Islet transplantation: immunological perspectives
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Clinical trials of islet transplantation are showing remarkable success, but they require administration of chronic immunosuppression, and are underscoring the large gap that exists between the number of human donors available and the number of patients that could benefit from the procedure. Recent progress has been made in the definition of key immunological mechanisms that are involved in determining islet transplant outcome. Clinical and preclinical studies, and studies in small animal model systems, will all eventually contribute to the definition of efficient and safe protocols for islet transplantation. If the use of xenografts is successful, it might represent a solution to the shortage of human organs.

Introduction
With the development of successful protocols of clinical islet transplantation, there is a new need for the definition of the immunological variables that influence graft survival and also for the development of efficient ways of manipulating them. It is clear that islet transplantation represents a viable option for the treatment of type 1 diabetes mellitus, but prolonged survival of the graft depends on the administration of chronic immunosuppression. Furthermore, a percentage of grafts fail in the long term, and this clearly shows the relative inefficiency of the currently utilized regimens of therapeutic modulation of the immune system.

The transplantation of islets of Langerhans for the treatment of type 1 diabetes mellitus faces intrinsic difficulties: it is not only allorejection (which inevitably ensues unless immunosuppression is administered) but also the recurrence of autoimmunity that contribute to graft failure. Additionally, islets are isolated and implanted in the liver microenvironment where, immediately after implant, they are subjected to inflammatory events triggered by ischemia/reperfusion-like phenomena occurring in situ. All of these characteristics of islet transplantation contribute to making it a particularly challenging chore. This review will summarize recent contributions to the advancement of knowledge in the field.

Clinical studies
The utilization of steroid-free immune suppression, together with sequential islet cell infusions, resulted in the first demonstration of reproducible insulin independence in human recipients of islet-only transplants [1]. This protocol has now enabled similar results to be reproduced at several centers worldwide. As reported in July 2002 by the Edmonton group [2], 54 islet transplant procedures had been completed on 30 subjects: detailed follow-up was reported for 17 of the patients who attained insulin independence subject to transplantation of a minimum of 9 000 islet equivalents (IEQs) per kilogram of recipient body weight. Data was available for 15 of the 17 patients who had completed one year of follow-up; and 12/15 patients (80%) remained insulin free at one year post-transplant. Three out of the 15 patients lost graft function, as determined by loss of c-peptide production at 7.5, 16 and 17 months, and two of these individuals are believed to have lost graft function because of recurrent autoimmune, as suggested by appearance of islet cell autoantibodies (ICAs) and glutamic acid decarboxylase (GAD) antibodies. For the 14/17 patients that demonstrated maintenance of c-peptide production beyond one year post-transplant, excellent glycemic control and decreased glycosylated hemoglobin (HbA1c), which gives a measure of glycemic control over a period of six weeks, were reported in the absence of hypoglycemia [2]. Complications from the 54 recorded transplant procedures included bleeding (five occurrences, with four requiring transfusion), transient elevation of liver enzymes (46% with >2 × normal levels), moderate abdominal pain (12), gallbladder puncture that resolved spontaneously (2) and thrombus (2). Portal venous pressure increased after islet infusion and significantly correlated with packed cell volume and the number of islets transplanted [3]. Basal portal pressure increased following infusion; liver enzymes were unaffected. Post-transplant portal pressures were greater with each subsequent infusion [3]. For the 17 patients that attained insulin independence, side effects of immune suppression included elevation of serum creatinine in two out of three patients...
that had pre-existing elevations, mouth ulcers (15), nausea and vomiting (2), diarrhea (10), anemia (8) and decreased white blood cell count in all patients (two required brief courses of granulocyte colony stimulating factor). Nine patients required initiation of, or increases in, antihypertensive therapy. Cholesterol rose in 15 of the patients and 11 required statin therapy. No lymphoproliferative disease or cytomegalovirus (CMV) infection were observed. The insulin response to metabolic challenge was not normal, but the transplant clearly enabled normalization of glycemic control [2]. Three hour stepped hypoglycemic clamp studies were undertaken on seven insulin-independent patients from this series [4]. Despite alleviation of actual hypoglycemic episodes in these patients, the clamp results demonstrated that hypoglycemic hormonal counter-regulation (glucagon and epinephrine production) and symptom recognition (ability to sense low glucose induced by the clamp) were not restored in islet transplant recipients and were similar to patients with type 1 diabetes [4]. Using steroid-free immune suppression, three patients have been reported to be insulin independent at Baylor College of Medicine after sequential infusions with islets isolated at the University of Miami [5]. For all islet transplant trials, a Food and Drug Administration (FDA)-approved investigational new drug is required to proceed [6].

