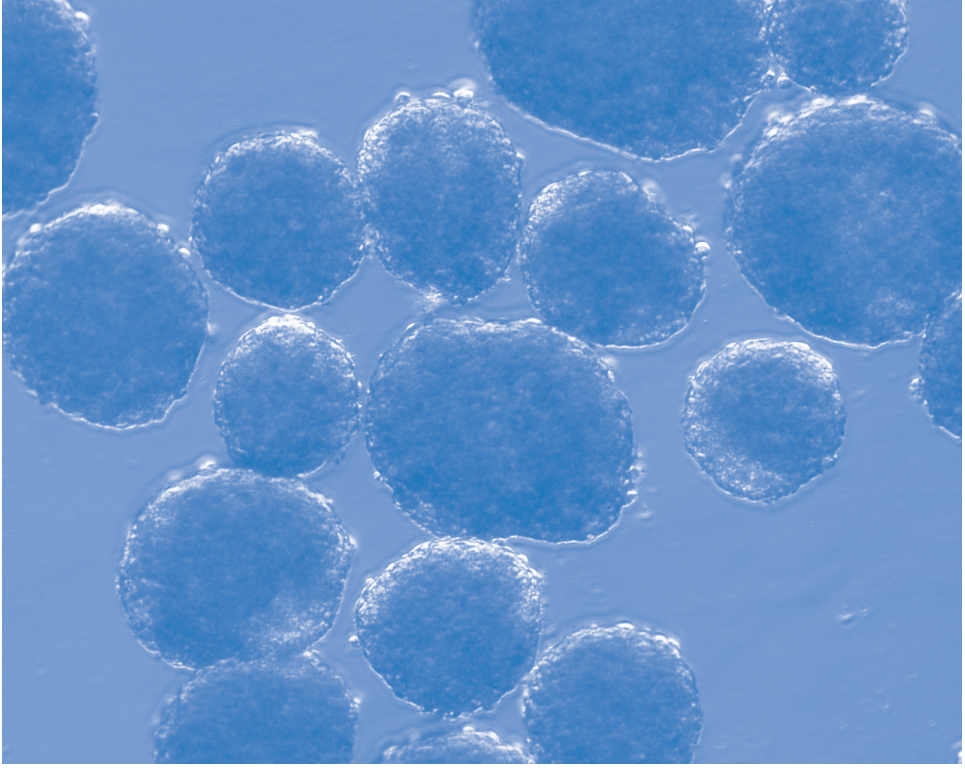

GOAL III:

DEVELOP CELL REPLACEMENT THERAPY

Recent Scientific Advances

Islet Transplantation: A Viable Therapeutic Alternative for Some Patients

- Infrastructure Creation To Improve Islet Isolation and Promote Basic Islet Research
- Achievement of Insulin Independence Using Islets from a Single Donor and Partial Pancreas
- Induction of Immune Tolerance and Development of Therapeutic Agent Pipeline
- Immune Monitoring for Early Diagnosis of Rejection and Tolerance

Islet Cell Biology

- New Technology To Study Developmental Biology of the Endocrine Pancreas
- Role of Master Control Genes in Regulating Formation of Pancreatic Beta Cells
- Recognition of the Regeneration Potential of Pancreatic Beta Cells
- Steps Toward the Creation of New Beta Cells from Stem Cells
- Imaging the Pancreatic Islet

Research Objectives and Strategies To Achieve Goals

Islet Transplantation

- Develop Novel Strategies and Infrastructure That Support Advancing Pancreas Procurement and Islet Processing
- Develop Improved Methods To Assess Islet Beta Cell Viability and Function That Predict Early Islet Function After Transplant
- Investigate the Use of Porcine Islets as an Alternate Source of Islets for Transplantation
- Improve Islet Transplant Procedures
- Develop Novel Methods To Accurately Assess the Post-Transplant Islet Mass
- Harness New Understanding of the Immune System To Develop Improved Clinical Monitoring and Immunotherapies

Pancreatic Development, Stem Cells, and Regeneration

- Grow a Renewable Supply of Pancreatic Beta Cells That Can Be Transplanted into Patients
- Understand How Mature Beta Cells Are Maintained and Replenished in the Adult Pancreas
- Develop Strategies To Regenerate Beta Cells Through Replication or Neogenesis

STRATEGIES TO DEVELOP CELL REPLACEMENT THERAPY

INTRODUCTION AND BACKGROUND

Islet transplantation has shown promise as a treatment strategy for type 1 diabetes, particularly in patients who suffer from recurrent bouts of hypoglycemia or who have already undergone a kidney transplant. Although the improvements in success rates with the therapy have brought tremendous hope for a cure, formidable obstacles impede widespread implementation of islet transplantation.

