Arch. Immunol. Ther. Exp. (2011) 59:421–429
DOI 10.1007/s00005-011-0143-0

REVIEW

Islet Transplantation: Factors in Short-Term Islet Survival

Natasha A. Deters · Rebecca A. Stokes · Jenny E. Gunton

Received: 28 January 2011 / Accepted: 25 May 2011 / Published online: 8 October 2011

Abstract Islet transplantation has the potential to cure type 1 diabetes. In recent years, the proportion of patients achieving initial insulin independence has improved, but longer term outcomes remain poor compared to those for whole pancreas transplants. This review article will discuss factors affecting islet yield and viability leading up to transplantation and in the immediate post-transplant period.

Keywords Islet transplantation · Apoptosis · Hypoxia · Type 1 diabetes · β cell

Abbreviations

ADSC	Adipose derived stem cells
IBMIR	Instant blood-mediated inflammatory reaction
MSC	Mesenchymal stem cells
SC	Sertoli cells
T1D	Type 1 diabetes

Introduction

Type 1 diabetes (T1D) mellitus is an autoimmune disorder in which the immune system destroys the insulin producing β cells of the pancreatic islets of Langerhans (Kahn et al. 2006). The disease most commonly has its onset before 30 years of age, but can be diagnosed at any age (Daneman 2006).

T1D was uniformly fatal before the purification of insulin by Banting and Best in 1922 (Banting and Best 2007; Banting et al. 2007; Bliss 1993). However, insulin must be administered parenterally, blood glucose levels need to be monitored to achieve tight control and dietary management is essential. Even with treatment, normal blood glucose levels cannot be achieved in the vast majority of T1D patients and chronic high blood glucose can damage organs, potentially leading to blindness, end-stage renal failure and amputations (1998; Atkinson and Eisenbarth 2001; Daneman 2006; Nathan et al. 2005). This affects quality of life and to this day, the average lifespan of people living with T1D is still markedly reduced (Brown et al. 2001).

Insulin is the major hormone which stimulates glucose uptake from the blood, predominantly into muscle and fat (Rhodes and White 2002). It is secreted only from pancreatic β cells which are located in the islets of Langerhans and which themselves make up 1–2% of the pancreas (Kloppel et al. 1985). As T1D is a direct result of β-cell destruction, the diabetes can be cured by replacing β cells. This can be achieved by whole pancreas or islet transplantation. Successful islet transplants are currently performed in many centres worldwide. However, it is a complex procedure with many factors affecting the outcome. Islet transplantation is still considered an experimental procedure and much research effort is currently being directed towards improvement and refinement of the current method for islet transplantation with the ultimate goal of long-term survival of islets. This review will discuss islet transplantation and the factors involved in short-term islet survival, including the islet isolation process and the immediate post-transplant period.

N. A. Deters · R. A. Stokes · J. E. Gunton (✉)
Diabetes and Transcription Factors,
Garvan Institute of Medical Research,
384 Victoria St, Darlinghurst,
NSW 2010, Australia
e-mail: j.gunton@garvan.org.au

Islet Transplantation

As with all human transplants, there is an imbalance between supply and demand. Therefore, islet transplant recipients are carefully selected and only a small percentage of people with T1D receive this treatment. Patients who are typically eligible for islet transplantation are those who have had T1D for more than 5 years, are aged between 18 and 65 and most importantly, have poor diabetes control including episodes of severe hypoglycaemia sometimes requiring external assistance.

Because the islets of Langerhans in the pancreas contain the insulin-secreting β cells, transplantation of only the islets is a theoretically very attractive approach for the treatment of T1D. As islets make up only 1–2% of the pancreas, islet transplantation provides a much smaller transplant mass than whole pancreas transplant and is therefore a much less invasive procedure, and presents a smaller load of immunogenic tissue. Most of the digestive enzyme secreting exocrine pancreatic tissue is removed.

Lacy and Kostianovsky (1967) were the first to develop a novel collagenase-based method for successfully isolating islets from a rat pancreas. During the 1960s and 1970s, successful non-human islet isolation and transplantation into animal models was established (Ballinger and Lacy 1972; Lacy and Kostianovsky 1967; Lillehei et al. 1969a, b; Reckard et al. 1973). Renal subcapsular transplants are a common animal transplant site, but are not suitable for human transplants due to volume issues. In an early paper where rodent islets were used, subcutaneous, free intraperitoneal and intraportal islet transplant sites were compared and the latter was found to be the only successful site (Kemp et al. 1973). It was this work and the success with autologous islet transplants in that site that led to the current method in which islet allografts are usually infused into the portal vein.