It has been reported that human islets express tissue factor [7], which may contribute to early islet loss by stimulating coagulation upon contact between islets and intraportal blood. The monitoring of thrombin–antithrombin complexes in the peripheral blood of islet transplant recipients in the early post-transplant period revealed that the level of thrombin–antithrombin complexes rose and peaked at 15 minutes after islet infusion and then tapered off. In vitro studies revealed that inhibitors of coagulation, such as inactivatized factor VIIa, effectively prevented islet-induced coagulation in allo-geneic blood, thereby suggesting that recipient treatment with such inhibitors may aid in the prevention of early islet loss and enable insulin independence with fewer islets [7]. Many studies are ongoing to assess immune parameters that may facilitate determination of the recipient’s ability to respond to transplanted islets and to differentiate between graft loss due to rejection, recurrent autoimmunity or islet exhaustion. Both molecular and cellular techniques are being utilized but no detailed publications of the data are available yet.

Preclinical studies
Preliminary data regarding islet rejection versus acceptance has been reported for nonhuman primate islet allograft recipients treated with various immune intervention protocols. Recently, investigators in Alabama reported the long-term follow-up of nonhuman primate recipients of islet allografts with operational tolerance [8]. Animals with streptozotocin-induced diabetes were treated with a tolerance protocol consisting of tapering doses of methylprednisolone on postoperative days (PODs) 0, 1 and 2, CD3-immunotoxin on PODs 0 and 1, and deoxypergualin on PODs 0–14. No immune intervention was given after POD 15. Of seven monkeys treated with the tolerance protocol, one experienced acute rejection on POD 70 and one died euglycemic on POD 187. For the five remaining animals, a series of experiments was carried out to stimulate the animals’ immune system and study general immune competence (N8), including third-party skin allografting, hepatitis B vaccination and 1-chloro-2,4-dinitrobenzene (DNCB) administration. Two of the five monkeys required exogenous insulin after these challenges and were considered to have rejected the islets (PODs 353 and 560). The other three animals maintained operational tolerance and stable allograft function (all POD >830). None of the animals experienced infections, malignancy or autoimmunity due to the transient T-cell depletion. Peripheral and lymph node T cells recovered fully within 6–12 months of treatment and animals responded normally to vaccination [8]. No alloantibody was detected, although the authors have previously reported immune deviation (measured by high levels of plasma IL-10 and IL-4, and low levels of IFN-γ) in rhesus monkey renal allograft recipients treated with this tolerance induction regimen [8]. The data are consistent with reports of immune deviation in tolerant rodents.

Studies in small animals
Studies in mice represent a critical component of islet transplantation research. This is particularly true if one considers that the non-obese diabetic (NOD) mouse is one of the few available models of islet-specific autoimmunity. For a long time there has been an impasse in islet transplantation research in NOD mice; all of the methods used to induce long-term survival of allogeneic islets in NOD mice were not clinically applicable, as they were based on the use of lethal or sublethal conditioning followed by bone-marrow transplantation to induce tolerance. Conversely, there was no clinical intervention that resulted in reproducible long-term islet survival in patients and that could be tested in NOD mice. Therefore, validation of the NOD mouse as a clinically relevant model of islet transplantation was not possible, other than on a purely theoretical basis.

This impasse was broken by the advent of the Edmonton protocol. We have demonstrated that a protocol closely resembling the Edmonton regimen of immunosuppression results in significantly prolonged survival of allogeneic islets in spontaneously diabetic NOD mice, providing a strong experimental rationale for the use of this animal model to study islet allotransplantation in autoimmune diabetes [9]. We have defined two additional immunosuppressive regimens that result in highly significant prolongation of islet allograft survival in NOD
The cytoprotection of islets is becoming an important field of study, as it links inflammation and immunology in a novel fashion. The premise of many studies addressing cytoprotection was that the use of free radical scavengers, anti-apoptotic compounds and selected growth factors might result in protection from the early post-transplant events of inflammation, resulting in better performance of the grafts in terms of islet mass requirement. This was confirmed by numerous authors [21–24] and quite recently by Andy Stewart’s group [25, 26], who showed that both hepatocyte growth factor (HGF) and parathyroid hormone-related protein (PTH-RP) can protect islets from death both in vitro and in vivo.

