Title
Beta-cell replacement for type 1 diabetes

Journal
Annual Review of Medicine, 55

Authors
Stock, P G
Bluestone, Jeffrey A

Publication Date
2004

DOI
10, 1146/annurev.med.

Peer reviewed

Annu. Rev. Med. 2004. 55:133–56
doi: 10.1146/annurev.med.55.091902.103539

BETA-CELL REPLACEMENT FOR TYPE I DIABETES

Peter G. Stock[1] and Jeffrey A. Bluestone[2]
[1]Department of Surgery, Division of Transplantation, University of California, San Francisco, San Francisco, California 94143; [2]Diabetes Center, Department of Medicine, Pathology, Microbiology and Immunology, University of California, San Francisco, San Francisco, California 94143; email: stockp@surgery.ucsf.edu, jbluest@diabetes.ucsf.edu

Key Words pancreas, islet, transplantation, tolerance, stem cell

■ **Abstract** The ability to achieve insulin independence with either solid-organ pancreas or islet transplantation has increased the number of patients seeking beta-cell replacement as an alternative to insulin therapy. Despite dramatic improvements in the ability to achieve insulin independence following solid-organ pancreas transplantation, the secondary complications of long-standing diabetes are frequently irreversible by the time surgical intervention is justified based on the risk of this procedure. Pancreatic islet transplantation provides a safer and less invasive alternative for beta-cell replacement that could be justified earlier in the course of diabetes to prevent the development of secondary complications. Recent advances in the technology of islet isolation, as well as the ability to prevent the alloimmune and recurrent autoimmune response following islet transplantation with immunosuppressive regimens that are not toxic to beta cells, have rekindled an interest in this field. Widespread application of islet transplantation will depend on further improvements in selective immunosuppression, development of immunologic tolerance, and finding new sources of beta cells.

INTRODUCTION

Dramatic improvements in the success of the transplantation of pancreatic tissue, either the whole organ or pancreatic islets, have sparked a renewed interest in transplantation as a treatment for diabetes mellitus. Pancreas transplantation has become a widely accepted treatment for type I diabetic patients who have undergone a previous or simultaneous kidney transplant. The success rate (success defined as normoglycemia and insulin independence) is currently >80% at 3 years (1, 2). However, solid-organ pancreas transplantation in the preuremic recipient is not widely accepted mainly because of the associated surgical complications and the need for vigorous immunosuppression, both of which contribute considerably to the overall morbidity and costs of this procedure (3). The secondary complications of long-standing diabetes are frequently irreversible by the time surgical intervention is justified based on the risks of the procedure, underscoring the

necessity of a safer and less invasive procedure for beta-cell replacement that could be justified prior to the development of the secondary complications. The efficacy of pancreatic islet transplantation, a significantly less invasive procedure, was only recently demonstrated by the achievement of insulin independence in seven consecutive type I diabetic recipients after percutaneous portal-vein transplantation of pancreatic islets (4). As a result of the significant improvements attained in the "Edmonton protocol," multiple centers across the United States and worldwide are developing programs to transplant pancreatic islets as an alternative method of beta-cell replacement. This review addresses the development of the current protocols for islet transplantation, as well as future strategies for extending the application of this new technology.

BACKGROUND: THE EVOLUTION OF BETA-CELL REPLACEMENT

The Problem

The incidence of diabetes mellitus is predicted to increase significantly in the next decade, and it already affects an estimated 130 million people worldwide. It affects 16 million Americans and consumes one out of every eight health care dollars. Despite the efficacy of insulin therapy, the devastating secondary complications, including nephropathy, neuropathy, retinopathy, and cardiovascular disease can shorten life expectancy by as much as one third. The Diabetes Control and Complications Trial demonstrated that tight regulation of blood sugars with intensive insulin therapy significantly lowered the level of the glycosylated hemoglobin (HbA_{1C}) and minimized the progression of the secondary complications. Nonetheless, even intensive therapy did not abrogate the development of secondary complications, and tight control resulted in a significantly higher risk of severe hypoglycemic reactions leading to seizure or coma (5, 6).

Solid-Organ Pancreas Transplantation

Solid-organ pancreas transplantation has undergone significant progress in the past decade. It has been the most consistent method of beta-cell replacement, resulting in sustained euglycemia, insulin independence, and normalization of HbA_{1C}. The most important advances have been in preventing rejection of this highly immunogenic transplant. The addition of mycophenolate mofetil and tacrolimus to immunosuppressive regimens decreased the incidence of rejection following pancreas transplantation from 80% to $<20\%$ at most centers performing this procedure. Antibody induction therapy with antilymphocyte preparations, along with the addition of sirolimus, an immunosuppressive agent that lacks nephrotoxicity or beta-cell toxicity, has permitted steroid-free maintenance therapy following solid-organ pancreas transplantation with continued low rejection rates. Despite the elimination of steroids from maintenance regimens, the incidence of rejection

of this highly immunogenic organ is currently reported to be <10% (7, 8). The dramatic improvement in the ability to prevent rejection of pancreas transplants is reflected in the results reported by the International Pancreas Transplant Registry (9). The three-year graft survival, as defined by insulin independence, is ~80% for pancreas transplants performed simultaneously with a kidney transplant as well as for pancreas transplants performed after a successful kidney transplant. Progression of the secondary complications of diabetes is curtailed by the presence of a functioning pancreas transplant, and the development of diabetic nephropathy is prevented in the simultaneously transplanted kidney (10). Similarly, there are gradual improvements in neuropathy (11) and overall improvements in the quality of life (12).

These highly significant improvements in success have been the impetus to proceed with solitary pancreas transplantation in the preuremic patient with life-threatening diabetes. Although pancreas allografts in the preuremic diabetic recipient are more vulnerable to rejection (rejection loss at one year equals 8% for these patients versus 2% for those who had simultaneous pancreas and kidney transplants), the improved results have increased the frequency of this procedure (2). Nonetheless, because of the invasive nature of the surgery and long-term complications of the rigorous immunosuppressive regimens, this procedure remains limited to patients with hypoglycemic unawareness or metabolic lability despite intensive insulin therapy. The transplantation of pancreatic islets provides an attractive and less invasive alternative to solid-organ transplantation. In addition to avoiding the technical complications of solid-organ transplantation, related to thrombosis of the blood supply to the whole-organ allograft and the danger of activation of the digestive enzymes associated with the exocrine function, pancreatic islet transplantation provides the opportunity to manipulate the islets prior to transplantation in order to decrease immunogenicity of the allograft. Of equal significance, this technique has the potential to provide an unlimited source of beta cells that would ultimately be independent of the limited donor pool. The proliferation of beta cells from either embryonic or adult stem cells is most promising, although xenogeneic islets may prove to be an attractive alternative source pending further refinement in immunosuppressive protocols and resolution of concerns related to endogenous animal viruses.

The History of Islet Transplantation

The first successful transplantation of pancreatic islets in experimental animal models was performed in 1972 (13, 14) and the first clinical islet allograft in a diabetic recipient of a previous kidney transplant in 1974 (15–17), but pancreatic islet transplants did not immediately emerge as a successful alternative to solid-organ transplantation. Despite enthusiasm for this procedure and promises that islet transplantation was just around the corner, the success of islet transplantation as defined by insulin independence was minimal. Over the 25 years following the first clinical attempt at islet transplantation, over 300 alloislet transplants in type I

diabetic patients were performed and described in a comprehensive report by Hering & Ricordi (18). Although many of the islet allografts had some function as evidenced by C-peptide, few of the transplants provided even transient states of insulin independence despite the use of potent immunosuppressive drugs. This comprehensive report also reviewed over 3000 fetal grafts, neonatal grafts, and xenografts performed worldwide during the same time period and reported to the International Islet Registry; 96% of these reports came from China and Eastern Europe. Although there were occasional reports of insulin independence (18, 19), the vast majority of these cases failed to demonstrate insulin independence or long-term engraftment as defined by presence of C-peptide.

Disappointment in islet transplantation based on the low frequency of insulin independence as compared to solid-organ transplantation was tempered by the success of islet autotransplantation to prevent diabetes following total pancreatectomy for chronic pancreatitis (20). The long-term efficacy of islet autotransplants has been reported by the transplant group at the University of Minnesota, who had successfully performed this procedure since the 1970s. The intraportal infusion of pancreatic islets isolated from nondiabetic patients suffering from pancreatitis has produced states of insulin independence for as long as 13 years following total pancreatectomy. This finding demonstrates that islet transplantation can be a viable alternative to solid-organ transplantation (21).

