

Reviews

Islet cell transplantation as a cure for insulin dependent diabetes: current improvements in preserving islet cell mass and function

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OBJECTIVE: To review the current progress of islet cell transplantation in patients with insulin-dependent diabetes, emphasizing on the difficulties with recovering and preserving islet cell mass and function, 30% of which is lost during the peri-transplantation period.

RESULTS: The islet-cell isolation technique is perfected, but improvements are still progressing in two major directions: preservation of islet cells and tolerance induction. Optimum islet cell viability and function depends on appropriate revascularization of the islet graft and blockade of thrombus formation as well as cytokine and free radical release. Conditioning the islet cells in-vitro prior to transplantation to either upregulate VEGF expression or downregulate NF-kappa B transcription factor has proven to improve revascularization and to prevent islet cell apoptosis and cytokine-mediated damage. Tolerance induction is currently being best achieved by selecting and combining immunosuppressive agents such as monoclonal antibodies which target the major signaling molecules during immune activation, but which are least toxic to islet cells.

CONCLUSIONS: Patients with insulin-dependent diabetes will greatly benefit from current developments in effective approaches to protect islets during the peritransplant period. Emerging interest in stem cell biology and differentiation may provide the ultimate solution to the problem of organ scarcity and islet cell protection from the peritransplant induced damage.

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Key words: islets of Langerhans; transplantation; graft function; graft preservation; type I diabetes

Introduction

When Langerhans first described pancreatic islets in 1869, he had successfully accomplished the first transplantation of pancreatic islets in dogs and initiated the first experiments involving transplantation of pancreatic fragments to reverse diabetes mellitus.^[1] The procedure was then performed in a child with diabetes in 1890.^[2] Three

decades later, insulin was discovered and defined as a “sugar destroying substance” secreted by pancreatic islets.^[3] Subsequently, clinicians assumed that daily injections of insulin would provide a cure for the diabetic state and its complications. Nevertheless, patients continued to suffer from the secondary disorders of diabetes, which remains the most frequent cause of end-stage-renal-failure and blindness in the United States. Therefore, various investigators have sought new therapeutic approaches for the management of diabetes mellitus.^[4–6] In 1966 the first whole organ pancreas transplant was performed at the University of Minnesota. The procedure was promoted as applicable to patients with “juvenile” or insulin-requiring type I diabetes mellitus (IDDM-I) to allow for sustained euglycemia and insulin independence and reported to the

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International Pancreas Transplant Registry, from which Sutherland et al^[7] have determined the morbidity and mortality rate of whole organ pancreas transplantation to be 70% and 9%, respectively. These results and the need for a difficult surgical procedure prompted a proposal to treat IDDM-I with isolated and injected pancreatic islets of Langerhans.^[6]

In this review following our introductory historical perspective we focus on the difficulties with recovering and preserving islet cells, as these have hampered progress in islet transplantation. We examine the latest developments on islet cell preservation during the peritransplantation period for the purpose of uniformly curing patients with type I diabetes, but we do not expand on the promises of xenotransplantation that may be the answer to a short organ supply for islet isolation. Finally, we briefly approach the promises of stem cell therapy as a cure for type I diabetes.

Current status of islet cell transplantation

In 1974 Sutherland et al^[7] started the first human clinical trials of pancreatic islet transplantation at the University of Minnesota. Although approximately 1000 patients with IDDM-I have received pancreatic islet allografts since 1974, the results remain disappointing. In a 1999 report by Hering and Ricordi,^[5] 70% of reported islet allografts transplanted worldwide into recipients with IDDM-I lost function completely within the first year post-transplant, and only about 10% of the recipients were still insulin-independent at one year.