Reliable human islet isolation proved more difficult because of a number of technical issues (Warnock et al. 1988), including the lack of a discrete capsule around the islet and the more variable size of human islets compared to animal islets. The eventual method for human islet isolation and the subsequent modified method were developed by Ricordi (1991) and Ricordi et al. (1988, 1990). Occasional reports of successful allogeneic human islet transplants appeared over the years (Gores et al. 1993; Scharp et al. 1991), but it was not until the landmark report from Shapiro et al. (2000) of seven T1D patients achieving insulin independence following islet transplantation that the field was truly re-vitalised.

Islet Transplant Success Rates

The Collaborative Islet Transplant Registry (CITR), founded in 2001, collects and analyses islet transplant data from all transplant centres in the US and Canada, as well as some European and Australian transplant centres. Their aim is to identify critical risk factors and key determinants of islet transplant success and thereby help to develop and refine islet transplant procedures. CITR's 2009 annual report comprises data from 412 allograft recipients (CITR 2009). The majority of islet recipients require additional islet infusions, with some receiving up to four islet infusions. Regardless of the total number of infusions received, at the 3-year follow-up approximately 27% of recipients are insulin independent, 30% are insulin dependent with detectable C-peptide and 27% have no detectable C-peptide (not all islet recipients have had their 3-year follow-up data entered into CITR). Interestingly, recipients transplanted since 2005 retained insulin independence significantly longer than those transplanted between 1999 and 2004, most likely due to improved islet isolation methods and post-transplant islet survival. The percentage of all islet recipients that are insulin independent declines steadily from 55% at 6 months post-transplant to just 16% at 4 years. Conversely, the proportion with complete loss of islet function increases from 12% at 6 months to 42% at 4 years. Overall, the reported 5-year rate for insulin independence is approximately 10% (Langer 2010; Shapiro et al. 2006).

Donor and Retrieval Factors

As with any organ transplant, the overall health of the donor and condition of the pancreas play a major role in transplant success or failure. The vast majority of islets for transplantation derive from donors who have been declared brain dead (Grundfest-Broniatowski and Novick 1986; Ridgway et al. 2010; Shapiro et al. 2000, 2006). Jung et al. (2007) have shown that islet yield from living donors is higher compared to cadaveric donors, and cerebrovascular stroke as cause of death and intracranial haemorrhage as mechanism of death are significantly associated with unsuccessful islet isolation (Takita et al. 2010). Studies using animal islets have reported an increased expression of tissue factor in islets isolated from brain dead rats compared to living rats (Saito et al. 2009). Tissue factor has been identified as the main trigger of the instant blood-mediated inflammatory reaction (IBMIR).

The overall importance of donor health is suggested by many factors. If the donor is in good health at the time of donation, as in the setting of islet auto-transplantation or in the report of a living-related transplant (Matsumoto et al. 2005; Robertson 2001), outcomes are generally improved. Conversely, outcomes are substantially worsened for non-heart beating donors (Jung et al. 2007; Kenmochi et al. 2008). Donors who have poor glucose control during their

intensive care unit admission are less likely to provide a successful islet yield, as are those who require high levels of inotropes to attempt to maintain blood pressure (Brandhorst et al. 1994, 1995; Lakey et al. 1995; Ridgway et al. 2010).

Most reports suggest donor age plays a role in islet yield. Kaddis et al. (2010) report that older donors give poorer islet yields. In a multi-centre analysis of ~800 preparations, older donors had an odds ratio of 0.61 for a good islet isolation yield (>315,000 IEQ) (Kaddis et al. 2010), though, islets from type 2 donors are not used for transplantation because of their known functional defects. However, Niclauss et al. (2011) recently reported no difference between islet yield from donors younger than 45 years compared to those older than 45, however, the graft function was significantly reduced in older donors. One explanation is that the collagen composition of the extracellular matrix of the pancreas changes with age, thus impacting collagenase affinity during the digestion phase of the isolation process (Lakey et al. 1996; Sabek et al. 2006). This may partially help to explain why no clear consensus has emerged as to ideal age boundaries for optimal donors.

Conversely, overweight or obese donors give better islet yields (Brandhorst et al. 1995; Ridgway et al. 2010). Pancreata with fat on the surface, or fatty infiltration of the pancreas also give better islet yields (Kaddis et al. 2010). These weight-related factors are helpful in directing use of organs: fatty pancreata perform poorly in whole-organ transplants and increase morbidity and mortality in the organ recipients so in fact are relatively contra-indicated for whole-organ transplants. Fatty pancreata give better islet yields, and are preferred for islet transplants.

Pancreas Perfusion and Transport Factors

The first step after surgical dissection is to flush the extracted pancreas with a chilled preservation solution. This is most commonly University of Wisconsin (UW) solution. UW solution gives equivalent or superior results to other perfusates in many but not all studies, by preventing loss of amylase and inducing a slight shrinkage of the acinar cells, which in turn improves the density separation of islets and viability of the cells (van der Burg et al. 1994). Other preservation solutions used include Celsior, HTK (histidine–tryptophan–ketoglutarate), Institut-George-Lopes (IGL)-1 and others (Hubert et al. 2007; Kaddis et al. 2010; Manrique et al. 2006; Noguchi et al. 2008). The flushing step cools the pancreas and removes debris and thrombi (Baertschiger et al. 2008; Hubert et al. 2007; Kinasiewicz and Fiedor 2003; Salehi et al. 2006; White et al. 2001; Wojtusciszyn et al. 2005).

