REVIEW

Current status of pancreatic islet transplantation

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ABSTRACT

DM (diabetes mellitus) is a metabolic disorder of either absolute or relative insulin deficiency. Optimized insulin injections remain the mainstay life-sustaining therapy for patients with T1DM (Type I DM) in 2006; however, a small subset of patients with T1DM (approx. 10%) are exquisitely sensitive to insulin and lack counter-regulatory measures, putting them at higher risk of neuroglycopenia. One alternative strategy to injected insulin therapy is pancreatic islet transplantation. Islet transplantation came of age when Paul E. Lacy successfully reversed chemical diabetes in rodent models in 1972. In a landmark study published in 2000, Shapiro et al. [A. M. Shapiro, J. R. Lakey, E. A. Ryan, G. S. Korbutt, E. Toth, G. L. Warnock, N. M. Kneteman and R. V. Rajotte (2000) N. Engl. J. Med. 343, 230–238] reported seven consecutive patients treated with islet transplants under the Edmonton protocol, all of whom maintained insulin independence out to 1 year. Substantial progress has occurred in aspects of pancreas procurement, transportation (using the oxygenated two-layer method) and in islet isolation (with controlled enzymatic perfusion and subsequent digestion in the Ricordi chamber). Clinical protocols to optimize islet survival and function post-transplantation improved dramatically with the introduction of the Edmonton protocol, but it is clear that this approach still has potential limitations. Newer pharmacotherapies and interventions designed to promote islet survival, prevent apoptosis, to promote islet growth and to protect islets in the long run from immunological injury are rapidly approaching clinical trials, and it seems likely that clinical outcomes of islet transplantation will continue to improve at the current exponential pace.

INTRODUCTION

Diabetes

DM (diabetes mellitus) is a metabolic disorder of either absolute or relative insulin deficiency. Worldwide, the impact of diabetes is far reaching: the WHO (World Health Organization) estimated that 177 million people were living with diabetes worldwide in 2000 [1]. Today, it is estimated that 194 million people live with diabetes, representing 6% of the population of developed countries. The WHO expects this number to rise to 300 million by 2025. Societal costs of diabetes range

Key words: immunosuppression, pancreatic islet transplantation, Type I diabetes mellitus.

Abbreviations: BMI, body mass index; CD40L, CD40 ligand; CITR, Collaborative Islet Transplant Registry; CTLA4, cytotoxic T-lymphocyte attenuator protein 4; CMV, cytomegalovirus; DCCT, Diabetes Control and Complications Trial; DM, diabetes mellitus; DP, donor point; FDA, Food and Drug Administration; GAD, glutamic acid decarboxylase; GLP-1, glucagon-like peptide-1; GRAGIL, Groupe de Recherche Rhin Rhône Alpes Genève pour la transplantation d’Îlots de Langerhans; HbA1c, glycosylated haemoglobin; HYPO score, composite hypoglycaemic score; IBMIR, instant blood-mediated inflammatory response; IE, islet equivalent; IL-2, interleukin-2; LI, lability index; MAGE, mean amplitude of glycaemic excursions; MRI, magnetic resonance imaging; NIDDK, National Institute of Diabetes and Digestive and Kidney Diseases; NIH, National Institutes of Health; PET, positron emission tomography; PFC, perfluorochemical; P02, partial pressure of oxygen; PTLD, post-transplantation lymphoproliferative disease; T1DM, Type 1 DM; mAb, monoclonal antibody; SI, stimulation index; UNOS, United Network on Organ Sharing; UW, University of Wisconsin; WHO, World Health Organization.

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between 5 and 15% of healthcare expenses in developed nations: estimated to be over $100 billion in the U.S.A. alone for 2005.

The American Diabetes Association classifies diabetes as Type I, Type II, gestational or other. Although the aetiology of T1DM (Type I DM) is incompletely understood, autoimmune destruction of β-cells is the resulting pathological finding. T1DM is genetically associated with certain HLA alleles [2]; however, environmental factors such as exposure to bovine milk protein may increase the risk of T1DM [3]. Historically, diabetes was an acutely fatal illness ending in hyperglycaemic coma. With the discovery of insulin and its therapeutic application just over 80 years ago, diabetes has become a chronic illness. Diabetic patients today can expect longer lives, but now face the long-term complications of hyperglycaemia, including diabetic retinopathy, nephropathy, neuropathy and vasculopathy.

Optimized insulin injections remain the mainstay life-sustaining therapy for patients with T1DM in 2006, with regular monitoring of blood glucose levels. A small subset of patients with T1DM (approx. 10%) are exquisitely sensitive to insulin and lack counter-regulatory measures, putting them at higher risk of neuroglycopenia. Hypoglycaemic episodes may become increasingly difficult to manage as patients lose their ability to predict the onset of hypoglycaemia based on adrenergic symptoms (sweating, tremor, tachycardia and anxiety) and start to exhibit dizziness, confusion and blurred vision. In severe cases, hypoglycaemia can lead to coma, seizure or death. Alternative forms of insulin administration, which more closely mimic physiological insulin secretion, such as implantable insulin pumps, can improve glycaemic control and reduce hypoglycaemic episodes, but are not suitable for all patients [4].

The importance of glycaemic control in the development of long-term complications of diabetes was most elegantly shown in the DCCT (Diabetes Control and Complications Trial) [5]. Alternative strategies to injected insulin therapy are slowly emerging and include inhaled insulins, insulin pumps, whole pancreas transplantation and now islet transplantation.

The first whole pancreas transplant was carried out by Kelly and Lillehei in 1966, but early outcomes were appalling; with less than 3% of transplants functional by 1 year and a mortality rate of 60%, whole pancreas transplant was a risky undertaking [7,8]. Failure of these preliminary attempts was probably the result of the mandatory use of high-dose steroids as an antirejection therapy, as this led to poor surgical healing of the duodenal anastomosis as well as subjecting the patient to increased risk of opportunistic infections.