Originally islet allograft rejection was thought to be the major barrier to islet engraftment.^[7] Thereafter most investigators attempted to propose new strategies for recipient immunosuppression. In fact, a recent preliminary report from the Edmonton Group (Edmonton, Canada) showed a 100% success in achievement of insulin-independence in patients with IDDM-I who received a steroid-free, immunosuppressive regimen utilizing induction with the humanized anti-IL-2 receptor monoclonal antibody daclizumab and a combination of tacrolimus and sirolimus as calcineurin inhibitors.^[8] Even

though the steroid-free regimen was used, two or more transplants were necessary to achieve normoglycemia. Because this success depended on a large islet mass, the scarcity of cadaveric pancreases will greatly restrain investigators from offering islet transplantation to the majority of patients with IDDM-I. Further research needs to be done to prevent early islet loss in order to sustain insulin-independence with only one transplant.^[8]

The mechanisms explaining the relative failure of pancreatic islet engraftment have not been completely elucidated. The portal venous tract of the liver is recognized as the optimum site of islet implantation for the direct delivery of insulin into the systemic circulation. But many factors may alter pancreatic islet engraftment such as toxicity of the preparation reagents, ischemia due to the low oxygen content of the portal vein, transplantation rejection, and the recurrence of the autoimmune disease associated with IDDM-I.^[5] Interestingly, early islet graft loss has occurred both in syngeneic islet transplant and in models of T-cell inactivation, suggesting that mechanisms other than immunological rejection such as apoptosis may be involved in this phenomenon.^[9] Apoptotic cell death has been shown to occur after islet isolation and as a response to cytokine stimulation, explaining at least partially the loss of functional islet mass during the early post transplant period.^[10-12]

Optimized islet isolation technique

Although islet cell isolation techniques have greatly improved since the preliminary work by Moskalewski in the 1960s, optimization of the purification process is still needed. Originally Moskalewski et al^[13] cut and diced a resected pancreas followed by collagenase digestion to disperse tissue.^[14] In the mid 1980s, intraductal collagenase infusion supplanted the older method, and thereafter the pancreas was incubated at 38 °C for about 50 minutes.^[15] The digested tissue was then passed through a 400 µ screen, after which the resulting tissue was washed in cold RPMI solution and purified through ficoll gradient centrifugation if needed.^[16] More recently, Ricordi^[17] has developed a

technique, which gives the highest possible yield of pancreatic islet tissue. The pancreas is first infused with a collagenase solution and then placed in a digestion chamber for a continuous digestion process. Warm balanced salt solutions circulate through the chamber in which the pancreas is progressively dispersed. The digested islets are released from the chamber and saved for additional washes and ficoll gradient separation. A major contribution of the recent studies from Berney et al^[18] demonstrates the induction of intra islet cytokine release by the presence of endotoxin in collagenase. The use of a new endotoxin free digesting enzyme, liberase, has now become the gold standard. In addition introduction of oxygenated perfluorocarbon (PFC) plus oxygen into the cold ischemic preservation phase of the pancreas in University of Wisconsin (UW) solution, also referred as the two layer (UW/PFC) method, has tremendously improved the quality of the islets to be transplanted.^[19]

Improving this current isolation process is the highest priority for the investigator committed to improve islet transplantation. However, as researchers work toward improvement it is important as well to focus on islet cell preservation.

Preservation of islet cell mass and function

Preventing inflammation mediated injury, immune rejection, and the recurrence of the autoimmune response remains a challenge. But the islet cell mass and function can be preserved by adopting various protective measures which have been investigated in animal models and more recently in clinical trials. These measures are categorized as follows:

Stimulating islet-graft revascularization

The success of pancreatic islet engraftment and function relies on a proper reorganization and revascularization of the implanted islet cells. Korsgren et al^[20] found that transplanted islet cell clusters remain autonomous and function as single endocrine units. But the origin of the endothelium

from these units composing the islet graft has been debated. Vajkoczy et al^[21] had shown through intravital microscopy that newly formed blood vessels in the islet cell unit grow primarily from the host arterial capillaries. The revascularization process is most likely founded on intra islet and/or localized release of VEGF. VEGF expression and intra islet release can be enhanced by exposing the islet cells in vitro to hypoxic conditions prior to transplantation.^[22] In addition to VEGF, the expression of its tyrosine kinase receptors (flt and flk-1) is essential to enhancing the islet graft vascularization.^[23] The Fltk-1 receptor may also play a role in the extracellular signaling of beta cell maturation by allowing beta cell regeneration and improving islet cell mass and function after transplantation.^[24] The addition of a population of endocrine non-beta-cells to the purified islet cell grafts partially accelerated the revascularization of pure beta-cell grafts, indicating that other factors besides the presence of VEGF, its receptors, and endothelial cells, may be crucial in recovering islet revascularization and function. Furthermore, protecting the islets from hyperglycemia during the early phase of engraftment seems to not only prevent early apoptosis and loss of beta-cell mass,^[25] but also to improve islet graft revascularization. Indeed exposure of the beta cell to hyperglycemia in the diabetic recipient may delay the expression of VEGF receptors in-and-around the islet cell and impair angiogenesis necessary for proper islet engraftment and function.^[26]