Successful states of insulin independence required the successful isolation of a significant number of islets following pancreatectomy, which underscores the necessity of a sufficient mass of islets to achieve insulin independence. Achieving insulin independence following allogeneic islet transplantation into a type I diabetic recipient is further complicated by the necessity for preventing both alloimmune and autoimmune destruction. The historic success of alloislet transplantation for nondiabetic patients with diabetes precipitated by surgical removal of the pancreas may imply the existence of an additional barrier to autoimmune disease directed against beta cells, which presumably recurs following islet transplantation. In the largest series, reported from the University of Pittsburgh (22, 23), simultaneous islet-liver allotransplantation after upper abdominal exenteration including total pancreatectomy resulted in insulin independence in seven patients. Several patients were able to sustain insulin independence until death secondary to malignancy, including one patient who was insulin-independent for five years until his death from malignancy. The recurrence of malignant disease following this procedure has prevented the continued application of this strategy. Nonetheless, the success of islet transplantation in this setting relative to islet allotransplants performed into type I diabetic recipients during the same era provided indirect evidence of the additional immunologic barrier in patients with autoimmunity against beta cells. Further reports of success following islet allotransplantation into patients without autoimmune disease, including diabetes secondary to cystic fibrosis (24) and hemochromatosis (25), underscored the importance of developing immunosuppressive strategies effective against both autoimmune and alloimmune responses.

The strength of the autoimmune response contributing to beta-cell destruction was conclusively demonstrated in the landmark paper by Steffes et al. (26), in which a living-donor pancreas transplant between identical twins was rejected by the recipient. Pathologic confirmation that the recurrent autoimmune process specifically destroyed beta cells, but left the rest of the pancreas intact, attested to the potency of the autoimmune response leading to the destruction of beta cells.

While the field of solid-organ transplantation for the treatment of type I diabetes enjoyed increasing success throughout the 1990s related to improvement in surgical techniques and immunosuppressive regimens, islet transplantation stagnated as a result of its relative failure to achieve insulin independence. The international registry, maintained in Giesen, Germany, demonstrated that as of 1990, $<10\%$ of the 447 islet allografts performed worldwide were able to sustain insulin independence (9). These disappointing results were attributed to the difficulty of obtaining satisfactory yields of functional islets following isolation, the toxic effects of the available immunosuppressive agents known to have beta-cell toxicity, and the inability to prevent alloimmunity as well as recurrent autoimmunity. In the majority of these procedures, the regimen of immunosuppression consisted of antibody induction with an antilymphocyte globulin combined with cyclosporine, azathioprine, and glucocorticoids. However, two centers, the University of Giesen (27) and the University of Alberta in Edmonton, Canada (28), persisted in perfecting the technology and immunosuppression essential for successful islet transplantation for patients with type I diabetes. The Giesen team's meticulous attention to optimal conditions for pancreas procurement, preservation, islet isolation, islet engraftment, and immunosuppressive strategies demonstrated in the late 1990s that clinical islet transplantation could produce states of insulin independence (29). This rekindled the interest in islet transplantation and prompted several centers worldwide to reinitiate clinical islet transplantation programs (Figures 1 and 2).

The Breakthrough of the Edmonton Protocol

In 1999, the University of Alberta group established that islet transplantation could consistently produce states of insulin independence (4). Two unique changes were responsible for the dramatic and consistent success observed in the Edmonton protocol. The first consisted of the intraportal infusion of freshly isolated islets, followed by a second and sometimes third infusion of additional islets from different donors (at a later date) in order to achieve insulin independence. Based on a review of their data, it appears that $\sim$8000–9000 islet equivalents per kilogram (Ieq/kg) were necessary to achieve a state of insulin independence, and this yield was rarely obtained with one donor. Thus, the majority of recipients required the infusion of islets isolated from at least two cadaveric donors. The other significant change from previous unsuccessful protocols was the use of effective immunosuppressive agents that do not cause toxicity to pancreatic islets (Figure 3). It has long been recognized that steroids and calcineurin inhibitors are toxic to beta cells. Although these agents have provided adequate immunosuppression for solid-organ

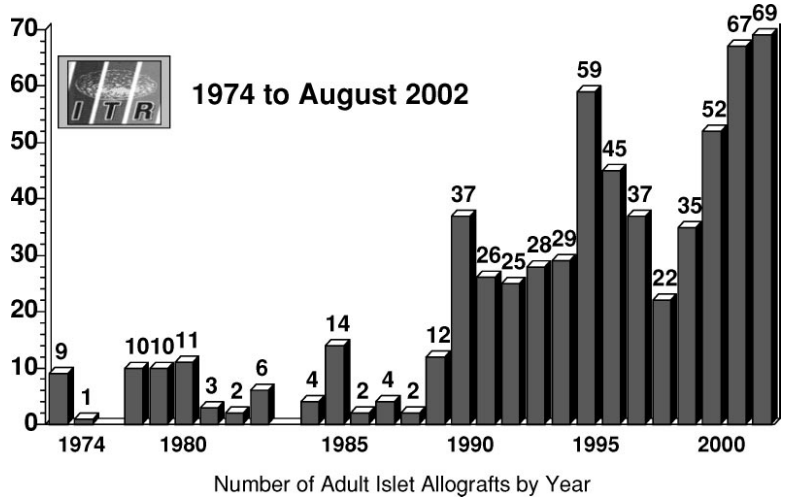

Figure 1 Number of islet allografts over time (1974–2002). There has been a dramatic increase in islet allotransplants in the past decade. However, the number decreased significantly after 1995 until 1999 when the success of the Edmonton Protocol led to a significant increase of transplants each year. These data were compiled by the Islet Transplant Registry (ITR) and kindly provided by Dr. James Shapiro, Edmonton, Canada.

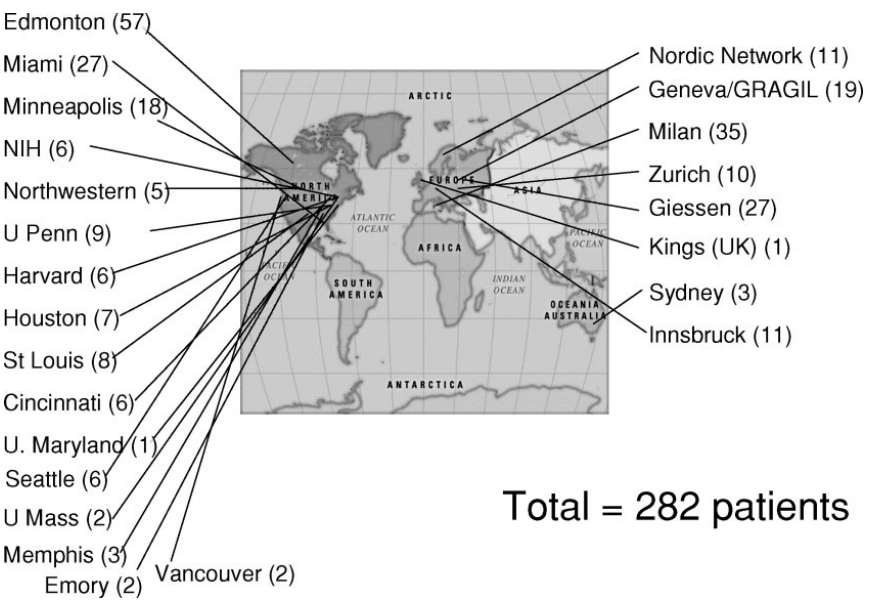

Figure 2 Number of individual sites throughout the world actively engaged in islet transplantation (1999–2002) with a total of 282 (as of early 2003). The number of transplants per site is shown in the parentheses. (Figure kindly provided by Dr. James Shapiro, Edmonton, Canada.)

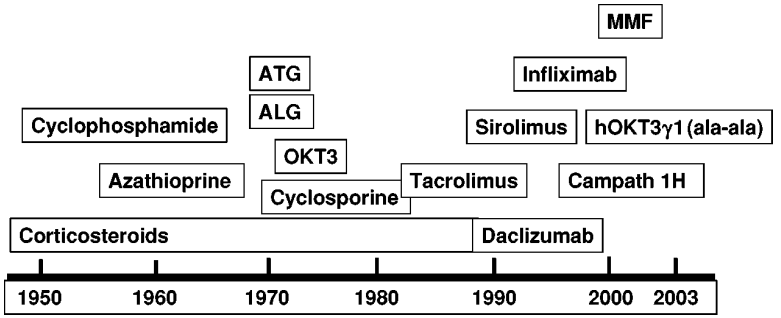

Figure 3 There has been a significant change in the range and number of immunosuppressive drugs used in the islet transplant setting, as depicted in this timeline chart. Importantly, in the late 1990s there was a shift from the use of the highly diabetogenic corticosteroids to cocktails of immunosuppressive drugs that rely on direct anti–T-cell activity, sirolimus and low-dose calcineurin inhibitors. ATG, anti-thymocyteglobulin; ALG, anti–lymphocyte globulin; OKT3/OKT3γ1 (ala-ala), anti-CD3; other names defined in text.

pancreas transplants, the quantity of beta cells transplanted with a whole pancreas was not sufficient to tolerate the toxic insult from the diabetogenic agents. By eliminating steroids and decreasing the dose of calcineurin inhibitors, Shapiro and colleagues provided effective immunosuppression for the decreased islet mass infused with islet transplants (4).