Islet transplantation came of age when Paul E. Lacy successfully reversed chemical diabetes in rodent models in 1972 [9]. This prompted enthusiasm that islet transplantation could move into the clinic as a viable option for T1DM. In 1990, a successful report of 1 month of insulin independence following islet transplant in a T1DM patient was reported by Scharp and co-workers [10]. However, technical limitations in islet isolation and immunosuppression prevented immediate wide-scale applicability. Over the next 10 years, approx. 450 attempts at clinical islet transplantation were carried out, but fewer than 8% of subjects were rendered insulin free, and enthusiasm for the approach waned. Notable exceptions included the series of cluster islet–liver transplants carried out in Pittsburgh, where 50% of recipients were insulin free; however, most of these subjects had surgical-induced diabetes following pancreatectomy rather than autoimmune T1DM. Groups in Milan and Giessen also reported approx. 50% of their subjects achieving insulin independence under cyclosporine and steroid-based immunosuppressive strategies and most of these transplants were in patients that already had a kidney transplant.

In a landmark study published in 2000, Shapiro et al. [11] reported seven consecutive patients treated with islet transplants, all of whom maintained insulin independence out to 1 year. The approach, dubbed the Edmonton protocol, included: (i) selection of T1DM subjects for ‘islet alone’ transplantation (before renal failure had set in) who suffered from severe hypoglycaemia unawareness or labile diabetes; (ii) the immunosuppressive protocol was steroid free, consisting of dacluzimab [an anti-CD25 mAb (monoclonal antibody)] induction, the newly introduced antirejection drug sirolimus and low-dose calcineurin inhibitor tacrolimus; (iii) islets were prepared for transplant in the absence of xenogeneic proteins using human albumin rather than bovine albumin; and (iv) 10000 IEs (islet equivalents)/kg of body weight was the minimum islet transplant administered to each patient, often administered as two or sometimes three infusions from sequential donors. Since the original report of the Edmonton protocol in 2000, an estimated 500 islet transplants have been conducted worldwide using variants and further advances, including the use of islet culture, pancreas transportation on ‘two-layer’ oxygenated perfluorodecalin and the use of an infusion bag.
Landmarks in Pancreatic Islet Transplantation

1893
1972
1977
1990
1996
2000
2004
2005

Williams and Harsant; Bristol, UK - First attempt of xenotransplantation of segments of sheep pancreas subcutaneously.

Lacy; Washington, USA - Successful reversal of hyperglycaemia in chemically induced animal model of diabetes using islet transplantation.

GRAIL Consortium; Giessen, Germany and Geneva, Switzerland - Series of islet allografts in diabetic patients, 20% insulin independence on 1-year follow-up.

Minnesota, USA - Clinical series of patients receiving islet autografts following therapeutic pancreatectomy.

Pittsburg, USA - Series of successful islet allografts to treat diabetes in pancreaticomized patients.

Edmonton, Canada - 100% insulin independence in 7 consecutive patients using the Edmonton.

Immune Tolerance Network - Multicenter trial of islet transplantation in T1DM patients.

Kyoto, Japan - First successful living donor islet transplant.

Minnesota, USA - Series of successful single donor islet transplants.

Houston and Miami, USA - Shipments of islets for clinical transplantation.

Figure 1 Landmarks in pancreatic islet transplantation

for gravity infusion rather than syringe injection into the portal vein. Although the current state of islet transplantation represents substantial and almost exponential progress, the necessity of life-long immunosuppression, the scarcity of cadaveric donors and technical inefficiencies in islet isolation preclude widespread use of this therapy today as a replacement for insulin. In the setting of unstable glycaemic control despite optimized insulin management, islet-alone transplantation has provided substantial benefit in these selected patients and major efforts are now underway in North America to seek licensure for islet transplantation by the FDA (Food and Drug Administration) in the U.S.A.

CURRENT STATUS OF ISLET TRANSPLANTATION

Indications for islet transplantation

The islet transplantation procedure and the necessary graft-sustaining immunosuppressive therapy administered thereafter carries risks that must be weighed against potential benefits of islet transplantation. Careful patient selection and an individual risk–benefit analysis are necessary to decide which patients are suitable for transplantation. Risks of islet transplantation include procedure-related risks as well as immunosuppressive-related risks.
Procedure-related risks include bleeding, thrombosis, biliary puncture, discomfort, transient rise in serum transaminase and arteriovenous fistulae. Immunosuppressive-related risks include oral ulcers, anaemia, diarrhoea, weight loss, fatigue, LDL (low-density lipoprotein) elevation, hypertension, renal dysfunction and peripheral oedema.

Islet autotransplantation has been used for almost three decades in patients who required total or near-total pancreatectomy for chronic pancreatitis [12,13]. Patients have avoided ‘brittle’ diabetes when endogenous islet function is restored and approx. 70% of subjects receiving more than 300,000 IEs have shown sustained insulin independence, in some cases as long as 21 years. Although islet transplantation can easily control diabetes in pancreatectomized patients, failure of islet allotransplantation in T1DM patients is likely due to (i) the diabetogenic toxicity of immunosuppressive therapy, and (ii) the autoimmune destruction of transplanted islets.

The DCCT report and subsequent data have shown that intensive management of blood glucose using insulin and hypoglycaemic agents can limit the progression of diabetes complications in T1DM patients [5]. Islet transplantation offers both short- and long-term benefits to diabetic patients [14].