Another approach to improve revascularization and engraftment was reported by Hirshberg et al^[27] who questioned and compared the traditional portal vein approach to the intestinal arterial system. They showed that a neo-endothelial cell layer from the hepatic arterial supply usually surrounds islet cells, implanted in the portal venous tract of the liver with better revascularization compared to other implantation sites such as the mesentery.^[27]

Preventing thrombus formation during islet graft portal vein injection

Injecting the islet cells into the portal vein does bear complications. Indeed, an instant blood-mediated inflammatory reaction (IBMIR) occurs

when the isolated pancreatic islets come in contact with ABO-compatible blood.^[28] Korgsgren et al^[20] developed an *in vitro* islet perfusion system that mimics *in vivo* transplantation of the islet cells as they are introduced into the blood stream. IBMR results in rapid activation of circulating platelets with β -thromboglobulin (β -TG) release. Concomitantly, the platelet as well as polymorphonuclear cell and monocyte count decreases. Histologic analysis of the islet cell cluster after its introduction into the blood stream shows it is entrapped in a platelet and fibrin thrombus infiltrated by leukocytes. This islet cell entrapment not only blocks the process of revascularization necessary for engraftment but also triggers a strong inflammatory reaction with cytokine release which damages islet cell viability and function.^[28]

To counteract this activation of coagulation most centers performing allogeneic islet transplantation today use heparin at the time of transplantation.^[29] Heparin does reduce platelet consumption, but does not completely prevent platelet and fibrin thrombi formation. On the other hand, the use of melagatran, a specific thrombin inhibitor, significantly reduces IBMR.^[29] In addition tissue factor (TF) inhibitor may also be required as TF is shown to be greatly upregulated in the transplanted islet cluster and to be a key activator of thrombin in this situation.^[29]

Preventing cytokine-mediated islet-cell damage

During the peritransplantation period, multiple factors such as ischemic injury, immunologic response, and inflammatory response may be responsible for islet graft loss in the early phase of pancreatic islet (PI) engraftment in the portal venous tract of the liver. The loss of approximately 30% of the islet mass may result from apoptosis.^[5] Indeed, studies by Thomas et al^[30] suggest that during the islet isolation and the peritransplant period, tumor necrosis receptor-apoptosis-inducing-ligand (TRAIL)-receptors-induced apoptosis is probably the main mechanism inducing pancreatic islet apoptosis associated with tumor necrosis factor (TNF)- α release. But other inflammatory cyto-

kines produced from intra-islet-activated macrophages are also recognized as cytotoxic to pancreatic islets *in vitro*. Indeed, IL-1, IL-6, and TNF- α usually added in combination inhibit insulin synthesis and secretion and also have cytotoxic effects on beta cells.^[31]

From these studies, it appears that primary intra-hepatic PI graft damage is mainly cytokine-mediated. Most inflammatory cytokines such as IL-1 and IL-6 are multifunctional cytokines, involved in the regulation of the immune response, hematopoiesis, inflammation, and cellular differentiation and could possibly provide a stimulus to recruit and to stimulate the proliferation of inflammatory cells and to enhance further the activation of T and B lymphocytes.^[32]