CURRENT STATE OF THE ART

Pancreas Procurement, Preservation, and Islet Isolation

Several important conclusions can be inferred from reviews of the largest series of islet isolations performed at centers that persisted in improving the technology of islet isolation (28, 30–33). Perhaps the most important observation is that successful isolation of pancreatic islets depends on the careful procurement of the donor pancreas. Many of the pancreases obtained for islet isolation were removed after procurement and preservation of other organs to be used in solid-organ transplant, with minimal attention dedicated to the procurement of the pancreas. Consequently, the initial preservation was less than optimal, and the initial insult ultimately translated into the poor quality and yield of the isolated islets (Figure 4).

A second conclusion drawn from these large series of human islet isolation is the importance of minimizing cold ischemia time following the procurement of the donor pancreas. Pancreases procured from deceased donors can tolerate cold ischemic times of up to 24 h and consistently produce states of insulin independence following transplantation in patients with insulin-dependent diabetes (2).

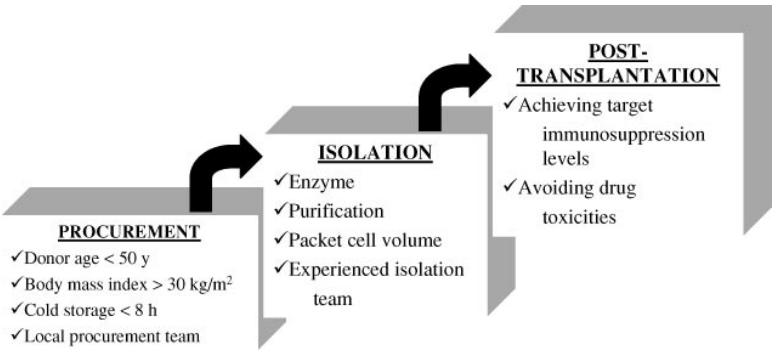

Figure 4 Essential elements of successful islet transplantation. Recent abstracts presented by James Shapiro and Jonathan Lakey at the American Society of Transplantation Conference (May 31 and June 2, 2003) were used to develep this list of criteria that influenced the outcome of islet transplantation during a controlled multicenter trial sponsored by the Immune Tolerance Network/JDRF/NIH (adapted figure kindly provided by Barbara DiMercurio, NIAID).

However, islets cannot tolerate the same amount of cold ischemia as their solid-organ counterpart. In the most complete review of worldwide experience with islet transplantation, Hering & Ricordi documented 16 cases of insulin independence following islet transplantation from a single donor prior to 1996 and observed that the ischemic time in 15/16 of these cases was <8 h (18). Subsequent trials of successful islet transplantation resulting in insulin independence since 1990 (4, 34, 35) correlate successful islet isolation and transplants to cold ischemic times of <8 h. The fact that islets have a lower tolerance for cold ischemia than the solid-organ pancreas is undoubtedly related to the multiple insults an islet must overcome during the isolation process.

Important progress in minimizing the detrimental effect of cold ischemia by using a two-layer preservation technique for storing the solid-organ pancreas prior to the digestion process has been reported (36–38). The two-layer technique utilizes a perfluorochemical preservation fluid that improves oxygen delivery to the stored pancreas and increases islet yields after prolonged storage (36, 37).

The isolation of human pancreatic islets from the solid-organ pancreas was significantly improved by the use of an automated digestion protocol (39, 40) and large-scale purification by continuous ficoll gradients on the Cobe 2991 blood-cell processor (41–43). Despite significant improvements, there continue to be unpredictable variables resulting in inconsistent yields of viable islets. The current isolation technology remains dependent on the distension of the pancreatic duct of the procured solid-organ pancreas with collagenase in order to dissociate the pancreas to free the imbedded islets.

A significant Achilles heel of this procedure, however, relates to the constitution, consistency, and consequent reliability of the digestive enzymes required

to free the islets from the surrounding islet tissue. If the collagenase (a mixture of bacterially derived enzymes) is too potent and the exposure time too long, the digestion process will destroy the islets. If the enzymatic activity is weak or the exposure time too short, the islets will not be freed from the surrounding acinar tissue, resulting in poor yields. An advance in the ability to isolate islets was the development of Liberase (Boehringer-Mannheim, now Roche, Indianapolis, IN). Liberase is a blend of three distinct collagenase enzymes purified from bacteria. It is constituted to be low in endotoxin content, consistent in enzyme activity, and low in contaminants. The use of this consistent cocktail minimizes the variability between different preparations of the enzyme (43, 44). However, different lots of Liberase continue to demonstrate significant variability. This variability is reflected in continued inconsistency in yields of functional islets necessary for transplantation. In preliminary reports for several institutions performing islet transplantation, centers are reporting that among isolations performed with the intent to transplant, $\sim$50% are achieving islet preparations suitable for transplantation.

The inconsistency in achieving adequate yields of islets is further complicated by the variability in the quality of the donor pancreas. In the most extensive analysis of the effect of donor variables on islet yield, Lakey et al. found that high donor body mass index (BMI), increased donor age, and procurement of the pancreas by an experienced local team improved yields of functional islets (28). Poor yields of functional islets correlated with hyperglycemia or hemodynamic instability of the donor prior to death, as well as increased duration of cold ischemia of the donor pancreas prior to the isolation procedure. Other analyses of the effect of donor variables have consistently correlated high donor BMI and donor hemodynamic stability with successful islet isolation (30, 32, 33, 46). Unfortunately, the current state of the art in terms of islet isolation suggests that only one third to one half of the preparations of islets isolated with the intent to transplant will yield enough functional islets ($>$250,000 Ieq) for transplantation. If up to three successful isolations are necessary to achieve an islet yield adequate for transplantation, as many as four to six donors may be necessary to achieve insulin independence for a given recipient. Clearly, the widespread application of islet transplantation as a treatment for diabetes mellitus will depend on improvements in the current technology of islet isolation and purification in order to produce more consistent yields of functional islets.

It is important to note that some pancreases which are not suitable for solid-organ transplantation may provide excellent quality and quantity of pancreatic islets. For example, donors with a high BMI ($>$30) are rarely used for solid-organ transplantation because of the high risk of postoperative complications, yet are ideal for islet isolation (28, 33, 46, 47).

Transplant groups from the University of Minnesota (34, 48) and University of Pennsylvania (49) have demonstrated consistent ability to achieve insulin independence from a single donor. Hering et al. (34, 48) have achieved insulin independence from a single donor by culturing the islets prior to transplantation, permitting pretransplant immunosuppression with immunodepleting agents to

facilitate engraftment. However, even this practical method for treatment of an increasing number of diabetic patients will rapidly be challenged by a severely limited donor pool.

Patient Selection for Trials

Successful islet transplantation requires the prevention of the alloimmune response as well as the recurrent autoimmune response, so it remains dependent on immunosuppressive agents. Despite the ultimate possibility of transplantation tolerance (see below), current recipients of islet transplants will be subjected to the long-term sequelae of immunosuppressive agents, including increased risks of infections and malignancies. Therefore, as in whole-organ pancreas transplantation, the exchange of insulin therapy for immunosuppressive agents must be justified by an assessment of the risk/benefit ratio. For this reason, subjects included in the majority of the current trials are limited to type I diabetic recipients with hypoglycemic unawareness or metabolic instability despite intensive insulin therapy (34, 48–52). In addition to the requirements for "life-threatening" diabetes, most current trials limit the weight of the recipients, as a result of the reports from the large Edmonton trial demonstrating the requirement for 8000–9000 Ieq/kg to achieve states of insulin independence. The ITN [sponsored by the National Institutes of Health (NIH) and Juvenile Diabetes Research Foundation (JDRF)] is the first large multicenter trial designed in an attempt to confirm the Edmonton protocol at nine sites (45). Patients weighing more than 70 kg are excluded from the trial. Another technique for predicting the potential for success as defined by insulin independence could be pretransplant daily insulin requirements, although the inclusion/exclusion criteria for the ITN and other trials continue to rely on absolute weight.