Inclusion factors for islet-alone transplantation have largely focused on subjects with unstable T1DM. Certain patient factors may preclude enrolment. These criteria have evolved jointly by the islet transplant and diabetology communities and are summarized in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Indications</th>
<th>Exclusions</th>
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<tbody>
<tr>
<td>T1DM</td>
<td>HbA1c &gt; 10%</td>
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<tr>
<td>Negative stimulated C-peptide (&lt; 0.3 ng/ml)</td>
<td>Uncreated proliferative retinopathy</td>
</tr>
<tr>
<td>Intensive diabetes management*</td>
<td>BP &gt; 160/100 mmHg</td>
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<tr>
<td>One or more severe hypoglycaemic events‡</td>
<td>GFR &lt; 70 (female) or &lt; 80 (male) ml·min⁻¹·1.73 m⁻²</td>
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<td>Plus one of the following three:</td>
<td>Present or history of macroalbuminuria</td>
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<tr>
<td>Reduced awareness of hypoglycaemia∥</td>
<td>PRA &gt; 20%</td>
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<tr>
<td>Marked glycaemic lability§</td>
<td>Severe cardiac disease</td>
</tr>
<tr>
<td>Composite score &gt; 75th percentile</td>
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<tr>
<td>Patient satisfaction with current management</td>
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* Glucose testing three or more times/day, three or more insulin injections per day or on an insulin pump, as directed by an endocrinologist, diabetologist or diabetes specialist.
† In the past 3 years requiring assistance, blood glucose < 50 mg/dl, intravenous glucose or glucagon.
‡ Clarke score > 4 and HYPO score > 90th percentile (≥ 1047) within the recent 6 months.
∥ Clarke score > 4, HYPO score > 90th percentile (≥ 432) and LI > 75th percentile (≥ 229).
§ Wide swings in blood glucose levels despite optimal diabetes therapy, defined as a glycaemic lability index > 90th percentile (≥ 432) within or recent 6 months.
|| Clark score > 4, HYPO score > 75th percentile (≥ 422) and LI > 75th percentile (≥ 329).

Objective measures to quantify the severity of both hypoglycaemia and metabolic lability have recently been introduced to ensure that islet transplantation is offered appropriately to patients with unstable T1DM, after all alternative measures to correct instability have been exhausted by multiple attempts to improve insulin regimens. A recent report by Ryan et al. [15] described quantitative measures of hypoglycaemia and glycaemic lability which have been helpful in screening for potential islet transplant recipients and to monitor outcomes post-transplantation. The standard definition of severe hypoglycaemia is one which requires outside help by the patient [16]. This subjective measure has limitations based on personal variation and does not account for multiple less severe hypoglycaemic events. Ryan et al. [15] report computation of a HYPO score (composite hypoglycaemic score) based on patient self-reported events; greater points are given based on neuroglycopenic over autonomic symptoms.

Glycaemic lability is another term which has had multiple definitions, and various scoring systems are reported in the literature, including the M-value described by Schlichtkrull et al. [17], MAGE (mean amplitude of glycaemic excursions) [18], and LI (lability index) [15]. Ryan et al. [15] report that LI correlates much more closely than MAGE with clinical assessment of lability and, therefore, serves as a better quantitative scale for the assessment of glycaemic control in islet transplant patients.

Certain special cases may affect patient eligibility and clinical decision-making. One clear example is a prior renal transplant recipient who is already taking anti-rejection therapies to sustain the kidney graft; it is accepted that patients with T1DM and renal failure would benefit from a kidney–pancreas transplant or an islet after kidney transplant [19].

A role for islet transplantation in the paediatric population has yet to be defined. Hathout et al. [20] suggest that children already on immunosuppressive therapy and others who have a life-threatening risk of hypoglycaemia could potentially be eligible for islet transplantation, and careful selection of children at acute risk of death or irreversible brain injury from neuroglycopenia will be the way in which paediatric islet transplantation is initially developed. Important concerns raised in the report by Hathout et al. [20] include the inability to predict whether an islet graft will grow with a child’s growth and the ability to ensure immunosuppressive compliance with age, especially in the teenage years. The ethical implication of the cumulative teratogenicity of immunosuppressive agents increases the complexity of this issue.
ABO (A-, B- and O-blood group) compatibility and negative donor–recipient cross-match remain part of the release criteria for islets destined for transplantation. The risk of infection of the recipient has been minimized through careful screening of donors for hepatitis B and C, and HIV. CMV (cytomegalovirus) infection has previously been a challenge in solid organ donation, but is experienced extremely rarely in the case of islet transplantation. Ganciclovir or valganciclovir prophylaxis is offered to CMV-negative recipients when a CMV-positive donor is used.

**Islet isolation and donor considerations**

Methods for isolation of the endocrine portion of human pancreas have improved rapidly over the last three decades. Staining of islets with the dye Neutral Red and microscopic dissection in historical attempts failed to purify a large number of viable islets [21]. Currently, digestion of pancreatic exocrine tissue with collagenase and subsequent density centrifugation for isolation using Ficoll or other non-ionic radiological contrast gradients (e.g. iodixanol) have yielded progressively better islet yields [22] (Figure 2). The availability of low-endotoxin reagents, including Liberase-HI and now Liberase-CI (Roche Biochemicals), and the introduction of automated procedure have ensured more predictable yields without compromising islet viability.

The most fundamental advance in islet processing came with the introduction of the semi-automated method for controlled pancreatic digestion by Camillo Ricordi. The ‘Ricordi Chamber’ is now used universally by islet-processing centres to optimize islet yield and consistency [23]. Although a detailed consideration of the techniques of islet processing are beyond the scope of the present review, recent developments in islet isolation, considerations in donor pancreas and islet transportation and known predictive factors of islet yield and viability are reviewed in [24].

Several factors must be considered when selecting and producing enzymes for digestion of the exocrine pancreas. Lot-to-lot variation in enzyme products has been an ongoing challenge in standardization of islet isolation; recently, the use of a two-component enzyme preparation for human islet isolation has produced favourable results [25]. To minimize lot-to-lot variability and control endotoxin content, a blend of Collagenase NB1 and Neutral Protease NB (SERVA) was compared with Liberase HI in human islet isolation. Bucher et al. [25] report higher IE yield, higher IEs/g of pancreatic tissue, improved islet function and decreased rate of
Methods of reducing pancreatic injury in the pre-isolation period, particularly cold ischaemic injury, have been the focus of attention in the past several years. The Euro-Collins solution initially introduced in 1991 as a solvent of Ficoll prevented cold storage oedema of exocrine tissue and improved yield of islet isolation [26]. Around the same time, the UW (University of Wisconsin) solution was introduced as a transport media for pancreases destined for the islet isolation laboratory [27]. Cold storage of pancreas for up to 26 h was reported to yield viable islets [28]. More recently pre- and post-purification islet yields have improved using the two-layer cold-storage method in which the pancreas is stored at the interface of UW solution and PFC (perfluorochemical) on ice with constant 95% oxygen perfusion of the PFC layer [29]; islet recovery from pancreases stored using the two-layer method was on average 349 × 10^3 IEs/pancreas compared with 214 × 10^3 IEs/pancreas when using UW solution alone.