First, there is an inadequate supply of donor pancreata for the number of potential recipients. Researchers are seeking ways to optimize both the organ procurement and the islet isolation processes from these precious and finite resources. Consonant with these efforts, research is in progress to determine whether progenitor/stem cells[2] or genetically modified pancreatic cells could be "coaxed" to develop into islets or beta cells, and thus provide an unlimited source of cells for transplantation. Ongoing research in regeneration is also determining if adult beta cells could be coaxed to form more beta cells (replication) or if other resident cell types could be directed toward a beta cell fate (neogenesis). Researchers are also investigating the potential use of porcine islets as an alternative to human islets.

Following transplantation, substantial islet cell death and dysfunction occur within the first few hours and days. Unlike whole organ transplantation, in which blood flow to the transplanted organ is immediately restored, islets do not have an intact blood vessel system (vasculature). A rapid new growth of blood vessels is an absolute requirement for islet survival and function. Furthermore, early inflammation at the site of transplantation, and even the diabetic environment itself, could contribute to rapid programmed cell death (apoptosis). It is important for researchers to identify ways to promote successful islet engraftment and survival so that patients require fewer islets and/or transplants to produce sufficient amounts of insulin.

Another major obstacle to islet transplantation is the requirement for lifelong immunosuppression. Drug intervention is required to prevent rejection of transplanted islets and to prevent recurrence of the underlying autoimmunity that initiated the disease. However, these drugs can cause serious and adverse side effects. Research is crucial to identify less toxic methods to prevent islet rejection and the recurrence of autoimmunity, in order to realize the promise of islet transplantation. Currently, only adult patients with exceptionally brittle diabetes and recurrent hypoglycemia, or patients with end-stage renal disease, are eligible for this experimental therapy due to the toxicity associated with the required immunosuppressive medicines.

New technologies on the horizon may dramatically improve the success of islet transplantation. For example, new *in vivo* imaging technologies are being developed that would allow transplant surgeons to "see" the islets after transplantation and monitor the effectiveness of transplantation therapy in patients. The translation of this technology from animal models to human patients would constitute a real breakthrough in the ability to detect disease and to monitor treatment in patients with type 1 diabetes.

Cell replacement therapy can eliminate not only the need for the endless finger sticks and needles, as well as anxiety, currently endured each day by type 1 diabetes patients, but also the devastating long-term disease complications. If cell replacement therapy could be done with reduced toxicity, many more people besides the limited group with exceptionally severe hypoglycemia or renal failure could benefit.
To make the widespread use of this therapy a reality, it is imperative to promote basic research in islet cell biology—to understand beta cell development and regeneration—as well as clinical research in islet transplantation. From both basic and clinical avenues of research will emerge strategies to permit long-term post-transplant function of the insulin-producing cells without the need for lifelong recipient immunosuppression.

[2] The NIH supports human embryonic stem cell research consistent with federal funding policies.

Islet Transplantation: A Viable Therapeutic Alternative for Some Patients

Within the past 5 years, transplantation of human pancreatic islets has been shown to reproducibly reverse debilitating severe hypoglycemic episodes and enable some patients to become insulin independent. These impressive findings, first reported by the Edmonton group, have led to a renewed interest in the islet transplantation field. The dissemination of the successful Edmonton Protocol from the original few centers to sites across the United States and throughout the world was an important research achievement. The Edmonton findings have now been confirmed by independent centers throughout North America and Europe. An international, multicenter trial of the Edmonton Protocol was completed by the Immune Tolerance Network at nine sites, showing that the protocol could be replicated with success, depending on the experience of the site. Worldwide, more than 500 patients have been treated since 2000 (19). The data obtained by the centers in North America are available through the Collaborative Islet Transplant Registry (CITR), which was established and funded by NIH to compile and analyze this information. Analysis of the data clearly shows that islet transplantation is a viable therapeutic alternative for some patients. The ability to successfully disseminate the Edmonton Protocol has also spurred the creation of a recently launched NIH-supported Clinical Islet Transplantation Consortium, which is building upon the success of the Edmonton Protocol to further improve the safety and long-term success of methods for islet transplantation. The number of transplant centers performing clinical islet transplantation continues to increase, and insulin independence without hypoglycemic episodes is now an achievable goal for some patients.