An important exclusion criterion for islet transplantation in the preuremic diabetic recipient is the presence of significant renal insufficiency. The current immunosuppressive strategies depend on the use of calcineurin inhibitors, which can further impair renal function and exacerbate existing diabetic nephropathy. For this reason, patients with significant impairment of renal function (creatinine clearance less than 60%–70%) are excluded from islet transplantation prior to receiving a kidney transplant. Given limited yields of islets per pancreas as well as the requirements for chronic immunosuppression, most type I diabetic patients selected for current trials of islet transplantation have life-threatening diabetes, with hypoglycemic unawareness or metabolic instability despite intensive insulin therapy. Although most of these patients have the secondary complications of neuropathy and retinopathy, the presence of significant nephropathy excludes patients from trials of islet transplantation in the absence of kidney transplantation. As islet trials are expanded, kidney transplant recipients will be simultaneously transplanted with islets, or islet transplantation will follow successful kidney transplant. A successful sequential kidney/islet transplant, dependent on steroid-free immunosuppression, was recently reported (53).

Many potential recipients for solid-organ pancreas transplantation cannot undergo the stress of the operative procedure because of advanced cardiovascular disease. Although the primary focus of islet transplantation is to provide a safer technique to justify earlier intervention prior to the development of secondary complications, it also allows beta-cell replacement in patients with life-threatening diabetes who cannot tolerate the cardiovascular stresses of solid-organ pancreas transplantation.

Techniques for Islet Transplantation

The intraportal infusion of islets is the only technique that has successfully led to insulin independence following islet transplantation in humans. The intrahepatic site is the only one currently being pursued in human trials, although other sites have been studied in animals, including the omental pouch and the submucosal space of the upper gastrointestinal tract (54–56).

Access to the portal system is accomplished with either percutaneous transhepatic cannulation (4, 49, 51, 52) or via branches of the mesenteric venous system cannulated by direct exposure using a mini-laparotomy (34). The advantage of the percutaneous approach is the avoidance of an abdominal wall incision, although the risk of postinfusion bleeding is greater. The risk of bleeding requiring transfusion is minimized by the administration of a hemostatic agent through the catheter tract at the conclusion of the islet infusion, as well as the use of a smaller 4Fr-gauge islet infusion catheter (50). The current infusion process requires anticoagulation with heparin to prevent thrombosis of the portal vein, although portal-vein thrombosis has been rare. In the most recent update from the Edmonton group, partial portal-vein thrombosis was detected following two of the 54 islet infusions. Five of the patients in this series had evidence of bleeding following the percutaneous infusion of the islets, three required transfusion, and one required transfusion and open surgery because of an expanding intrahepatic and subcapsular hemorrhage (52). Transient elevations in portal pressures have been noted during and immediately after the intraportal infusion of islets, but no long-term sequelae have been reported when purified islets have been infused (57).

Although some of the earlier trials in islet transplantation used nonpurified preparations of islets, most of these preparations were obtained from pancreatectomy specimens that were processed for autoislet transplantation (20). In these cases, the amount of exocrine and endocrine tissue obtained from diseased pancreases was smaller than that obtained from a normal pancreas, and purification was not attempted because of concerns about further islet loss. Of note, one of the earliest reports of insulin independence following islet transplants from a single donor was accomplished with nonpurified islets (58). Because a significant increase in portal pressures has been associated with infusion of tissue pellets greater than 10 cc, the current islet protocols used purified preparations of islets with significantly smaller tissue pellets. Most current strategies for portal-vein cannulation have adopted the percutaneous route now that

technologic advances have minimized the risks of postinfusion bleeding. The mini-laparotomy and transjugular cannulation of the portal system remain viable alternatives.

Immunosuppressive Strategies

A major advance toward achieving insulin independence following islet transplantation was the elimination or minimization of immunosuppressive agents known to have beta-cell toxicity. The success of the Edmonton protocol has in part been attributed to the elimination of steroids as well as the minimization of the calcineurin inhibitor tacrolimus. The ability to provide regimens that are not toxic to beta cells following islet transplantation has been facilitated by access to newer non–beta-cell–toxic agents, most notably sirolimus, a TOR inhibitor that blocks IL-2–dependent proliferation of T lymphocytes. Current immunosuppressive regimens depend on an induction agent used only around the time of the islet infusion, followed by maintenance immunosuppression. For the Edmonton trial as well as the NIH/JDRF ITN trial the induction agent was dacluzimab, a humanized monoclonal antibody directed against the IL-2 receptor (CD25) (4, 45). Maintenance immunosuppressive therapy has consisted of sirolimus and low-dose tacrolimus, thus minimizing the nephrotoxic and diabetogenic effects of tacrolimus but providing enough immunosuppression to protect the islets from the immune response. For the Edmonton protocol, islets were transplanted immediately following preparation without any culture period (4, 45). Although protocols utilizing fresh islets for immediate transplantation after islet preparation offer the benefit of minimizing further islet loss in culture, they do not allow functional testing of the isolated islets prior to transplantation, and there is some risk of transplanting impaired islets. Moreover, the immediate transplantation of the islets precludes any pretreatment of the recipient or the islets themselves.

A strategy that is gaining popularity includes a 48-hour period of islet culture prior to transplantation. This permits the pretransplant evaluation of the purified islets for purity, sterility, endotoxin content, and in vitro response to a glucose challenge. Of equal significance, the pretransplant culture period has permitted induction therapy with immunodepleting (i.e., anti-CD3) and anti-inflammatory (i.e., anti-TNF) agents prior to the infusion of islets, thus facilitating the engraftment of islets by minimizing the initial immune-mediated "hit" to the islet allograft. Another successful strategy, which has resulted in insulin independence following the infusion of islets isolated from a single donor, has utilized induction therapy with a nonmitogenic humanized antibody directed against CD3 (34) followed by maintenance therapy with sirolimus and low-dose tacrolimus (see below). As more protocols are developed, other promising immunodepleting agents being tested include Thymoglobulin (polyclonal anti–T-cell agent) and Campath-1H (monoclonal anti-CD52). In addition, non-nephrotoxic and non–beta-cell–toxic agents are being developed, including biologic agents that block costimulation (CTLA4-Ig,

anti-CD11a, anti-CD40L), anti-CD45RB, and FTY720. Despite the development of more selective immunosuppression and less toxic immunosuppression, any use of these agents will inhibit widespread application of islet transplantation regardless of islet availability, based on the risk/benefit ratio of immunosuppression versus insulin therapy.

RESULTS IN THE CURRENT ERA: RATES OF INSULIN INDEPENDENCE, ISLET FUNCTION AND LONGEVITY, COMPLICATIONS

Since the initial report (4), an increasing number of centers have reported achieving insulin independence following islet transplantation. Recent reports of success have come from Miami (59), the NIH (60), Philadelphia (49), Minnesota (34, 48), Milan (61), and the Swiss-French Group (35). The Minnesota and Philadelphia teams achieved insulin independence with islets isolated from a single donor most of the time. The long-term function and viability of islet transplantation remains to be determined; the longest published follow-up to date is that reported by the Edmonton group (52). Of the 17 consecutive patients who completed the protocol and obtained insulin independence, 15 were available for one-year follow-up. Of these patients, 12 (80%) remained insulin-independent and had normalization of HbA_{1C} levels. All patients required islets isolated from at least two donors, and a total islet mass of 8000–9000 Ieq/kg was necessary to achieve insulin independence. At the time of the most recent publication, the mean follow-up of the 17 patients who had obtained initial insulin independence was 20.4 months. Eleven of the 17 remained insulin-independent, although 2 of the 11 were started on an oral agent because of a rise in HbA_{1C} levels. C-peptide was lost in 3 of the 6 patients who returned to insulin therapy. Half of the patients who returned to insulin therapy had an increase in the titer of islet cell antigen (ICA) and glutamic acid decarboxylase (GAD) antibodies, which suggests recurrence of the autoimmune response. The etiology of the islet loss in the other patients remains speculative, but it could be related to "burnout" of the islet tissue versus the alloimmune response. In terms of function, only two patients with insulin independence had normal oral glucose tolerance testing (OGTT), despite stable glucose control and normalization of HbA_{1C}. The abnormal OGTT seen in the majority of the recipients presumably represents an islet mass that is marginal but sufficient to produce states of insulin independence. All patients with detectable C-peptide, regardless of insulin requirements, had resolution of glycemic instability and hypoglycemic unawareness.