Retrospective multifactor analysis of 437 donor pancreases that were processed for human islet transplantation reported by Nano et al. [30] shed light on how donor characteristics, pancreas condition, organ procurement method, digestion characteristics and purification procedure affect islet yield, purity and function in vivo. Donor factors, including donor age and BMI (body mass index), are significantly and positively correlated with islet yield [31]. Of pancreatic features important in predicting islet yield, pancreatic morphological condition and pancreas weight are most important. Nano et al. [30] reported further that collagenase activity and neutral protease activity are the most important digestion features that predict islet yield.

In an attempt to create a standard protocol for acceptance of donor pancreas for islet transplantation, O’Gorman et al. [32] proposed the use of a DP (donor point) system which gives a pancreas a score from 0–100 based on pancreas and donor criteria. The scoring system has been proven to be effective in assessing the potential for favourable isolation outcome and may streamline the acceptance procedures at centres worldwide. An acceptance criteria based on a DP-based system could potentially reduce islet isolation expenses lost to failed islet isolations.

Several methods of assessing viability and function of human islet preparations have emerged recently. Current practice at our centre includes islet quantification (including a measurement of purity and number of IEs) and islet viability measurement (assessed by membrane integrity using staining). Additionally, glucose-stimulated insulin secretion by purified islets can be assessed using static incubation assays or a perfusion study. The SI (stimulation index) is calculated as the ratio of insulin secreted during incubation of islets in medium containing 20 mM glucose to basal insulin secretion in medium containing 2.8 mM glucose. SI of transplanted islets typically ranges from approx. 2–4. Laser scanning cytometry has been proposed for assessment of composition and β-cell viability of human islet preparations, which offers improved specificity and sensitivity [33]. Extensive studies of islet graft cellularity and composition have been published elsewhere [34].

**Current islet transplantation procedure**

Currently, islets are delivered into the portal vasculature, and the liver has pragmatically been proven to be the site with clinical success to date. However, a perfectly hospitable site for the islet graft remains elusive. The naive pancreatic bed is relatively inaccessible and attempts to deliver islet grafts into the splenic vasculature resulted in significant morbidity, including infarction, rupture and gastric perforation [35]. Embolization of islets into the liver offers a physiological advantage, since the liver is the major site of insulin function and is physiologically consistent with pancreatic secretion of insulin directly into the portal vasculature. One disadvantage to the hepatic site is a relatively low oxygen tension: although the portal vein has a moderately low oxygen content [P\(O_2\) (partial pressure of oxygen), 10–15 mmHg], islets ultimately end up in smaller portal veins where oxygen content is even lower [P\(O_2\), 8–10 mmHg]; by comparison the intact pancreas offers a substantially higher content (P\(O_2\), 40 mmHg) [36,37].

Two approaches are currently used to access the portal vasculature: (i) cannulation of the mesenteric venous tributary of the portal system at time of surgical laparotomy or (ii) radiological percutaneous approach to the portal vasculature using fluoroscopic and ultrasound guidance. Once access has been obtained, islets are introduced using a closed infusion bag system that contains islets resuspended in approx. 200 ml of transplant medium. Unfractionated heparin is included in the infusion mix to reduce the occurrence of portal vein thrombosis (although this is rare, and most often associated with impure or only partially purified islets [38,39]). Portal venous pressure, measured by indirect pressure transducer, is used to monitor infusion and changes in portal pressure may be a limiting factor of proceeding with islet infusion. Procedure-related risks of islet transplantation include bleeding, thrombosis, biliary puncture, discomfort, a transient rise in serum transaminase and arteriovenous fistulae.

The surgical approach offers added control which helps to prevent bleeding, whereas the radiological approach avoids the risk of wound infection and wound herniation (which may be of increased risk because of sirolimus administration post-transplantation). Patients generally prefer the radiological method due to its relative simplicity, reduced pain and avoidance for general anaesthesia. However, with the radiological procedure
there still remains the potential risk of bleeding once the catheter has been withdrawn from the liver track. Bleeding after islet implantation may be avoided if the catheter track is sealed along its entire length, and a variety of approaches have been effective, including the use of D-STAT by the Miami group, the combination of coils and gelfoam by the Minnesota group, and the use of microfibrillary collagen (Avitene® paste, made up in radiological contrast agent) by the Kyoto and Edmonton groups.

During the immediate post-transplantation period, glycaemic control with exogenous insulin is used to maintain a stable and hospitable environment for newly engrafted islets. Experimental data from animal models have shown that islet engraftment is achieved more frequently in a euglycaemic than in a hyperglycaemic environment [40]. As islets engraft and begin to secrete insulin, exogenous insulin therapy must be tapered to prevent hypoglycaemia. In some cases, the release of insulin from apoptotic transplanted islets may create a hypoglycaemic environment which necessitates intravenous dextrose. No data from clinical trials are currently available relating to the superiority of intravenous insulin management compared with subcutaneous insulin if capillary blood glucose is higher than 8 mM before a meal or 10 mM at 2 h post-prandial.