Infrastructure Creation To Improve Islet Isolation and Promote Basic Islet Research: Pilot clinical trials have demonstrated that insulin independence and long-term islet graft function could be obtained not only with islets processed and transplanted at the same institution, but also with islets processed at regional NIH-funded Islet Cell Resource Centers (ICRs) and shipped for transplantation at remote institutions across the United States. This success has validated the concept that regional centers could be utilized for islet cell processing and distribution. Furthermore, the establishment of the ICRs has enabled an infrastructure that permits collaborative optimization of pancreas shipping devices, preservation media, islet isolation technology, and interim storage through comparative assessments. The collaboration has also led to the identification of salient roadblocks to large-scale islet production and transplantation. The ICRs provide resources, structure, and a coordinated community of investigators focused on enhancing the quality of isolated islets, promoting basic islet research, and enabling additional facilities to perform the procedures. The ICRs work closely with the CITR to collate and disseminate data on islet procurement and production, as well as on clinical outcomes following transplantation in North America. This joint effort facilitates comparative analyses that will eventually define the safest and most effective clinical protocols.

Achievement of Insulin Independence Using Islets from a Single Donor and Partial Pancreas: The improved success rates of islet transplantation have largely been achieved using islets isolated from two or three donor pancreata. Recently, success has been realized using both single donors and islets isolated from part of a pancreas taken from a living donor. One study tested a single-donor procedure on eight patients with type 1 diabetes and found that all patients achieved insulin independence and freedom from hypoglycemia. Five patients remained insulin-independent for more than 1 year. Another study showed that islets could be isolated from a portion of the pancreas of a closely related living donor and transplanted into a patient with type 1 diabetes. Successful transplantation with fewer islets can be attributed to improved isolation procedures resulting in increased islet viability and survival. Thus, improved islet isolation procedures in the future could help to overcome the current barrier regarding the shortage of islets available for transplantation.

Induction of Immune Tolerance and Development of Therapeutic Agent Pipeline: As described in the preceding chapters, the immune system has the complex task of recognizing foreign molecules, while disregarding molecules native to the host ("tolerance"). Long-term islet graft survival in animals without the requirement for long-term immunosuppression has been demonstrated using methods that suppress costimulatory signals (key signals for discriminating between foreign and self molecules) to immune cells. The success achieved in animal models showing sustained islet graft survival and reversal of diabetes after discontinuation of all anti-rejection drugs suggests that tolerance may also be achievable in humans. Monoclonal antibodies, other blocking agents, and selected cytokine combinations are under intense investigation to prevent immune activation and induce

immune tolerance in islet transplantation. Identification of novel therapeutic agents promises to overcome the current barrier imposed by the requirement for chronic immunosuppressive therapy after transplantation. These same therapeutic agents may also have added utility for preventing or reversing the initial autoimmune process. Some new agents studied in other diseases and/or animal models are already moving into pilot clinical trials of islet transplantation. Additional novel molecules and drugs are currently in the pipeline, or are already at the pre-clinical level of testing. These molecules may soon become available for pilot clinical trials of islet transplantation. These new therapeutic agents may enable more effective modulation of the immune response, while also decreasing the side effects associated with chronic administration of currently available anti-rejection drugs.

Immune Monitoring for Early Diagnosis of Rejection and Tolerance: Many clinical and biological markers can be used to determine if a solid organ graft is being rejected. In contrast, there is no biochemical marker for islet rejection that enables detection of islet loss early enough following transplantation to permit effective intervention and rescue. At the time of documented hyperglycemia and need for return to exogenous insulin administration, significant islet loss has already occurred. This observation is similar to the situation that occurs at the onset of type 1 diabetes, as described in the previous chapters. Scientists have recently demonstrated elevated expression of several key genes in the peripheral blood associated with inflammation—an event that precedes clinical evidence of post-transplant islet loss. Gene expression profiles may serve as molecular signatures that foretell impending graft rejection. In addition, these profiles may provide predictive guideposts for withdrawal of immunosuppression. Early detection of destructive processes will guide the development of effective intervention strategies to reverse immune activation after islet transplantation, before islet cell destruction occurs.

Islet Cell Biology

Many scientific advances over the past 5 years have markedly advanced the understanding of how pancreatic beta cells develop and function, as well as how they are adversely affected in type 1 diabetes. These advances have been due largely to new information flowing from completion of the human and mouse genome sequencing projects and the development of novel technologies that have enabled researchers to study the genes expressed in the pancreas—both in humans and in a variety of model organisms. Research progress in this field has also been accelerated by the creation of the Beta Cell Biology Consortium (BCBC), which consists of a team of researchers who generate publicly available resources and tools that could greatly advance the study of beta cell development and regeneration.