The most significant complications associated with the islet-transplant procedure have been related to the side effects of immunosuppression. In general, the procedure is well-tolerated and requires a single day of hospitalization if performed percutaneously. To date, none of the centers that perform islet transplantation

have reported any long-term sequelae to the liver from the intraportal injection of the islets, based on resolution of liver function tests. However, the development of portal hypertension and long-term effect of islets on the liver have yet to be formally addressed. As for toxicity from the immunosuppressive drugs, even low-dose tacrolimus (trough <5 ng/ml) has resulted in progression of renal insufficiency in 2 patients in the Edmonton trial who had evidence of pre-existing disease, as well as exacerbation of proteinuria in 4 other patients (52). Antihypertensive therapy was started in 53% of the patients in the Edmonton trial, presumably related to tacrolimus. A known complication of sirolimus therapy is hypercholesterolemia, and 53% of patients in the Edmonton trial required statin therapy (52). Anemia and leukopenia have also been seen in several protocols using sirolimus as maintenance therapy; patients have required granulocyte colony-stimulating factor to correct the leukopenia. A frequent complaint in all regimens utilizing therapeutic levels of sirolimus has been mouth ulcers, which resolve after decreases in the dosage of sirolimus. Diarrhea has also been a significant complaint of patients on sirolimus/tacrolimus-based therapy but has not been a long-term problem. No serious infectious complications have been reported, including infectious complications related to the infusion of contaminated islets. Of equal significance, there has been no evidence of cytomegalovirus (CMV) disease despite the transplantation of islets isolated from CMV-positive donors into CMV-negative recipients. None of the islet trials have reported lymphoproliferative disease.

There has been no sensitization of islet recipients to HLA antigen, despite the infusion of islets isolated from multiple donors (4, 34, 52). The lack of sensitization reflects effective immunosuppression from the alloimmune response, which is reassuring, since these patients are at significant risk for needing a kidney transplant. On the other hand, the necessity of reinstituting insulin therapy in 6/17 patients at a mean follow-up of 20 months in the Edmonton trial suggests that further improvement in immunosuppressive strategies may be necessary (52). This concern is confirmed by evidence of recurrent autoimmunity in half of the patients who returned to insulin therapy. The cause for graft failure in the other three patients remains unclear. The lack of the development of anti-HLA antibodies as well as autoantibodies suggests that the loss of islet function may be nonimmunologic; rather, the loss of insulin production may reflect "burnout" of the islets, as many of the patients have only a marginal islet mass (see above). Recent evidence suggests that neo-islet formation from islet progenitor cells in the ducts of the pancreas is necessary to maintain beta-cell mass (62). Further follow-up of the successful islet transplant will provide insight into the requirement for islet precursors to maintain long-term function. Although current reports suggest the safety and efficacy of islet transplants, the ultimate utility of this procedure will depend on finding a better source of islets, as well as even better immunosuppression to prevent alloimmunity and recurrent autoimmunity. For this reason, the next sections focus on two areas highly relevant to further development of islet-transplantation strategies: the development of transplantation tolerance and the potential for stem cell–derived islets for transplantation.

ISLETS AS A MODEL FOR TOLERANCE STRATEGIES

Islet transplantation has become an accepted treatment option for selected patients with inadequate glucose control even under stringent insulin therapy. However, the application of this procedure for the treatment of type 1 diabetes is limited by the need for potent nonselective immunosuppression, including nephrotoxic and diabetogenic calcineurin-inhibitor therapy. Islet transplantation has unique pluses and minuses as a venue for testing tolerogenic therapies (therapies that will lead to a rejection-free state without ongoing immunotherapies). On the plus side, diabetes is not an acutely life-threatening disease. Additionally, even though the graft might be lost should the tolerogenic therapies fail, even short-lived blood-sugar control has significant long-term benefit to diabetic patients, reducing complications and morbidity as reported by the Diabetes Control and Complications Trial Group (63). Moreover, in contrast to solid-organ transplantation, the risk of the transplant procedure itself is quite modest. There is no surgery, and the procedural risks are limited to portal hypertension and, in rare cases, bleeding. On the other hand, one weakness of choosing islet transplantation as a target for tolerance therapies in patients with type 1 diabetes is that, unlike other organ-transplantation settings, in type 1 diabetes the induction of potent and persistent tolerance must be considered in the context of an ongoing pathogenic autoimmune response. Thus, tolerogenic strategies for the treatment of type 1 diabetes are confounded by the memory T-cell response, the presence of autoantibodies, and the genetic immunologic abnormalities found in the autoimmune individual. As such, a series of novel therapeutic approaches must be developed to target clonal deletion of T and B cells, regulatory T-cell expansion, and altered T-cell receptor-mediated signaling.

Over the past few decades, a more detailed understanding of the molecular events associated with T-cell recognition and activation has led to various tolerance approaches in numerous models of both autoimmunity and transplantation (64). The results of these studies have suggested a number of sites for intervention in the immune response. For instance, it is clear that T lymphocytes require the engagement of both the T-cell receptor and a series of coreceptors, notably costimulatory signals, for complete activation. Blockade of these cell-surface molecules results in incomplete activation and T-cell anergy. Thus, costimulation antagonists present an attractive means of promoting tolerance (65).

Clinical trials of costimulation blockade have provided important data pertaining to the safety, efficacy, and mechanisms of tolerance induction. For instance, the kidney transplant program at the University of California, San Francisco has been involved in the initial trials of LEA29Y, a high-affinity mutant form of CTLA-4Ig that antagonizes the CD28 costimulatory pathway. Preliminary studies have shown that this therapy provides effective immunosuppression following renal transplantation in humans and islet allografts in nonhuman primates (66). Similarly, in the preclinical nonhuman-primate allotransplant setting, anti-CD154 monoclonal antibody (IDEC-131) and other CD28 antagonists—such as antibodies directed against the CD28 ligands, B7.1 and B7.2 (67)—have been effectively combined

with rapamycin for maintenance immunosuppression (68, 69). It should be emphasized that all of these preclinical and clinical studies successfully prevented rejection without corticosteroid and calcineurin inhibitors. Thus, the use of single or combination costimulation antagonists is likely to be a productive avenue of clinical research in the islet-transplantation arena.

The use of T-cell–depleting induction therapy has become widespread, and new trials have been designed with a focus on immunosuppressive drug withdrawal. The hypothesis behind the use of these drugs is that a transient but profound T-cell depletion can reset the immune system to a tolerized state in the presence of autoantigen and alloantigen expression of the foreign islets. Preclinical studies have shown that a depleting anti-CD3 immunotoxin, combined with more general macrophage inhibitor 15-deoxyspergualin, is an effective tolerogenic therapy (70). There has been increasing use of Campath 1H (Alemtuzumab), a humanized anti-CD52 monoclonal antibody (mAb), currently approved for the treatment of B-cell chronic lymphocytic leukemia. The mAb has been shown to rapidly deplete peripheral blood B cells and T cells. In some cases, it can take over a year to reconstitute the immune system.

A number of studies are testing the efficacy of Campath 1H in transplantation, with particular regard to calcineurin-free regimens. In one study, over a dozen patients received transplants under the cover of Campath 1H with encouraging results (71). Based on these results and others, the ITN (http://www.immunetolerance.org) has approved an islet-transplant protocol by the Edmonton group that will treat islet-transplant recipients with a combination of Campath 1H and sirolimus (rapamycin) with the intent to withdraw all drugs at $\sim$1 year, assuming that the patients meet certain requirements of operational tolerance.

Thymoglobulin, a polyclonal rabbit antihuman thymocyte globulin (SangStat), is approved for the treatment of acute renal-transplant rejection and is a powerful lymphocyte-depleting agent. At the 2002 American Transplantation Congress, the transplant group from the University of Pittsburgh reported a small clinical trial that used thymoglobulin as a pretreatment drug (72). The investigators reported weaning success in 64 kidney-transplant patients, 7 of whom took the calcineurin inhibitor (tacrolimus) just once weekly. In addition, of 18 lung-transplant patients put through the same protocol, 5 are on tacrolimus four times weekly, 5 on a once-daily dose, and 8 on a twice-daily dose. Instead of the usual three-drug combination, the lung-transplant recipients are receiving only the tacrolimus plus very-low-dose prednisone. It is anticipated that many islet-transplant trials in the next few years will focus on these T-depleting agents. As an example, an ongoing thymoglobulin-based islet-transplant trial at the University of Minnesota uses the thymoglobulin therapy in combination with low-dose conventional immunosuppressive drugs (48). Thus, it is likely that these types of therapies will be evolving and moving into the islet-transplantation area.