**Immunosuppression**

The Edmonton protocol (and more recent variants) use a glucocorticoid-free combination immunosuppressive pharmacotherapy typically including dacluzimab, sirolimus and tacrolimus. Dacluzimab (anti-CD25 mAb) is administered in the peri-transplant period as two intravenous infusions, whereas sirolimus (loading dose, 0.2 mg/kg of body weight and 0.1 mg·kg⁻¹ of body weight·day⁻¹ thereafter) and tacrolimus (initially 1–2 mg twice daily) are administered life-long following transplantation to prevent chronic rejection. Regular monitoring of sirolimus and tacrolimus levels guides fine-tuning of doses post-transplantation. Target predose trough levels are 3–6 ng/ml for tacrolimus and 12–15 ng/ml for sirolimus [11]. This ensures adequate and effective immunosuppression and avoids unnecessary overdosing, which could result in rapid and severe toxicities. In cases where there is an elevated panel reactive antibody or increased risk of donor sensitization, T-cell depletion therapies, including with rabbit anti-(T-cell globulin) (thymoglobulin) or an anti-CD52 T-cell-depleting antibody called alemtuzumab (Campath-1H) may be used. Alternative experimental T-cell modulatory therapies also include hOKT3γ1 (Ala-Ala) used successfully by Hering et al. [41] in their single donor islet infusion protocols (see below).

Side effects of sirolimus and tacrolimus include nausea, oral ulceration, diarrhoea, constipation, fatigue, anaemia, neutropenia, oedema, tumour, acne, hypertension and dyslipidaemia. When higher doses of tacrolimus are used, toxicities, including nephrotoxicity and tremor, may appear. Long-term immunosuppression may increase the risk of infection and certain malignancies. PTLD (post-transplantation lymphoproliferative disease) is seen in immunosuppressed recipients of solid organ and bone marrow transplants [42], but there have been no reports yet of PTLD in clinical islet transplant recipients – the estimated risk is approx. 1–2%, but may be lower where careful surveillance for Epstein-Barr virus using PCR assays, and possibly ganciclovir antiviral therapies, are used.

Although the current immunosuppressive therapies have improved outcome in transplant recipients at the 1- and 3-year time points post-transplantation, it is now emerging that insulin independence is not sustainable in most subjects once they reach 5 years post-transplantation [43]. Approx. 80% of grafts continue to function and secrete C-peptide, however, and patients benefit considerably from near-normal HbA₁c (glycosylated haemoglobin) and avoidance of hypoglycaemic reactions. The exact cause for progressive islet dysfunction is incompletely defined to date, but most likely reflects multifactorial aetiologies, including not only acute and chronic rejection, but the recurrence of autoimmunity and the fact that islets are placed in a non-physiological environment and are exposed further to chronic drugs that have diabetogenic and antiproliferative side effects. Furthermore islet exhaustion may set in when a subtherapeutic islet engraftment mass is forced to continually secrete insulin at maximum capacity.

**Clinical outcomes of islet transplantation**

Insulin independence (that is normal blood glucose levels without the need for exogenous insulin therapy) is perhaps an ultimate goal for patients with T1DM; however, most ‘brittle’ T1DM patients derive substantial benefit from even a partially functional graft in that endogenous insulin secretion (with C-peptide) is restored, and they no longer fear hypoglycaemic events and have reduced need for insulin injections. C-peptide and insulin independence following islet transplantation was reported recently by Ryan et al. [43] in a series of 47 islet transplant recipients transplanted in Edmonton, and is summarized in Figure 3.

HbA₁c is an important clinical marker of islet graft function and reflects the degree of long-term glycaemic control. Patients with failed islet transplants normally return to pre-transplant HbA₁c levels within 36 months, whereas patients with successful grafts can have normalized HbA₁c levels even 66 months post-transplantation [43] (Figure 3).

It has been difficult to develop a single measure that reflects the degree of islet function. HYPO and LI scores have been used for more objective selection of patients with unstable T1DM, and may be followed
post-transplantation for the degree of correction attained [43]. Ryan et al. [43] demonstrated a significant reduction in both HYPO and LI scores out to 5 years post-transplantation (Figure 4). Ryan et al. [44] also developed a β-score as a global measure of islet function post-transplantation.

Protective benefits of islet transplantation against the long-term complications of diabetes still remain to be determined. Although it can be predicted from the DCCT that any form of glycaemic and HbA1c control will reduce such complications, data from islet transplant patients are incomplete. Preliminary retrospective analysis by Fiorina et al. [14] exhibits improved cardiovascular and endothelial function after islet transplantation in T1DM kidney-transplanted patients. Evidence of recurrence or progression of autoimmune T1DM in transplanted patients has been limited, but a case report by Vantyghem et al. [45] suggests that anti-GAD (glutamic acid decarboxylase) antibody levels, a marker of autoimmune T1DM, increase in patients receiving islet transplantation. Although islet transplantation may restore endogenous regulated insulin secretion, recent studies show that intrahepatic islet transplantation fails to restore hypoglycaemic hormonal counter-regulation or symptom recognition [46].

Cumulative data from over 50 centres, which now participate in islet transplantation, are becoming available as collaborative efforts to pool patient information and islet transplant data emerge. Complicating analysis of such data are various confounding factors, including the numerous transplant and immunosuppressive protocols, and variability in islet preparation, culture, and purity [41,47]. A detailed CITR (Collaborative Islet Transplant Registry) was established in North America by the NIDDK (National Institute of Diabetes and Digestive and Kidney Diseases), and provides annual in-depth reports on islet transplant activity [48]. Multicentre networks have also published joint data on their clinical islet transplant programmes, including the GRAGIL (Groupe de Recherche Rhin Rhône Alpes Genève pour la transplantation d’Ilots de Langerhans) Swiss–French Multicentre Network, which recently released data on the outcomes of 260 pancreases donated between 2002 and 2003 for islet transplantation [49].