New Technology To Study Developmental Biology of the Endocrine Pancreas: A few key genes code for special transcription factor proteins that regulate the expression of many other genes. To understand the necessary steps for a progenitor/stem cell to develop into a beta cell, it is important to identify the transcription factors and the downstream target genes that mediate this transition. A major barrier to determining these steps is that these progenitor cells are transient in nature and are found in vanishingly small numbers. Furthermore, no methods exist to prospectively identify and isolate purified populations of pancreatic progenitor/stem cell populations. Recently, scientists in the BCBC have created mouse models that allow researchers to visually track the expression of transcription factors that characterize pancreatic progenitors at various stages of progression toward mature beta cells. Using these genetically engineered mice, researchers could isolate pancreatic beta cells using an experimental technique called fluorescence activated cell sorting (FACS). This advanced technology yields pure populations of mouse pancreatic beta cells at different stages of development. These cell populations can then be used to gain further insights into which genes regulate beta cell development and function. This approach will enable researchers to identify appropriate cell surface markers on pancreatic progenitor cells. Pursuit of this research avenue could pave the way to the isolation and prospective purification of human progenitor cell populations that will mature into insulin-producing beta cells.

Role of Master Control Genes in Regulating Formation of Pancreatic Beta Cells: Researchers have identified important transcription factors that have essential roles in either the formation or the function of the pancreas, pancreatic islets, or pancreatic beta cells. When mutated, some of the transcriptional regulators expressed in pancreatic beta cells during development have been found to cause rare forms of diabetes mellitus termed Maturity Onset Diabetes of the Young (MODY). Identification of many of these transcription factors was the result of years of systematic studies of the insulin promoter, the part of the insulin gene that regulates its expression. This research pinpointed specific regulatory DNA sequences within the promoter and the transcription factor proteins that bind to them. Other key genes were serendipitously discovered. For instance, the gene *neurogenin 3* (*ngn 3*), which was being studied for a possible role in brain development, was found to be essential for the formation of

pancreatic islets, including beta cells. The identification and molecular characterization of key transcription factors, such as *Pdx-1* (a master regulator for formation of the endocrine pancreas and a MODY gene), provide a starting point for understanding the complex gene regulatory networks that exist within both the pancreatic progenitor cells and the mature beta cells. These studies can help researchers identify the necessary steps to turn progenitor/stem cells into insulin-producing beta cells.

Recognition of the Regeneration Potential of Pancreatic Beta Cells: Evidence gained over the past 5 years indicates that both humans and animals have some ability to regenerate beta cells. Thus, it may be possible to restore beta cell mass in type 1 diabetes patients whose beta cells are not completely destroyed. Many tissues have been found to contain progenitor/stem cells that could restore lost cell types. However, it is not yet clear whether such a cell type exists in the pancreas or, if so, whether it can form new beta cells after all existing ones have been damaged or destroyed. Recent studies in mice imply that the proliferation of new beta cells after injury contributes predominantly to the new beta cell population. This observation contradicts older models of pancreas regeneration. These novel findings need to be expanded, in order to enhance understanding of the regenerative potential of beta cells and other resident pancreatic cells, as well as to determine whether regeneration is a clinically significant process. A dampening of autoimmunity through intervention with Freund's complete adjuvant has been reported to reverse autoimmune diabetes in mice. The reversal was attributed to a restoration of beta cell mass that occurred through regeneration and suggests that it may be possible to devise similar regeneration approaches in humans. In humans, there is morphological evidence for beta cell regeneration, even in patients with long-standing type 1 diabetes. Such studies are encouraging because, even if the number of residual beta cells is small, perhaps only a few cells may be needed to generate sufficient numbers of cells to restore lost beta cell function in a patient with type 1 diabetes.

Steps Toward the Creation of New Beta Cells from Stem Cells: In 1998, researchers reported deriving the very first human embryonic stem (ES) cells. Since then, the potential of inducing human ES cells to form a wide variety of other cell types has been demonstrated by several groups of researchers. With these remarkable advances, the possibility has emerged of generating from human ES cells large quantities of either pancreatic beta cells or whole pancreatic islets. However, several barriers have become apparent as a result of attempts to convert human ES cells into insulin-secreting beta cells. The major barrier is the lack of knowledge about how to direct the differentiation of ES cells, or any other progenitor/stem cell type, toward a pancreatic beta cell fate. It has become clear that ES cells, or any other starting cell type, may need to be induced to pass through many intermediate cell fates, just as occurs when pancreatic beta cells are formed during development. This avenue of research has the potential to create an unlimited supply of islets for transplantation, which can help to overcome the current clinical barrier created by an insufficient number of available cadaveric donor pancreata.