Preclinical studies have suggested that one approach to attaining tolerance is the creation of a chimeric state in which large numbers of donor cells are maintained in the recipient. The most clinically relevant approaches have been those that use nonmyeloablative host-conditioning regimens, since the whole-body irradiation

used in other regimens to allow donor bone marrow to become established carries excessive risks of toxicity. Nonmyeloablative mixed chimeric approaches have allowed complete immunosuppressive withdrawal in some limited cases (73). Thus, these therapies may provide a robust approach to tolerance in the islet-transplant setting. In this regard, Ricordi and colleagues were recently approved to perform allogeneic islet transplants using a combination of Campath 1H, sirolomus, and CD34+ stem cell therapy in preconditioned recipients (http://www.immunetolerance.org). The goal of this study is to determine whether this protocol will lead to chimerism that can be the basis for total drug withdrawal (74).

An approach that has been successful in moderating both the autoimmune and alloimmune responses is the use of T-cell receptor antagonists such as anti-CD4 or anti-CD3 mAbs. Over the past decade, the potential for anti-CD3 mAbs to induce tolerance in a safe and effective manner has been studied by several groups. In mice, a five-day course of anti-CD3 antibodies at the time of disease onset was sufficient to reverse the disease, induce long-term remission, and prevent recurrent immune responses even against transplanted syngeneic islets (75). Mechanistic studies demonstrated that the mAb has short-lived effects on naive T cells but delivers a partial signal in activated T cells, inactivating Th1 cells while permitting proliferation/cytokine production by Th2 cells and regulatory T cells (76). Thus, the antibody therapy "tips" the balance of immune homeostasis so that the Th2 and regulatory T cells block the residual pathogenic response.

Based on in vitro and small animal studies, a phase I/II trial of patients with new-onset type 1 diabetes was initiated. In this trial, a humanized FcR nonbinding anti-CD3 mAb was administered for two weeks at the time of disease onset. The FcR nonbinding anti-CD3 mAb, given without other immune-suppressive agents, halted the progression of disease for >1 year (74). Moreover, significant increases in IL-10 in the serum of approximately two thirds of treated patients and an IL-$10^{+}CD4^{+}$ T-cell population was observed in vivo after drug treatment. These studies led to a pilot trial in which "brittle" type 1 diabetic patients were transplanted with allogeneic islets under the cover of FcR nonbinding anti-CD3, low-dose tacrolimus, and sirolimus. Sixty-six percent of these patients maintained long-term insulin independence after a single-donor islet transplant (34). However, to date, the therapies have not been shown to be toleragenic in the allotransplant setting. In this regard, the ITN recently agreed to sponsor a drug-withdrawal trial in islet-transplant recipients at the University of Minnesota. Investigators will administer a combination of the FcR nonbinding anti-CD3 mAb in combination with sirolimus. Patients will be withdrawn from all drugs at $\sim$1 year, assuming that they meet certain requirements of operational tolerance (http://www.immunetolerance.org).

STEM CELL–DERIVED ISLETS FOR TRANSPLANTATION

The shortage of functional beta cells from available donors is becoming one of the major limiting factors for the treatment of diabetes by islet transplantation. Even if all the available cadaveric pancreases could be used effectively to prepare islets

and if each recipient needed only one pancreas equivalent, the supply of pancreases is believed sufficient to treat only a small fraction of all individuals with type 1 diabetes. A potential solution is the use of xenogeneic tissue (see above). However, barriers to successful xenotransplantation include the risk of transmitting infections agents from one species to another and the inherent increased immunological reactivity to tissues from other species. Given the significant hurdles facing these approaches, other possible sources for islet tissue are being sought. In this section, we describe two parallel approaches to developing islet cells: the use of adult and embryonic stem (ES) cells.

ES cells are derived from embryos that develop from eggs that have been fertilized in vitro. They are not derived from eggs fertilized in a woman's body. Human ES cells are derived from the blastocyst, which is a group of 32 cells. Because of ES cells' special properties of renewable growth and selective differentiation, deriving insulin-secreting cells from this source is an exciting prospect for generating an unlimited supply of specialized beta cells for transplantation. Additionally, ES cells are amenable to stable genetic modification, through which they could be manipulated so as to escape or inhibit the immune responses of the patient and prevent rejection. In fact, recent studies have suggested that stem cells themselves are protolerogenic, suggesting that these cells may be used as the tolerogen for subsequent islet-cell therapy (77). Finally, differentiated cell types derived from ES cells possess many of the physiologic and functional capacities of normal cells. Under certain conditions, with selected differentiation factors, the ES cells can be differentiated into insulin-producing beta cells (78, 79). More important, the long-term function or expansion of such cells when they are transplanted into diabetic animals has been demonstrated (79). Unfortunately, this field remains extremely controversial, with some scientists challenging the validity of the experimental results (80). Despite several publications suggesting that mouse and human ES cells can be used to develop islet cells, convincing evidence that this is feasible is still lacking.

An alternative possibility is that multipotent progenitor cells reside within the adult pancreas and that regulated differentiation of these cells could fill the role of ES-cell–derived beta cells. In the adult pancreas, beta cells have a limited life span, and cell replacement is critical to maintain glucose homeostasis (81). Regeneration of beta cells after tissue injury has been observed in several model systems, indicating that certain cells within the mature pancreas retain the ability to partially restore beta-cell mass after injury (82). Recent studies indicate that differentiated exocrine acinar and/or ductal cells may trans-differentiate into beta cells (81). In fact, recent studies have demonstrated progressive beta-cell regeneration in beta-cell–deficient mice (83). Whether this in vivo observation can be adapted to an in vitro system that will result in sufficient expansion for human transplantation remains to be seen. Nonetheless, it should be pointed out that even as few as five doublings of already mature beta cells could lead to a 30-fold increase in islet transplants.

Several studies over the past 4–5 years have suggested that stem cells for endocrine pancreas are present in distant organs such as bone marrow (84, 85).

A recent study has suggested that cells transferred during bone marrow transplantation can be detected in the pancreas and differentiate locally into functioning beta-like cells (84). However, there is evidence that the bone marrow cells fuse to endogenous tissues and may not differentiate into the target tissue (86). Still, it is encouraging that a unique population of bone marrow progenitors developed by Verfaillie and colleagues can be induced to differentiate to most somatic cells may provide a novel approach to developing a new islet source (85).

CONCLUSIONS: INSULIN THERAPY VERSUS BETA-CELL REPLACEMENT

The ability to achieve insulin independence with either solid-organ pancreas or islet transplantation has increased the number of diabetic patients seeking beta-cell replacement as an alternative to insulin therapy. Given the known complications of chronic immunosuppression, either transplantation procedure should be limited to patients requiring immunosuppression for a kidney transplant or patients with life-threatening diabetes mellitus. The latter category would include diabetic patients with hypoglycemic unawareness or metabolic instability/lability despite intensive insulin regimens.

However, significant achievements have been made in the field of clinical islet transplantation in the past three years. The demonstration of diabetes reversal on a consistent basis in islet-transplant recipients marked a turning point in the history of islet transplantation and cell-based diabetes therapies (4). These findings have now been confirmed at ~10 additional institutions. Yet, for islet transplantation to become the treatment of choice for type 1 diabetics, additional advances are necessary, including maximization of the islet preparation and the transplant protocol as well as the introduction of new, potentially tolerogenic drugs to control both alloimmune and autoimmune responses. Corticosteroids and calcineurin inhibitors must be avoided to circumvent inhibitory effects on insulin secretion and insulin action, which are both particularly deleterious if a marginal mass is present. Avoiding calcineurin inhibitors will further eliminate nephrotoxic side effects and increase the likelihood of successful tolerance induction. Most important, widespread use of the procedure will depend on new sources of islets either using adult or embryonic stem cells.

Meanwhile, many insulin-dependent diabetics meet the indications for beta-cell replacement today, so an algorithm based on the current state of the art must be developed. Because the success of islet transplantation as defined by insulin independence requires the infusion of ~9000 Ieq/kg, this procedure would be most effective in patients with low BMI or low insulin requirements. Islets are a viable option for such patients if they are hesitant to undergo an abdominal operation. For patients receiving transplants at experienced centers, the chance of insulin independence one year after the procedure is 80% for islet transplants versus

90% for solid-organ transplants. For larger patients with life-threatening diabetes, solid-organ pancreas transplant remains a better alternative, offering a high chance of insulin independence. The operative procedure for solid-organ transplantation is rigorous, and patients with significant cardiovascular risks would be better served by an islet transplant.