Use of remote islet-isolation facilities and transportation of pancreatic islets is a logical approach which maximizes expertise and efficiency in avoidance of setting up multiple clean room facilities, and may thereby improve patient access to islet transplantation [50–53]. The more specialized and experienced islet isolation centres have traditionally experienced better islet yield and viability. Centralized islet-isolation facilities reduce the cost of islet transplantation, since the establishment of an islet-isolation clinic which meets current good manufacturing practice (cGMP) guidelines is an expensive undertaking for any clinical institution. Advances in tissue culture media and assessment standards will undoubtedly foster the progression of remote islet isolation and transplant from isolated case reports to mainstream practice.

Islet engraftment

Although allograft rejection and autoimmune disease recurrence may lead to islet destruction in the long
term, there is strong evidence to suggest that immediate ‘innate immune’ responses to islets in the immediate post-transplantation period remain an important mechanism of islet graft destruction. It is estimated that 50–70% of islets are destroyed in the immediate post transplant period by either the IBMIR (instant blood-mediated inflammatory response) or by stress-induced apoptosis [54–55]. This immediate loss of islet not only means that excess islets must be transplanted in order to achieve a minimum amount of glucose-sensitive insulin-secreting tissue, but also inevitably leads to increased antigenic material being released in the host which may prime the adaptive immune response. Korsgren and co-workers [56–58] have extensively investigated the IBMIR and found that human islets excrete large amounts of tissue factor, which triggers platelet binding and activation. This then triggers coagulation and complement cascades, leading to granulocyte and monocyte infiltration and ultimately islet destruction [59,60]. Both tissue factor and selected chemokines [particularly MCP-1 (monocyte chemoattractive protein-1)] are known to trigger this reaction [61]. An array of potent inhibitors of IBMIR are now being explored in experimental and clinical islet transplantation to control IBMIR, and include low-molecular-mass dextran sulphate, nicotinamide and melagatan [57,62,63]. Another interesting compound, LSF (lisofylline), has anti-inflammatory properties and has been shown to reduce by 30% the number of human islets necessary to return a diabetic mouse to normoglycaemia [64].

Additionally, anti-apoptotic peptides, such as caspase-inhibitory peptides [IAP (inhibitor of apoptosis protein) family] [65] and hormones that promote islet growth and function in vivo (EGF (epidermal growth factor), gastrin, GLP-1 (glucagon-like peptide-1) and exendin-4) [66–68] are being investigated for their possible role to promote islet engraftment.

Reducing islet death and promoting engraftment are advantageous for two reasons at least: first, these protocols reduce the absolute number of islet equivalents necessary to render a recipient insulin independent, which addresses the shortage of islet donors, and secondly, reducing islet cell death reduces the amount of antigenic tissue which is released into the periphery, thereby reducing the priming of the adaptive immune response and ultimately impairing allograft rejection.

A clinical test that can predict the onset or risk of chronic rejection would be of major benefit. Several markers in peripheral blood have been investigated for use in such a way. The endogenous islet antigen GAD65 (65 kDa GAD) has been shown to be a poor marker of islet allograft rejection compared with the simplified glucose tolerance test [69,70].

Imaging modalities have been proposed to assess islet engraftment. A recent NIH (National Institutes of Health) workshop on β-cell imaging suggests the use of MRI (magnetic resonance imaging), PET (positron emission tomography) or optical imaging techniques [71]. Direct MRI contrast agents, such as lanthanides and manganese, MRI imaging probes, such as superparamagnetic iron oxide nanoparticles, PET using β-cell-specific antibodies or pharmacological agents, such as glyburide analogues or d-manoheptulose, and optical techniques using metabolic markers, such as calcium flux or insulin granular exocytosis, have been suggested as modalities of choice.

Currently, biopsy-based screening of islet recipients post-transplantation is more challenging than in other solid organ transplants, as it may be difficult to obtain adequate islet samples on a core liver biopsy specimen. Defining the molecular signature associated with dysfunctional islets may hold early clues to rejection events, however, and would be of enormous benefit [72,73]. Development of a technique which involves transcriptome microarray analysis of RNA purified from laser capture microdissection of islet biopsy samples may be a costly undertaking, but may hold clues that could be translated into PCR-based assays for islet rejection.

An ideal clinical assay of islet rejection would include one which could detect islet rejection early in its course or even before it happens, can be conducted on a routine basis, is relatively non-invasive, gives information regarding the stage of rejection and can be administered to patients who live at sites remote from dedicated islet transplant centres.

THE FUTURE OF PANCREATIC ISLET TRANSPLANTATION

Addressing a limited source of insulin secreting tissue

The UNOS (United Network on Organ Sharing) reports that 2019 pancreases were donated in 2004 in the U.S.A.; in the same year 604 pancreas transplants were performed. Only a limited number of pancreases were processed at islet-isolation laboratories and fewer still met clearance criteria for transplantation. The number of organs processed by centres in the U.S.A. is highly influenced by access to limited funding, and reflects the fact that islet transplantation is designated as an experimental procedure and necessitates an FDA-governed
of insulin secreting tissue.

**Single donor islet transplant and living donor islet transplant**
One strategy for increasing the number of successful islet transplants is to eliminate the need for multiple islet donors per recipient. Most centres currently use islets from two (or occasionally three) donors to treat a single patient. A minimum of 10 000 IEs/kg as an islet donor mass to relieve insulin dependence is the current accepted standard. As established islet transplant centres process more pancreases, improvements in purity, yield and viability of islet preparations are rendering single donor islet transplants sufficient for insulin independence in recipients [74,75]. Hering et al. [74] report that an average of 7271 IEs/kg, isolated from a single cadaveric donor, was sufficient for all eight recipients to achieve insulin independence, and that five of these patients remained insulin independent for longer than 1 year.

Living donor transplantation of kidneys, livers and lungs has substantially increased the number of patients who can receive transplants. Living donor kidney transplant is by far the most popular form of living donor organ transplantation, and approx. 50% of renal transplants conducted in the U.S.A. are from living donors. Although there is limited experience in regenerative potential and β-cell reserve of the healthy human pancreas, an early observation that patients receiving distal hemi-pancreatectomy for benign and malignant disease maintained glucose homoeostasis suggests that the distal hemi-pancreas could be donated for transplant by a healthy donor. Indeed, Sutherland and co-workers at the University of Minnesota have carried out more than 150 living donor segmental vascularized pancreas transplants and this programme remains active at their institution.