Preventive strategies to ameliorate PI primary non-function should target the production of inflammatory cytokines, TNF- α , IL-1 or IFN- γ . Faust et al^[33] have shown that treatments with the "suppressive cytokines" IL-4 and IL-10 decreased Th-1 cytokine production and enhanced PI graft survival in autoimmune nonobese diabetic mice. Furthermore, Rabinovitch et al^[34] presented evidence that IL-4, and to a lesser extent IL-10, improved the ability of cyclosporine A to prevent autoimmune destruction of β -cells in syngeneic islets transplanted into NOD mice. Nicotinamide has been shown by Kenmochi^[35] to partially reverse the effects of pro-inflammatory cytokines i. e., reduction of islet insulin content and induction of islet nitric oxide (NO) production. There is considerable controversy whether or not cytokine damage is mediated by induction of nitric oxide (NO) formation.^[36] Moreover, Yamada et al^[37] found that in the absence of L-arginine, a major precursor of NO, a combination of IFN- γ and TNF- α , highly cytotoxic to mouse islet cells, failed to destroy islet cells. However, Rabinovitch et al^[36] found that a NO synthetase inhibitor did not affect cytokine-induced reduction in the DNA and insulin content of the islet cell cultures. They concluded that cytotoxic mechanisms independent of NO were sufficient to destroy the islet beta cell. Cetkovic^[38] found that aminoguanidine, an inhibitor of NO production, prevented a combination of IL-1, plus IFN- γ , plus TNF- α from reducing islet DNA content, but failed

to prevent depletion in islet insulin content. He concluded that the major effect of these cytokines was an 80% decrease in islet insulin content, which was not mediated by NO. Stevens et al^[39] demonstrated that the use of L-N-G monomethyl-arginine, a competitive inhibitor of NO synthetase, did not improve intra-hepatic islet engraftment. Thus they also questioned the direct involvement of NO in islet primary non-function (PNF). On the other hand, Thomas et al^[40] recently showed that NO production by beta cells themselves is required for IL-1 and IFN- γ -mediated inhibition of islet function and islet damage and that NO produced by non-endocrine islet cells does not mediate beta cell damage. Burkart et al^[41] have used fusidic acid, a compound originally described as an antimicrobial drug, to suppress NO toxicity and to protect islet beta cells from destruction in IDDM-I. However, most of the NO synthetase inhibitors, which partially improved pancreatic islet engraftment in rodents, have not been tested clinically.

Down-regulating NF-kappa B

Release of various inflammatory cytokines and chemokines is largely regulated by nuclear factor-kappa B (NF- κ B) transcription factor, which requires phosphorylation of I κ B subunit for activation.^[42] In the context of islet cell transplantation pharmacological attenuation of NF- κ B transcription factor activation may also diminish cytokine release and improve islet cell survival and function.^[43] The family of drugs, statins, can block the activation and translocation of NF- κ B into the nucleus and inhibit the induction of proinflammatory cytokine production.^[44] More recently, pravastatin, a 3-hydroxy-3-methylglutarylcoenzyme A reductase inhibitor that suppresses inflammatory cell activity prevented islet isograft non-function in mice.^[45,46] Contreras et al^[43] also reported the importance of the phosphoinositide 3-kinase (PI3-K)-dependent serine-threonine protein kinase, called Akt, which inhibits apoptosis. After simvastatin treatment, Akt phosphorylation (i.e. activation) was observed in isolated pancreatic islets with increase in islet viability. This observation was associated with decreased Bad phosphorylation, low cytochrome c re-

lease, caspase-9 activation, and translocation of forkhead transcription factor (FKHR).^[43] Another molecule, 17 β -estradiol was shown by Contreras et al^[47] to inhibit NF- κ B nuclear translocation in islet cells and to prevent apoptosis by decreasing mitochondrial cytochrome c release and caspase 9 activation.

Blocking apoptosis by targeting inflammatory signaling pathways such as NF- κ B or PI3-K/Akt pathways is a first step in preserving isolated islet viability and function. Another important step is to identify and potentially restore the main protective factors provided normally by the exocrine tissue and/or the extracellular matrix which may be lost once the islets are isolated and disrupted from their native tissue.^[48]