Over 20 years ago, high hopes were pinned on islet transplantation as a cure for diabetes mellitus. Early results from the Edmonton trial suggest that we are closer to that goal but still have room for significant progress. The widespread application of islet transplantation will depend on further improvements in immunosuppressive strategies, advances in the area of transplantation tolerance, increases in the longevity of islet transplants, and development of an unlimited source of beta cells.

The *Annual Review of Medicine* is online at http://med.annualreviews.org

LITERATURE CITED

1. Gruessner AC, Sutherland DE, Dunn DL, et al. 2001. Pancreas after kidney transplants in posturemic patients with type I diabetes mellitus. *J. Am. Soc. Nephrol.* 12:2490–99
2. Gruessner A, Sutherland D. 2002. Pancreas transplant outcomes for United States (US) and non-US cases as reported to the United Network for Organ Sharing (UNOS) and the International Pancreas Transplant Registry (IPTR) as of October, 2001. In *Clinical Transplants 2001*, ed. M Cecka, P Terasaki, pp. 41–72. Los Angeles: Regents of Univ. Calif.
3. Sutherland DE, Gruessner RW, Najarian JS, et al. 1998. Solitary pancreas transplants: a new era. *Transplant. Proc.* 30: 280–81
4. Shapiro AM, Lakey JR, Ryan EA, et al. 2000. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N. Engl. J. Med.* 343:230–38
5. Nathan DM. 1993. Long-term complications of diabetes mellitus. *N. Engl. J. Med.* 328:1676–85
6. The DCCT Research Group. 1993. The effect of intensive treatment of diabetes on the development of long-term complications in insulin-dependent diabetes mellitus. *N. Engl. J. Med.* 329:977–86
7. Kaufman DB, Leventhal JR, Koffron AJ, et al. 2002. A prospective study of rapid corticosteroid elimination in simultaneous pancreas-kidney transplantation: comparison of two maintenance immunosuppression protocols: tacrolimus/mycophenolate mofetil versus tacrolimus/sirolimus. *Transplantation* 73:169–77
8. Freise C, Hirose R, Feng S, et al. 2002. Minimal rejection with a steroid sparing protocol in simultaneous pancreas kidney transplantation. *Am. J. Transplant.* 2(Suppl. 3): 203 (Abstr.)
9. Brendel M, Hering B, Schultz A, et al. 2001. International Islet Transplant Registry Report. *Int. Transplant Reg. Newsl.* 8:1–20
10. Fioretto P, Steffes MW, Sutherland DE, et al. 1998. Reversal of lesions of diabetic nephropathy after pancreas transplantation. *N. Engl. J. Med.* 339:69–75
11. Navarro X, Sutherland DE, Kennedy WR. 1997. Long-term effects of pancreatic transplantation on diabetic neuropathy. *Ann. Neurol.* 42:727–36
12. Gross CR, Limwattananon C, Matthees BJ. 1998. Quality of life after pancreas

transplantation: a review. *Clin. Transplant.* 12:351–61
13. Ballinger WF, Lacy PE. 1972. Transplantation of intact pancreatic islets in rats. *Surgery* 72:175–86
14. Reckard CR, Barker CF. 1973. Transplantation of isolated pancreatic islets across strong and weak histocompatibility barriers. *Transplant. Proc.* 5:761–63
15. Najarian JS, Sutherland DE, Matas AJ, et al. 1977. Human islet transplantation: a preliminary report. *Transplant. Proc.* 9:233–36
16. Matas AJ, Sutherland DE, Payne WD, et al. 1977. Islet transplantation. The critical period of donor ischemia in neonatal rats. *Transplantation* 23:295–98
17. Sutherland DE, Matas AJ, Najarian JS. 1978. Pancreatic islet cell transplantation. *Surg. Clin. North Am.* 58:365–82
18. Hering B, Ricordi C. 1999. Islet transplantation for patients with type I diabetes. *Graft* 2:12–27
19. Hu YF, Gu ZF, Zhang HD, Ye RS. 1992. Fetal islet transplantation in China. *Transplant. Proc.* 24:1998–99
20. Wahoff DC, Papalois BE, Najarian JS, et al. 1995. Autologous islet transplantation to prevent diabetes after pancreatic resection. *Ann. Surg.* 222:562–75; discussion 75–79
21. Robertson RP, Lanz KJ, Sutherland DE, et al. 2001. Prevention of diabetes for up to 13 years by autoislet transplantation after pancreatectomy for chronic pancreatitis. *Diabetes* 50:47–50
22. Ricordi C, Tzakis AG, Carroll PB, et al. 1992. Human islet isolation and allotransplantation in 22 consecutive cases. *Transplantation* 53:407–14
23. Tzakis AG, Ricordi C, Alejandro R, et al. 1990. Pancreatic islet transplantation after upper abdominal exenteration and liver replacement. *Lancet* 336:402–5
24. Cretin N, Buhler L, Fournier B, et al. 1998. Results of human islet allotransplantation in cystic fibrosis and type I diabetic patients. *Transplant. Proc.* 30:315–16
25. Brunicardi FC, Atiya A, Stock P, et al. 1995. Clinical islet transplantation experience of the University of California Islet Transplant Consortium. *Surgery* 118:967–71; discussion 71–72
26. Steffes MW, Sutherland DE, Goetz FC, et al. 1985. Studies of kidney and muscle biopsy specimens from identical twins discordant for type I diabetes mellitus. *N. Engl. J. Med.* 312:1282–87
27. Hering BJ, Bretzel RG, Hopt UT, et al. 1994. New protocol toward prevention of early human islet allograft failure. *Transplant. Proc.* 26:570–71
28. Lakey JR, Warnock GL, Rajotte RV, et al. 1996. Variables in organ donors that affect the recovery of human islets of Langerhans. *Transplantation* 61:1047–53
29. Bretzel RG, Brandhorst D, Brandhorst H, et al. 1999. Improved survival of intraportal pancreatic islet cell allografts in patients with type-1 diabetes mellitus by refined peritransplant management. *J. Mol. Med.* 77:140–43
30. Zeng Y, Torre MA, Karrison T, et al. 1994. The correlation between donor characteristics and the success of human islet isolation. *Transplantation* 57:954–58
31. Benhamou PY, Watt PC, Mullen Y, et al. 1994. Human islet isolation in 104 consecutive cases. Factors affecting isolation success. *Transplantation* 57:1804–10
32. Brandhorst D, Hering BJ, Brandhorst H, et al. 1994. Influence of donor data and organ procurement on human islet isolation. *Transplant. Proc.* 26:592–93
33. Brandhorst H, Brandhorst D, Hering BJ, et al. 1995. Body mass index of pancreatic donors: a decisive factor for human islet isolation. *Exp. Clin. Endocrinol. Diabetes* 103(Suppl. 2):23–26
34. Hering B, Kandaswamy R, Harmon J, et al. 2001. Insulin independence after single-donor islet transplantation in type I diabetes with hOKT3 (ala-ala), sirolimus, and tacrolimus. *Am. J. Transplant.* 1(Suppl. 1):180A
35. Benhamou PY, Oberholzer J, Toso C, et al. 2001. Human islet transplantation network