With the potential for diabetes induction in hemi-pancreas donors, living donors for segmental vascularized pancreas or indeed islet transplantation must be chosen carefully to minimize risk of diabetes in the donor. Donor workup for pancreas transplantation is reviewed comprehensively by Gruesnner et al. [76]. Similar criteria may be potentially applied in segmental living pancreas donation for islet isolation. Choosing donors with higher BMI has traditionally yielded better results in terms of islet yield; however, targeting donors with high BMI in the living donor case may be potentially hazardous, since high BMI is associated with greater susceptibility to Type II DM in the donor. Appropriate screening and follow up of donors, including blood glucose measurement, glucose tolerance test and test of anti-GAD and anti-insulin antibodies will be essential in the stringent selection process of candidate living islet donors. In addition to the risk of diabetes, living pancreas donors face surgical risks of the procedure, including pancreatic fistula, pancreatitis, wound infection and bleeding. Based on case reports, hand-assisted laparoscopic distal pancreatectomy has been suggested to be a safe and efficient route for pancreas harvesting for living pancreas donation [77]. Distal pancreas donors could also potentially benefit from some of the innovative β-cell regenerative therapies, including GLP-1 analogues (e.g. Exenatide), and these could be administered both pre- and post-donation.

The first clinical attempts at living donor islet transplantation were conducted by Sutherland and co-workers [78] at the University of Minnesota in the early 1980s. Initial attempts of living donor islet transplantation were unsuccessful. Twenty-five years later, the islet isolation procedure has improved substantially with the Ricordi chamber, liberase collagenase and continuous gradient centrifugation systems. The first successful living donor islet transplantation was reported by the group from Kyoto, Japan collaborating with Edmonton, in 2005 [79]. In this report, 408 144 IEs were isolated from the freshly harvested distal pancreas of a mother of a ‘brittle’ diabetic and were transplanted in an unpurified state shortly after islet processing was complete. The recipient became insulin independent 22 days post-transplantation and had a normal oral glucose tolerance test 37 days post-transplantation. The donor also had stable glucose homoeostasis post-donation. It should be noted that, in this first case, the recipient suffered from chronic pancreatitis with islet ‘burn-out,’ but did not have autoimmune T1DM.

Several advantages exist to living donor islet transplantation: use of more strict criteria of HLA and PRA (panel-reactive antibody) matching reduces the risk of rejection, living donor pancreas not exposed to haemodynamic instability and pro-inflammatory cytokines, common in non-heart-beating and brain-dead donors on ionotrophic agents, yields better and more islets for transplantation, and the optimal timing of harvest and transplantation reduces warm and cold ischaemia time.

**Alternatives to human islet donation**
Numerous alternative forms of insulin-secreting tissue have been suggested to replace human islets as a form of β-cell-replacement therapy [80]. One promising alternative is xeno-islets for transplantation, and the focus has rested on the feasibility of porcine islets for this purpose. If suitable for transplantation into humans, porcine islets...
Addressing immunosuppression and tolerance

Immunosuppression in islet transplantation

Prior to the Edmonton protocol, immunosuppression of islet transplant recipients was governed by what was felt to be optimal therapy to sustain the existing solid organ graft. In that era, a combination of azathioprine, cyclosporine and corticosteroids was administered to islet–kidney transplant recipients and to a limited number of islet-alone transplant recipients [92].

Cyclosporine is an effective blocker of IL-2 (interleukin-2) and IL-2R (IL-2 receptor) transcription; however, in the 1980s it was reported that cyclosporine induced insulin resistance [93], and later it was revealed that cyclosporine was actually diabetogenic due to its toxicity towards β-cells. Corticosteroids have a widespread immunosuppressive impact and are also particularly diabetogenic [94]. The β-cell toxic effects of cyclosporine and corticosteroids led many clinical islet programmes to pursue steroid-free immunosuppression for islet transplantation.

The discovery of specific immunosuppressive agents and the insight to combine several agents at low dose to avoid toxicity forged the next generation of immunosuppression in islet transplantation. The Edmonton protocol addressed several pitfalls in the islet transplantation procedure, the major one being the diabetogenic properties of immunosuppressives. The combination of sirolimus and tacrolimus, along with anti-CD25 mAb induction, improved outcomes of clinical islet transplantation, showing insulin-independence in seven subsequent islet recipients.

Advances in immunosuppression towards tolerance

Recent modifications of the Edmonton protocol have employed experimental compounds of the following categories: (i) T-cell-depleting agents, (ii) T-cell co-stimulatory receptor-blocking agents, and (iii) lymphocyte trafficking blockade.

T-cell-depleting agents, such as alemtuzumab (Campath-1H; anti-CD52 mAb), hOKT3y1 (Ala-Ala) (anti-CD3 mAb), anti-T-cell globulin (polyclonal antibody) and diptheria immunotoxin anti-CD3, are currently being investigated in primates and plans are progressing to evaluate these in human islet transplantation. Alemtuzumab is an anti-CD52 antibody found to deplete lymphocytes and prevent T-cell activation through the CD45 pathway. Alemtuzumab is effective in the management of autoimmune diseases, including acute vasculitides and multiple sclerosis, and has been shown to be effective as an induction agent for renal transplantation when used alongside either low dose cyclosporine [95] or sirolimus (although not as effective as calcineurin inhibitors) [96,97]. An alternative strategy to depleting T-cells is the use of an Fc-receptor non-binding humanized anti-CD3 mAb [hOKT3y1 (Ala-Ala)], which has been used clinically in the single donor islet transplantation protocol in Minnesota with promising preliminary results [41].