Imaging the Pancreatic Islet: Since 1999, there has been significant progress toward directly visualizing the pancreatic beta cells, transplanted islets, and inflammation of type 1 diabetes using imaging technologies, particularly positron emission tomography (PET) and magnetic resonance imaging (MRI) (see Goal VI). Isolated human islets have been labeled with nontoxic imaging agents that allow them to be seen after transplantation into animals. Targeting molecules are being developed that can carry imaging agents directly to proteins on the beta cell surface, in order to count the number of beta cells in people. The visualization of early beta cell loss would enable imaging to be used as a noninvasive diagnostic tool for type 1 diabetes, as well as a means to follow the progression of the disease and monitor the effectiveness of transplantation therapy in patients. When the pancreas is under attack by the immune system, its blood vessels become "leaky." This process can be visualized through use of an imaging molecule that moves from the blood into the inflamed tissue. This ability to actually see the cells and processes associated with disease *in vivo* could help researchers better understand the life cycle of the islet and how it is damaged in diabetes.

Islet Transplantation

The overall clinical experience in islet transplantation has highlighted the complexity of a sequential approach that can last days between pancreas procurement, islet processing, pre-transplant culture, recipient immune-conditioning, and the final islet infusion. To maximize the use of scarce donor islets and minimize the risk of procedure-related complications, highly specialized and multidisciplinary teams are required. It is necessary not only to obtain uniformly high-quality islet cell products, but also to ensure optimal islet infusion techniques, infusion sites, and effective post-transplant patient management. A major barrier to transplantation is the immune response of the recipient against the donor islets. The toxicity associated with current immunosuppression makes islet transplantation appropriate for only a very limited subset of patients with type 1 diabetes. Moreover, although current protocols achieve remarkable success in achieving immediate insulin independence and/or improved glucose control without severe hypoglycemia, longer-term follow-up suggests that this success wanes over time. Chronic rejection and recurrent autoimmunity are major challenges to be overcome in this regard. The following research objectives are critically important for a stepwise, integrated approach to develop successful cell replacement strategies to treat diabetes.

Research Objective—Develop Novel Strategies and Infrastructure That Support Advancing Pancreas Procurement and Islet Processing:

- *Study potential donor interventions that minimize the negative effects of brain death and ischemia (low blood supply)/hypoxia (low oxygen) on islet survival and function.*
- *Develop improved preservation medium, shipping containers, and monitoring technologies to improve pancreas preservation during transport.*
- *Develop improved islet isolation and purification methods and novel methods for tissue processing, beyond the currently available enzyme-blend techniques.*
- *Develop new strategies to improve pre-transplant islet culture that will sustain graft survival and function.*

Prior to transplantation, islet grafts have been exposed to a series of nonphysiological conditions that sequentially contribute to a progressive reduction of the original islet cell mass. This loss of insulin-producing tissue results from the collective incremental effects of many factors, including the molecular effects of donor injuries preceding islet isolation associated with brain death and hypoxia; prolonged time of pancreas cold preservation and shipment to the islet cell processing center; enzyme-based tissue digestion techniques; and islet purification steps and pre-transplant culture/shipment to the final transplant facility. Improvement at all phases of these processes and technologies will be critically important to minimize islet loss and maximize the potential use of each donor pancreas. For example, potential strategies could include the delivery of anti-inflammatory agents and/or agents that enable survival of the pancreatic islets prior to procurement. These improvements may ultimately allow transplantation of sufficient numbers of islets obtained from only a segment of donor pancreas, such as in the case of living donor islet transplantation.

Research Objective—Develop Improved Methods To Assess Islet Beta Cell Viability and Function That Predict Early Islet Function After Transplant:

- *Define and implement novel strategies and methods for assessment of beta cell-specific viability and function.*
- *Develop predictive tests to determine the suitability of an islet cell product for clinical use (i.e., tests predictive of post-transplant survival and function).*