Down-regulating adhesion molecules ICAM-1 and MHC

ICAM-1 and MHC molecules also play an important role in signaling pathways during inflammatory cell and lymphocyte recruitment.^[49] In vivo, Coddington et al^[50] documented evidence of ICAM-1 and class II MHC enhanced immunohistochemistry staining on subcapsular kidney histologic sections within days of alloislet engraftment. Moreover, the blockade of ICAM-1/LFA-1 with monoclonal antibody treatment prolonged islet allograft survival in both the kidney capsule and portal venous sites of the liver in the mouse model.^[51] Instead of blocking the expression of antigens such as MHC molecules and/or ICAM adhesion molecules, Kuttler et al^[52] have been able to decrease expression of these same molecules on islet cell surfaces maintained in long-term culture. Therefore, initiation of rejection and generation of cytotoxic cells might be reduced when transplanting long-term cultured islets. Indeed Gaber et al^[53] showed improved viability and in vivo function of human islets maintained in culture for at least 7–14 days prior to transplantation into nonobese diabetic-severe combined immunodeficient mice.

Blocking free radical production

Finally, blockage of free radical production by leukocytes and by the islet cells themselves un-

dergoing oxidative stress has to be applied as well. Pileggi et al^[54] recently reported that the up-regulation of the ubiquitous free radical-scavenging stress protein heme oxygenase-1 protected both rodent islets and beta cell lines from apoptosis and improved their *in vivo* function. Furthermore, expression of the enzyme manganese superoxide dismutase (SOD) in insulinoma cells is known to prevent IL-1 β -induced cytotoxicity and to reduce NO production.^[55] Therefore, targeting the oxidative stress injury is another approach to scavenge free radicals and to protect islet cells, and Bottino et al^[56] have reported significant islet cell mass preservation using a novel SOD mimic compound in culture after isolation. Preservation of islet cell mass can also be achieved by enhancing the regenerative potential of the beta cell. Indeed beta cell exposure to growth factors such as IGF-1 have not only had an effect on preventing apoptosis but also on inducing islet cell growth and regeneration.^[57]

From *in-vitro* conditioning to gene therapy

Most of these conditioning approaches can be done *in vitro* prior to islet cell infusion, allowing the islet cells to recover from isolation-induced stress and also to be manipulated for regeneration or tolerance induction through anti-inflammatory conditioning or gene therapy.

Gene therapy approaches such as Bcl-2 gene transfection *ex vivo* prior to transplantation have shown significant cytoprotection of macaque isolated pancreatic islet during the peritransplantation period.^[58] Adenoviral gene transfer of the interleukin-1 receptor antagonist protein to human islets has also prevented IL-1 β -induced beta cell apoptosis.^[59] Additionally, adenoviral gene transfer of an I κ B repressor protected human islets from interleukin-1 β .^[60] Weber et al^[61] had examined the safety of adenoviral gene transfection on beta cell function and survival and there did not appear to be a significant damaging influence.

Inducing tolerance

Tolerance is the lack of an immune response against an antigen and is physiological in the con-

text of self-tolerance. But in the context of allogeneic islet transplantation for the treatment of type I diabetes, the challenge is to induce tolerance to alloantigen in addition to restoring self tolerance to beta-cell, preventing graft rejection and recurrence of autoimmunity, respectively.

Some of the classical immunosuppressive agents such as steroids and calcineurin inhibitors are known to be diabetogenic. However immunosuppressive regimens have been improved in order to properly prevent immune rejection without damaging islet cell function. Shapiro et al^[8] reported the first successful study without steroids including an IL2-receptor antibody (daclizumab), sirolimus (rapamycin), and low dose tacrolimus. But the draw back from the Edmonton protocol is that most recipients required two to three islet grafts to maintain insulin independence. More recently insulin independence was achieved from a single donor using a new immunosuppressive agent OKT 3 Gamma Ala, which decreases early inflammatory response, in combination with antithymocyte globulin (ATG) combined daclizumab, rapamycin, and mycophenolate.^[62] But the toxicity of some of these immunosuppressive drugs on β -cell function remains a key problem in the poor success of islet allotransplantation. A study by Paty et al^[63] examined the effects of current immunosuppressive agents on insulin secretion from culture islet cell line HIT-T15. By comparing their results with other studies, they concluded that cyclosporin and tacrolimus (FK506), both calcineurin inhibitors that block IL-2 production, and mycophenolate, a reversible inhibitor of purine synthesis, consistently reduced insulin secretion. On the other hand, they concluded that sirolimus, which blocks IL-2 T-cell proliferation, and daclizumab, an IL2-receptor antibody, had minimal to no detrimental effect on islet function and glucose metabolism.^[63] A new immunomodulator, FTY720, which increases lymphocyte homing, can prevent the islet-graft infiltration by lymphocytes without direct toxicity to the islets.^[64] In any case, it is now the recommendation to use low-dose immunosuppressive protocols for islet transplantation.^[62]