Several compounds that specifically target T-cell co-stimulatory molecules, including the CD28 and CD40L (CD40 ligand (CD154)) pathways, have been explored in islet transplantation. Two strategies of steric interference between co-stimulatory receptors and their ligands have been proposed: a non-depleting mAb to block receptors or receptor–Ig fusion proteins, which bind co-stimulatory ligands thereby precluding their binding to cognate co-stimulatory receptors on the T-cell. Initial studies of an anti-CD40L mAb in non-human primates promised long-term allograft survival including in islet transplantation [98,99]. However, clinical trials in islet transplantation were not pursued following Phase I trials which revealed unanticipated microthrombotic events and the death of one subject [100]. It has been suggested that the thromboembolic complication, which is also seen in non-human primates treated with humanized anti-CD40L, results from platelet activation (platelets express a high level of CD40L) and aggregation rather than a response to T-cell co-stimulation blockade [101]. A new wave of interest in co-stimulation blockade has
emerged from the fusion protein CTLA4 (cytotoxic T-lymphocyte attenuator protein 4)-Ig and its even more potent analogue LEA29Y (Belatacept) [102]. These fusion proteins bind to CD80 and CD86, blocking their interaction with the co-stimulatory receptor CD28 on T-cells. Results of a Phase III clinical trial in renal transplantation showed reduced chronic allograft nephropathy in patients treated with LEA29Y when compared with cyclosporine [103]. Clinical studies of LEA29Y in islet transplantation are currently underway.

An alternative approach to traditional immunosuppression, which has generally targeted lymphocyte activation, is to inhibit lymphocyte migration to their site of activation or effector function. It is now well understood that lymphocyte activation and effector responses occur in distinct anatomical compartments, the migration to which is controlled by chemokines [104]. Inhibitors of lymphocyte trafficking have been gaining popularity as immunomodulatory agents. Emerging compounds of interest include FTY720, a potent inhibitor of lymphocyte egress from the thymus and lymph nodes, which depends on several chemokine systems [105]. FTY720 has been investigated in primate models of islet transplantation with promising results in terms of safety and efficacy when combined with basiliximab and everolimus (a corticosteroid- and calcineurin-inhibitor-free immunosuppressive regimen) [106]. FTY720 is a non-specific antitrafficking agent; newer agents which specifically target one chemokine receptor subsystem either in the form of antibody blockade of chemokine receptors or small molecule chemokine receptor antagonists are currently being tested in preclinical models of islet transplantation [107,108].

Discovery and use of novel immunosuppressive and inflammatory blockade agents in the field of islet transplantation have made significant improvements to the outcome of various clinical islet transplant programmes. Those agents which are successful share four features in that they (i) are non-diabetogenic or reduce the need for more diabetogenic immunosuppressive agents, (ii) reduce initial damage of islet cells and promote engraftment, (iii) induce a functional tolerance, and (iv) aim to manage the underlying autoimmune nature of T1DM in addition to stopping allograft rejection processes.

Ultimately the induction of immunological tolerance would be a highly desirable state not only in islet transplantation, but also in solid organ transplants. This would avoid any potential long-term risks and side effects that patients face today when they exchange insulin for antirejection therapies. Extensive investigation of tolerance induction has occurred over more than 50 years since the first report of tolerance induction in mice by Billingham, Brent and Medawar [109]. Tolerance has been readily achievable in small animal models using two alternative approaches. Central tolerance with myeloablation and thymic and peripheral re-education has been achieved using donor-derived bone marrow transplantation. Peripheral tolerance has been achieved by a variety of strategies, including co-stimulatory blockade of either the CD80/86–CD28/CTLA4 pathway or the potent anti-CD40L antibodies (discussed above). Recent findings also suggest that regulatory T-cell populations may play a pivotal role in controlling graft stability [110]. Although experimental tolerance induction has been tantalizing in both small and large animal transplant studies, significant challenges remain for clinical translation. A limited number of renal transplant recipients have achieved chimaerism and no longer require chronic immunosuppression after bone marrow transplantation for treatment of leukaemia, followed by a subsequent kidney transplant from the same donor [111].

**CONCLUSIONS**

Progress in islet transplantation has improved glycaemic control, corrected HbA1c, and protected approx. 80% of subjects from severe hypoglycaemic reactions. Although insulin independence has been achieved in approx. 70% of subjects receiving completed islet infusions at the 1-year mark, this has tended to fade over time using current antirejection protocols. The jury is still out in terms of overall impact of islet transplantation in protection from secondary diabetic complications, but a large body of literature in whole pancreas transplantation and early clinical studies in islet-transplanted patients suggest benefit in terms of cardiovascular and renal risk.

Substantial progress has occurred in aspects of pancreas procurement, transportation (using the oxygenated twolayer method) and in islet isolation (with controlled enzymatic perfusion and subsequent digestion in the Ricordi chamber). Clinical protocols to optimize islet survival and function post-transplantation improved dramatically with the introduction of the Edmonton protocol, but it is clear that this approach still has serious potential limitations. Newer pharmacotherapies and interventions designed to promote islet survival, prevent apoptosis, to promote islet growth and to protect islets in the long run from immunological injury are rapidly approaching clinical trials, and it seems likely that clinical outcomes of islet transplantation will continue to improve at the current exponential pace. There are clearly several obstacles ahead, including addressing better ways to maintain islet function without deterioration beyond 5 years and reducing the cost of such therapy (currently estimated at approx. Canadian $140000 per patient). Addressing the scarcity of cadaver human islet donation and managing international demand for islet transplantation from a select number of dedicated clinical islet isolation centres remain at the forefront of issues that must be resolved.

Islet transplantation was considered an experimental procedure up till 2000. In 2001, based on the results of the
Edmonton protocol and the replication of this protocol in international centres, islet transplantation became designated as 'non-research' in Canada. Since that time, data have been accrued on over 500 islet transplants internationally and, through the support of the NIH and the Juvenile Diabetes Research Foundation, islet transplantation will probably receive a biological license status from the FDA in the U.S.A.

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48 Collaborative Islet Transplantation Registry (http://spitfire.emory.edu/study/isl/index.html)


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