To predict transplant success, improved assays to assess islet quality are needed that report on beta cell-specific parameters, rather than general traits, such as oxygen consumption. Such assays are needed because the ratio of beta cells to other pancreatic cells can vary greatly among preparations, and robust pancreatic exocrine cells in a partially purified islet preparation can easily utilize enough oxygen to mask damaged beta cells. The NIH has established ICRs across the country to provide high-quality human islets for treatment of type 1 diabetes and for basic research. The ICRs share a mission to improve the quantity and quality of available human islet tissue. They have been pivotal in establishing some uniform measures of "functional" testing of islets. These measures already include testing of glucose-stimulated insulin release, but research would benefit from new surrogate markers of islet quality, which are beta cell-specific and correlate well with engraftment potential. Toward this end, research teams that include cell biologists and clinicians should work together to establish and standardize functional testing, and to identify islet factors that predict success or failure in achieving insulin independence. Candidate assays include apoptosis markers and measures of cell function, measures

of beta cell antigenicity, as well as characterization based on proteomic or genomic technologies. An adequate measure of beta cell function would correlate well with the ability of the islets to restore insulin independence in an appropriate animal model (e.g., NOD-scid mice) and in human transplantation. Once such tests are established, it will be important to identify factors in the islets that contribute to engraftment success and failure.

Research Objective—Investigate the Use of Porcine Islets as an Alternate Source of Islets for Transplantation:

- *Develop strategies to overcome hyperacute rejection.*
- *Address immunological barriers to xenotransplantation.*
- *Pursue regimens for immune tolerance induction to xenografts.*

Xenotransplantation offers a potential solution to the severe shortage of pancreata needed to treat patients with type 1 diabetes. Currently, the swine is the primary species of interest, due to its favorable reproductive capacity, as well as anatomical and physiological similarities to humans. However, xenotransplantation currently poses significant challenges, including: the immune response of the recipient against the xenograft; the physiological limitations of proper functioning of the transplanted organ/tissue; and potential transmission of infectious agents, such as porcine endogenous retrovirus, from the graft to the recipient. Recently, researchers have genetically engineered pigs whose organs are not subject to hyperacute rejection when transplanted into patients and that can be used for future research studies of xenotransplantation. The NIH is supporting a research consortium studying pre-clinical, porcine to non-human primate models of xenotransplantation. Future efforts will build upon novel preclinical findings toward a goal of making xenotransplantation a viable therapeutic strategy for patients with type 1 diabetes.

Research Objective—Improve Islet Transplant Procedures:

- *Determine the optimal sites for islet transplantation.*
- *Develop novel islet survival strategies.*

Currently, the liver is the preferred site for islet transplantation, in which islets are infused into the portal vein. However, the liver is a suboptimal or even hostile environment for transplanted islets, which may contribute to limited islet longevity. After infusion into the liver and upon entering the blood stream, islets are immediately exposed to a chemical assault. For example, islets embedded in the liver are exposed to above normal amounts of metabolic toxins and are subjected to high levels of toxic immunosuppressive drugs. Prior to engraftment, islets must also survive without the assistance of an intact network of blood vessels (in contrast with whole organ transplants). Therefore, novel approaches are needed to ensure engraftment immediately after transplant, as well as to increase the lifetime of the graft. Islets transplanted into the liver have been shown to lose their ability to respond to hypoglycemia (termed a "counter-regulatory response"). This impaired response is not seen when the islets are placed in other sites. Furthermore, changes in liver structure have been recently described following some cases of islet transplantation. Possible alternatives to the liver as a site for islet engraftment include the spleen, omentum, pancreas, and muscle. Several of these alternative sites would have the added advantage of enabling clinician scientists to retrieve and replace grafts. Preventing early post-transplant islet loss remains a challenge, but is critical to graft survival.

Research Objective—Develop Novel Methods To Accurately Assess the Post-Transplant Islet Mass:

- *Define and implement post-transplant metabolic testing of the transplant recipients to estimate: (1) functional islet mass that successfully engrafted, and (2) eventual changes in functional islet mass in long-term post-transplants.*
- *Develop novel strategies for imaging islet cells post-transplant and/or in the native pancreas (PET, MRI, video-endoscopy, in vivo microscopy).*

It is essential to develop novel methods to accurately assess the mass of insulin-producing tissue that successfully engrafts post-transplant. Both metabolic and imaging strategies are needed to define islet survival during the early post-transplant period as compared to the islet mass initially infused. These methods could permit monitoring of long-term changes in islet mass not only at the transplant site, but also in the pancreas of the patient, in order to assess, for example, the effect of therapeutic strategies on beta cell regeneration in the native pancreas. Achieving this objective will require collaboration among physicians, imaging experts, chemists, and biologists (discussed more fully under Goal VI).