for the treatment of type I diabetes: first data from the Swiss-French GRAGIL consortium (1999–2000). Groupe de Recherche Rhin Rhjne Alpes Geneve pour la transplantation d'Ilots de Langerhans. *Diabetologia* 44:859–64
36. Matsumoto S, Kandaswamy R, Sutherland DE, et al. 2000. Clinical application of the two-layer (University of Wisconsin solution/perfluorochemical plus O_2) method of pancreas preservation before transplantation. *Transplantation* 70:771–74
37. Hering BJ, Matsumoto I, Sawada T, et al. 2002. Impact of two-layer pancreas preservation on islet isolation and transplantation. *Transplantation* 74:1813–16
38. Kuroda Y, Kawamura T, Suzuki Y, et al. 1988. A new, simple method for cold storage of the pancreas using perfluorochemical. *Transplantation* 46:457–60
39. Warnock GL, Ellis D, Rajotte RV, et al. 1988. Studies of the isolation and viability of human islets of Langerhans. *Transplantation* 45:957–63
40. Ricordi C, Lacy PE, Finke EH, et al. 1988. Automated method for isolation of human pancreatic islets. *Diabetes* 37:413–20
41. Olack B, Swanson C, McLear M, et al. 1991. Islet purification using Euro-Ficoll gradients. *Transplant. Proc.* 23:774–76
42. Finke E, Marchetti P, Falqui L, et al. 1991. Large scale isolation, function, and transplantation of islets of Langerhans from the adult pig pancreas. *Transplant. Proc.* 23:772–73
43. Lake SP, Bassett PD, Larkins A, et al. 1989. Large-scale purification of human islets utilizing discontinuous albumin gradient on IBM 2991 cell separator. *Diabetes* 38(Suppl. 1):143–45
44. Linetsky E, Bottino R, Lehmann R, et al. 1997. Improved human islet isolation using a new enzyme blend, liberase. *Diabetes* 46:1120–23
45. Shapiro J, Hering B, Ricordi C, et al. 2003. International multicenter trial of islet transplantation using the Edmonton protocol in patients with type I diabetes. *Am. J. Transplant.* 3 (Suppl. 5):A3 (Abstr.)
46. Rastellini C, Rilo HH, Fontes P, et al. 1995. The effect of donor age on the outcome of islets isolated from human pancreata. *Transplant. Proc.* 27:3257–58
47. Une S, Atiya A, Ohtsuka S, et al. 1997. Re-evaluation of donor factors affecting islet isolation from human pancreas using a two-step digestion method. *Transplant. Proc.* 29:1969 (Abstr.)
48. Hering B, Kandaswamy R, Ansite J, et al. 2003. Successful single donor islet transplantation in type I diabetes. *Am. J. Transplant.* 3(Suppl. 5):A567 (Abstr.)
49. Markmann JF, Deng S, Huang X, et al. 2003. Insulin independence following isolated islet transplantation and single islet infusions. *Ann. Surg.* 237:741–49; discussion 49–50
50. Shapiro AM, Ryan EA, Lakey JR. 2001. Pancreatic islet transplantation in the treatment of diabetes mellitus. *Best Pract. Res. Clin. Endocrinol. Metab.* 15:241–64
51. Ryan EA, Lakey JR, Rajotte RV, et al. 2001. Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol. *Diabetes* 50:710–19
52. Ryan EA, Lakey JR, Paty BW, et al. 2002. Successful islet transplantation: continued insulin reserve provides long-term glycemic control. *Diabetes* 51:2148–57
53. Kaufman DB, Baker MS, Chen X, et al. 2002. Sequential kidney/islet transplantation using prednisone-free immunosuppression. *Am. J. Transplant.* 2:674–77
54. Yasunami Y, Lacy PE, Finke EH. 1983. A new site for islet transplantation—a peritoneal-omental pouch. *Transplantation* 36:181–82
55. Ao Z, Matayoshi K, Lakey JR, et al. 1993. Survival and function of purified islets in the omental pouch site of outbred dogs. *Transplantation* 56:524–29
56. Tchervenivanov N, Metrakos P, Kokugawa Y, et al. 1994. Submucosal transplantation of pancreatic islets. *Transplant. Proc.* 26:680–81

57. Casey JJ, Lakey JR, Ryan EA, et al. 2002. Portal venous pressure changes after sequential clinical islet transplantation. *Transplantation* 74:913–15
58. Gores PF, Najarian JS, Stephanian E, et al. 1993. Insulin independence in type I diabetes after transplantation of unpurified islets from single donor with 15-deoxyspergualin. *Lancet* 341:19–21
59. Alejandro R, Ferreira J, Froud T, et al. 2003. Insulin independence in 13 patients following transplantation of cultured islets. *Am. J. Transplant.* 3(Suppl. 5):A565 (Abstr.)
60. Rother K, Hirshberg B, Gaglia J, et al. 2002. Islet transplantation in patients with type I diabetes. NIH experience in 6 patients. *Am. J. Transplant.* 2(Suppl. 3):A359 (Abstr.)
61. Luzi L, Perseghin G, Brendel MD, et al. 2001. Metabolic effects of restoring partial beta-cell function after islet allotransplantation in type 1 diabetic patients. *Diabetes* 50:277–82
62. Butler AE, Janson J, Bonner-Weir S, et al. 2003. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* 52:102–10
63. Nathan DM, Lachin J, Cleary P, et al. 2003. Intensive diabetes therapy and carotid intima-media thickness in type 1 diabetes mellitus. *N. Engl. J. Med.* 348:2294–303
64. Matthews JB, Ramos E, Bluestone JA. 2003. Clinical trials of transplant tolerance: slow but steady progress. *Am. J. Transplant.* 3:794–803
65. Masteller EL, Bluestone JA. 2002. Immunotherapy of insulin-dependent diabetes mellitus. *Curr. Opin. Immunol.* 14:652–59
66. Adams AB, Shirasugi N, Durham MM, et al. 2002. Calcineurin inhibitor-free CD28 blockade-based protocol protects allogeneic islets in nonhuman primates. *Diabetes* 51:265–70
67. Lenschow DJ, Walunas TL, Bluestone JA. 1996. CD28/B7 system of T cell costimulation. *Annu. Rev. Immunol.* 14:233–58
68. Kirk AD, Tadaki DK, Celniker A, et al. 2001. Induction therapy with monoclonal antibodies specific for CD80 and CD86 delays the onset of acute renal allograft rejection in non-human primates. *Transplantation* 72:377–84
69. Hausen B, Klupp J, Christians U, et al. 2001. Coadministration of either cyclosporine or steroids with humanized monoclonal antibodies against CD80 and CD86 successfully prolong allograft survival after life supporting renal transplantation in cynomolgus monkeys. *Transplantation* 72:1128–37
70. Contreras JL, Jenkins S, Eckhoff DE, et al. 2003. Stable alpha- and beta-islet cell function after tolerance induction to pancreatic islet allografts in diabetic primates. *Am. J. Transplant.* 3:128–38
71. Knechtle SJ, Pirsch JD, Fechner JH, et al. 2003. Campath-1H induction plus rapamycin monotherapy for renal transplantation: results of a pilot study. *Am. J. Transplant.* 3:722–30
72. Murase N, Metes D, Zeevi A, et al. 2003. Immunomonitoring in abdominal whole organ recipients treated with a tolerance-enhancing regimen of immunosuppression. *Am. J. Transplant.* 3(Suppl. 5):A1156 (Abstr.)
73. Spitzer TR, McAfee SL, Dey BR, et al. 2003. Nonmyeloablative haploidentical stem-cell transplantation using anti-CD2 monoclonal antibody (MEDI-507)-based conditioning for refractory hematologic malignancies. *Transplantation* 75:1748–51
74. Herold KC, Hagopian W, Auger JA, et al. 2002. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N. Engl. J. Med.* 346:1692–98
75. Chatenoud L. 2003. CD3-specific antibody-induced active tolerance: from bench to bedside. *Nat. Rev. Immunol.* 3:123–32
76. Smith JA, Bluestone JA. 1997. T cell inactivation and cytokine deviation promoted by anti-CD3 mAbs. *Curr. Opin. Immunol.* 9:648–54
77. Fandrich F, Lin X, Chai GX, et al. 2002. Preimplantation-stage stem cells induce long-term allogeneic graft acceptance

without supplementary host conditioning. *Nat. Med.* 8:171–78
78. Hori Y, Rulifson IC, Tsai BC, et al. 2002. Growth inhibitors promote differentiation of insulin-producing tissue from embryonic stem cells. *Proc. Natl. Acad. Sci. USA* 99:16105–10
79. Lumelsky N, Blondel O, Laeng P, et al. 2001. Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. *Science* 292:1389–94
80. Rajagopal J, Anderson WJ, Kume S, et al. 2003. Insulin staining of ES cell progeny from insulin uptake. *Science* 299:363
81. Bonner-Weir S. 2000. Islet growth and development in the adult. *J. Mol. Endocrinol.* 24:297–302
82. Hayashi KY, Tamaki H, Handa K, et al. 2003. Differentiation and proliferation of endocrine cells in the regenerating rat pancreas after 90% pancreatectomy. *Arch. Histol. Cytol.* 66:163–74
83. Pelengaris S, Khan M, Evan GI. 2002. Suppression of Myc-induced apoptosis in beta cells exposes multiple oncogenic properties of Myc and triggers carcinogenic progression. *Cell* 109:321–34
84. Hess D, Li L, Martin M, et al. 2003. Bone marrow-derived stem cells initiate pancreatic regeneration. *Nat. Biotechnol.* 21:763–70
85. Verfaillie CM, Schwartz R, Reyes M, et al. 2003. Unexpected potential of adult stem cells. *Ann. NY Acad. Sci.* 996:231–34
86. Wurmser AE, Gage FH. 2002. Stem cells: cell fusion causes confusion. *Nature* 416:485–87