Somehow unexpectedly, cytoprotection can also result in prolongation of islet survival both in autoimmune mice and in a fully allogeneic setting (L. Inverardi, NS Kenyon, C. Ricordi, unpublished data; [27]). Administration of manganese superoxide dysmutase (MnSOD), cobalt protoporphyrin (CoPP), or enforced deficiency of c-REL (a member of the NF-κB transcription family), result in protection from either autoimmune recurrence or allo-rejection [28]. Interestingly, viral-mediated delivery of the immunomodulatory cytokine IL-4 in a syngeneic marginal islet mass transplant had no protective effect [29].

**Xenotransplantation**

The shortage of human islets for transplantation is likely to become even more dramatic, as success in clinical protocols and the number of procedures performed increases. Alternative sources of insulin-producing tissue are needed and xenogeneic islets might represent a viable alternative to human islets. Although there has been substantial progress in our knowledge of the mechanisms of xenogeneic islet recognition and rejection, there are many immunological hurdles that still need to be addressed. Olack et al. [30] have shown that pig islets are rejected by CD4⁺ lymphocytes activated via the indirect pathway of antigen recognition, and Koumoundouros et al. [31] have recently reported that pig islets are protected from autoimmune recognition in NOD mice after treatment with anti-CD4 antibodies, contributing to elucidation of the mechanisms of islet-specific autoimmune aggression in a xenogeneic combination. Omer et al. [32] have suggested that encapsulation might work in a remarkable manner, as it does in allografts, in xenogeneic islet transplantation. In support of this report is the observation that pig islet function and survival is improved following encapsulation and culture preconditioning, as reported by Sato et al. [33]. Lastly, the transplant of encapsulated pig islets into dogs was reported by Kin et al. [34], who used an agarose–polystyrene mixed gel for prolonged graft survival in the absence of immunosuppression, and were successful in a sizable percentage of pancreatectomized recipients. Although there is still debate on the impact of the ζ-galactosyltransferase (ζ-Gal) epitope on occurrence of islet xenograft rejection,
the recent availability of α-Gal knockout pigs will help to definitively address its role [35*].

Quite interestingly, a clinical trial of islet transplantation in diabetic patients is ongoing in Mexico, and results will be soon available. Initial reports suggest a potential effect of the procedures, in which pig neonatal islets are transplanted together with Sertoli cells in a device that is implanted subcutaneously [36]. This clinical trial has stirred substantial controversy on the safety of xenogeneic transplants in humans, especially because of concerns on the transmission of porcine endogenous retrovirus (PERV) across this species barrier. The most recent reviews, have been highlighted as:

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

★ of special interest
★★ of outstanding interest


The authors have pioneered the important observation that isolated islets, when exposed to blood, trigger coagulation, complement activation and polymorphonuclear cell recruitment. They show in this paper that the probable culprit for this series of events, which leads to significant islet damage, is tissue factor, expressed on the islet surface. This observation has profound clinical implications.


Few studies are performed in nonhuman primates, and even fewer have described the achievement of transplantation tolerance. In this paper, the long-term metabolic follow-up of primates tolerant to transplanted allogeneic islets shows stable function of the islets. Recipients were made tolerant with a short course of prednisone, CD3 immunodepletion and donor-specific transfusions.


The authors demonstrate that a protocol closely resembling the Edmonston clinical protocol of immunosuppression efficiently prevents allogeneic islet rejection in diabetic NOD mice. This observation provides support for the use of the NOD mouse model in studies of the immunobiology of islet graft survival in an autoimmune background for the first time.


The expression of Fas ligand on splenocytes is obtained by biotinylation of the cell surface followed by use of a recombinant chimeric molecule made by coupling the ectodomain of Fas ligand to streptavidin. Fas ligand-expressing splenocytes have powerful immunomodulatory characteristics in vitro and in vivo, promoting prolonged graft survival of cotransplanted allogeneic islets. This novel strategy for displaying cell surface molecules in a rapid and efficient way has tremendous therapeutical potential.


38. Clemenceau B, Jegou D, Martignat L, Sai P: Microchimerism and transmission of porcine endogenous retrovirus from a pig cell line or specific pathogen-free pig islets to mouse tissues and human cells during xenografts in nude mice. Diabetologia 2002, 45:914-923.