Research Objective—Harness New Understanding of the Immune System To Develop Improved Clinical Monitoring and Immunotherapies:

- *Identify markers of immune rejection and recurrent autoimmunity.*
- *Define effective strategies for immunomodulation of the recipient immune response and for tolerance induction following islet transplantation.*

- *Develop effective strategies for T cell regulation.*
- *Develop novel strategies for costimulatory blockade and expansion of candidate humanized monoclonal antibodies for costimulatory blockade.*
- *Employ tissue engineering strategies to protect transplanted islets from immune cell destruction.*

There is a great need for biochemical markers that are accessible in the peripheral blood and can be used for the timely detection of islet loss; assessment of inflammation; and effective intervention and rescue, when needed. Research is warranted to assess lymphocyte gene expression in blood as a means to monitor rejection. More research is also needed to develop complementary methods for monitoring early immune activation, such as noninvasive imaging before islet cell destruction occurs. As described in Goal II, promising areas of research include the development of cellular functional assays, as well as biomarkers based on broad measurements of gene expression, including proteomics. Such assessments could help provide the scientific rationale and guidance for the optimal time to adjust and eventually discontinue immune therapy. Novel strategies of immunomodulation have recently demonstrated in animals that islet transplantation could reverse diabetes without a requirement for continuous immunosuppression. Long-term survival of mismatched islets that enabled 100 percent insulin independence with normal blood glucose levels has been achieved in diabetic non-human primates treated with a brief course of costimulatory blockade. If comparable success using these reduced levels of toxic immunosuppression could be reproduced in patients with diabetes, clinical islet transplantation may ultimately provide a curative therapy. The limited exposure to immunosuppression is key because, as previously discussed in Goal II, risks accompanying continuous immunosuppression limit the numbers of potential beneficiaries for this procedure. Therefore, extension of translational research efforts is still required to define novel immune interventional strategies aimed at blocking costimulation. Efforts must be expanded to understand relevant mechanisms of immune regulation, especially those that induce specific tolerance to the graft. Additionally, researchers should explore novel strategies, such as those that exploit T cell regulation and the infusion of donor immune and progenitor cells to achieve tolerance. Tissue engineering strategies that incorporate materials or devices that keep the islets isolated from the immune system could also be enormously helpful in shielding islets from rejection (see Goal VI). Collectively, these objectives represent overlapping aims also shared with the prevention or reversal of type 1 diabetes in its early stages.

Pancreatic Development, Stem Cells, and Regeneration

Furthering basic research in developmental and stem cell biology of the pancreas would greatly enhance efforts to produce an abundant supply of pancreatic beta cells for transplantation, or to restore to normal the mass of damaged or destroyed beta cells in individuals with type 1 diabetes. It is currently unknown which stem/progenitor cells may be the most useful as a possible source of islets for transplantation. Thus, it is important to support research using different types of stem cells, including embryonic, adult, and cord blood, as well as studies of both human and animal cells, because research on each type will build knowledge of how beta cells are formed and maintained.

Of paramount importance is the creation and free distribution of reagents of particular usefulness to beta cell biology research, such as polyclonal and monoclonal antibodies and relevant animal models and cell lines, as well as the application of tools of functional genomics and proteomics. These key reagents and resources are needed to accelerate research focused on identifying, isolating, and differentiating islet stem/progenitor cell populations to generate beta cells, as well as to propel research on understanding mechanisms of beta cell regeneration. The creation and utilization of these key resources will open new avenues toward the development of cell-based therapies in diabetes.

Research Objective—Grow a Renewable Supply of Pancreatic Beta Cells That Can Be Transplanted into Patients:

- *Identify and characterize genes that play particularly critical roles in the formation of the pancreas.*

By understanding pancreatic development, researchers may be able to recapitulate normal development of beta cells in tissue culture using stem cells obtained from the patient or from other human donors. It will be necessary to perform more standardized and highly defined studies of both the gene and protein expression profiles of pancreatic beta cells and their progenitors during development. One strategy is the application of bioinformatics tools, as they become available, for modeling the sequential activation or repression of genes during pancreatic organogenesis. These studies should be directed at identifying, defining, and then characterizing the changes that occur in gene regulatory networks. Detailed genetic studies are also needed to understand the functional

importance of these regulatory genes in a mammalian model organism such as the mouse. While the mouse should remain the primary model system, other genetic model systems such as zebrafish could be exploited for discovering novel genes and pathways involved in pancreatic development. Characterizing the regulatory mechanisms that underlie the formation of the endocrine pancreas will provide the basis for understanding how to grow pancreatic islets in the laboratory for ultimate use in islet transplantation. Another fundamental question to address is how newly forming beta cells acquire their antigenicity. Identifying and characterizing genes that play a role in this process will allow investigators to test whether type 1 diabetes is initiated by a defect in the beta cell—research that could provide new information about the etiology of type 1 diabetes.