The ultimate goal is tolerance induction without the use of toxic immunosuppressive drugs, ma-

king islet cell transplantation a safer therapy for type I diabetes particularly in children. Two major approaches to tolerance induction have shown some success primarily in primate models.^[66,66] The first approach immunomodulates the host response through the selective alteration of costimulatory pathways necessary for the initiation of a competent alloimmune response, targeting both CD28 and CD40 molecules (anti CD154) and inducing peripheral anergy of existing T cells.^[65] The second approach involves the induction of haemolymphopoietic chimerism, using allogeneic bone marrow stem cell transplantation, that triggers central tolerance controlling future alloreactive T cells.^[67] Both of these approaches still need improvement. Indeed the anti CD154 antibody may also have a receptor on platelet surfaces; therefore, the risk of thromboembolic events has hampered its use. Furthermore, the induction of mixed chimerism generally requires aggressive myeloablative preconditioning regimens, often requiring total body irradiation, with significant side-effects.^[68]

Immunoisolation by encapsulation of pancreatic islets allows for transplantation without immunosuppression and may be an alternative to these tolerogenic regimens. A semi-permeable membrane forms a mechanical barrier separating the islet graft from the host antibodies and immune cells but allows for the diffusion of glucose, insulin, nutrients, and islet waste products.^[69] The two major encapsulation systems described by Vos et al^[69] are macroencapsulation and microencapsulation. Macroencapsulation refers to intravascular macrocapsules containing groups of islets implanted in either the peritoneal cavity, the subcutaneous site, or the renal capsule in rodent models in most studies.^[69] Microencapsulation refers to islets individually enveloped by a semi-permeable membrane, the approach currently preferred by researchers. Indeed the microcapsules are often more stable and have a better diffusion capacity. Problems with both systems are still being investigated before they can be applied clinically: the macrocapsule presents with aggregation of the encapsulated islets into a platelet and fibrin clot, preventing islet function; the microcapsules induce a non-specific fibrotic reaction surrounding the capsule and preventing diffusion of

glucose, insulin and nutrients.

In any case, the scarcity of human pancreases for pancreatic islet isolation to treat patients with type I diabetes is a major obstacle. Indeed, many investigators are now focused on regenerating β -cells from either fetal pancreatic stem cells, embryonic stem cells or hematopoietic stem cells.^[70,71]

Conclusion

The ultimate goal of pancreatic islet preservation is to block cell death and to restore glucose sensitivity. During the peritransplant period, the pancreatic islet undergoes a tremendous amount of stress: the islet is exposed 1) to ischemia, 2) to disruption of the extracellular matrix, 3) to mechanical and enzymatic injury, during its isolation from the whole organ of the pancreas, and 4) finally to the toxicity of immunosuppressive agents during the early engraftment phase. This research is complex and complicated by many factors that decrease islet mass and function including cytotoxic/pro-apoptotic molecules released from the islets or surrounding tissues and the lack of protective factors normally supplemented by the non-beta cells of isolated islets. Currently, targeted therapies to prevent apoptosis and block inflammatory signaling pathways appear most promising. For example, in our laboratory we investigated the role of portal vein endothelial cells in producing IL-6 and TNF- α , promoting an inflammatory process that may lead to destruction of the pancreatic β -cell graft.^[72] The challenge is to prevent the production of toxic inflammatory cytokines during the islet-cell peritransplant period. But in order to increase the chances for success in islet cell preservation, research should remain multi-focused. Therefore, better immunosuppression, better tolerogenic regimens, and developments in stem cell biology and differentiation also have to be achieved to find an ultimate cure for diabetes.

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirect-

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