- *Develop reagents and protocols for isolating pancreatic endocrine progenitor cells.*

The development of monoclonal antibodies to cell surface markers of beta cell precursors would allow the rapid isolation of target populations after ES cells have been induced to differentiate. To this end, key resources need to be developed, including a collection of mouse and human ES cell lines that are genetically tagged with markers that faithfully report the expression of genes specific to pancreatic progenitor cell types. Together, these research resources would enable the prospective isolation of progenitor intermediates, one of the first steps toward making pancreatic islets in culture.

- *Identify growth conditions that permit the stepwise differentiation of beta cells from stem cells or precursor cells.*

A multitude of signaling pathways is active during formation of the endocrine pancreas. However, new research is needed to determine the correct combinations of growth factor signals required at each stage of development. Furthermore, high-throughput screens are needed to identify novel, small molecules that would enhance stage-specific differentiation of the endocrine pancreas and expansion of progenitor cells. Accruing this missing knowledge would allow the creation of a protocol to both expand and differentiate cells—a key step in the development of a stem/progenitor cell-based replacement therapy.

- *Develop animal models to test the engraftment, survival, and metabolic impact of beta cells or islets derived in culture from stem/progenitor cells.*

Quantitative transplantation assays are needed to assess the efficacy of cell replacement therapies. Mouse models that are engineered to accept human cells or non-human primate models for safety testing are of particular importance. The development of appropriate animal models for testing potential cell replacement therapies is a critical step before human therapies can be realized.

- *Determine if multipotent cells from fetal and adult tissue could be viable sources for beta cell replacement therapy.*

Many potential sources of cells could be used as a starting point for generating pancreatic islets. These sources could include cells from pig, human amniotic cells, and adult human multipotent cells isolated from bone marrow, liver, pancreas, or gut. Additional research is needed to determine which cell type(s) could be the most therapeutically useful sources.

Research Objective—Understand How Mature Beta Cells Are Maintained and Replenished in the Adult Pancreas:

- *Determine the mechanism by which beta cell number is restored after beta cell loss.*

A new challenge in beta cell research is to understand aspects of "islet maintenance," in particular, how an islet regulates its mass. Assessing beta cell mass and turnover quantitatively will require the development of better assays. The generation of monoclonal antibodies to cell surface markers on mature islet cell types, including beta cells, will aid in the development of new imaging methodology to assess beta cell mass *in vivo*. Researchers should investigate in animal models whether proliferation of existing beta cells, or alternatively, the proliferation of other cells in the pancreas, contributes significantly to the formation of new beta cells.

- *Identify factors and agents for enhancing beta cell division or decreasing cellular apoptosis.*

Testing the ability of hormones and peptides to increase beta cell proliferation and/or induce autologous beta cell regeneration should be systematically explored in animal models, both in rodents and in large animal models, such as pigs and non-human primates. High-throughput screens could be developed to identify combinations of growth factors or agents that enhance beta cell growth. A project has been initiated to develop immortalized human pancreatic beta cell lines that are functionally equivalent to primary beta cells. The transplantation of human beta cells derived from these lines into appropriate type 1 diabetes animal models will be an important first step toward the development of novel cell therapies. Investigators should determine why there are functional differences between isolated beta cells and the beta cells located within a pancreatic islet. Understanding the basic mechanisms that regulate and maintain beta cell number and

function could lead to strategies for preserving and/or restoring lost beta cells in individuals whose cells have come under attack by their own immune systems.

Research Objective—Develop Strategies To Regenerate Beta Cells Through Replication or Neogenesis:

- *Enhance understanding of the regenerative potential of beta cells.*
- *Determine whether beta cell replication or neogenesis is a clinically significant process.*
- *Develop therapeutic strategies to promote beta cell regeneration.*

Clinical research studies have demonstrated that even patients with long-standing type 1 diabetes still have some remaining beta cells in their pancreata. Furthermore, recent research has shown that both humans and animals have some ability to regenerate beta cells. Therefore, a possible therapeutic approach for treating type 1 diabetes is to promote regeneration of patients' remaining beta cells. Research is needed to fully understand the regenerative potential of beta cells before it can be determined whether *in vivo* regeneration could be explored as a potential therapeutic approach. It is also possible that, with additional insights into beta cell regeneration, islets isolated from human pancreata could be coaxed to form more beta cells in the laboratory, which would be another approach to increasing the number of islets available for transplantation. The knowledge gained through studies of the underlying molecular mechanisms of beta cell development and function, as described previously, is essential to developing strategies for the regeneration of beta cells and may also provide clues for increasing the beta cell mass in people with type 1 and type 2 diabetes.