Cold storage and transport of the pancreas is vital to islet yield and viability; warm ischaemia has been found to damage tissue (Brandhorst et al. 1994; de Gruyl et al. 1977; Florack et al. 1983), however, cold ischaemia time beyond 8 h also results in reduced yields and quality of human islets (Caballero-Corbalan et al. 2007; Kuhtreiber et al. 2010; Pileggi et al. 2009). In most studies, shorter cold ischaemic time associates with better islet yields (White et al. 2001). Transport using a two-layer method where the pancreas rests on a substrate in the top layer of a perfluorocarbon "bath" appears to give slightly improved outcomes (Scott et al. 2010a) and showed promising results in pancreata from humans (Scott et al. 2010b).

Islet Isolation Factors

The islet isolation process remains a major challenge. As discussed above, islets only make up a small fraction of the pancreas (Kloppel et al. 1985), and the isolation process is designed to remove the exocrine part of the organ while preserving structurally intact islets. Enzymatic digestion, followed by mechanical separation and a density gradient are required and all of these steps are detrimental to islets.

Using the modified Ricordi protocol (Froud et al. 2005; Ricordi et al. 1988), the pancreatic duct is injected with a digestive enzyme cocktail (e.g. Liberase, Collagenase NB1, Collagenase NB8) (Bucher et al. 2005; O'Connell et al. 2006; Ricordi et al. 1988; Shapiro et al. 2000). This step is followed by mechanical dissociation in a temperature-controlled Ricordi chamber. Originally the pancreas was placed in a metal chamber with metal or glass spheres and shaken repeatedly manually (Ricordi et al. 1988); this process has now been automated. Sample aliquots collected at intervals through the process are used to check separation and the progress of the digestion. Subsequently, islets are separated through sieves and density gradient centrifugation steps. The IBM 2991 cell separator (COBE Laboratories), used for over a decade to separate blood leucocytes by blood banks, was modified to purify islets (Lake et al. 1989). The density gradient steps need to be performed as quickly as possible, because all known density gradient media are toxic to islets (Scharp et al. 1990), although there does not appear to be substantial difference between the different density gradient options (Kaddis et al. 2010).

The islet isolation procedure exposes islets to mechanical, enzymatic, osmotic, and ischaemic stress, and as a consequence, a large proportion of islets are destroyed or rendered non-viable. Furthermore, digestion and fragmentation of islets is a major contributor to low islet yield for transplantation and it is highly dependent upon the particular batch of enzyme used (Johnson et al. 1996; Nano et al.

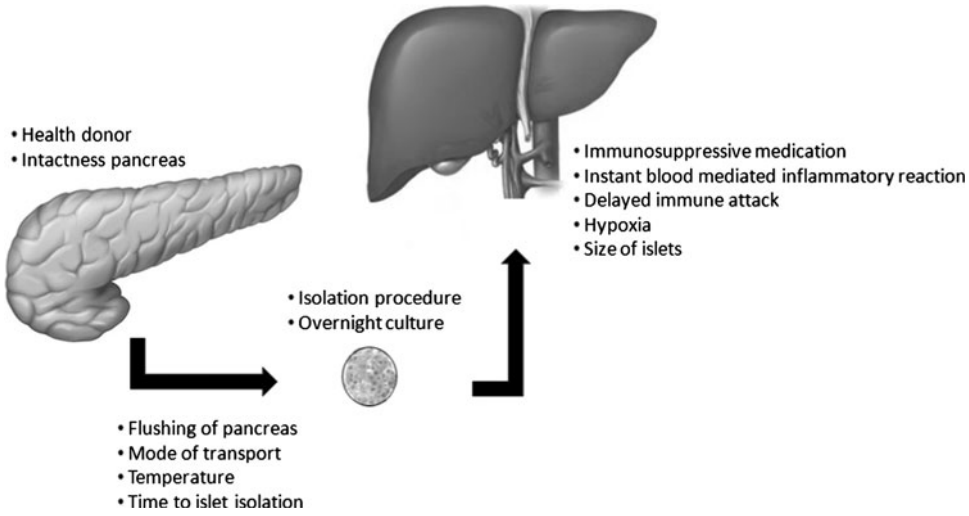

Fig. 1 Factors that play a role in islet yield and survival during the early post-transplant period

2005). Disruption of normal cell–cell relationships leads to an increase in apoptosis (anoikis) in islet β cells (Thomas et al. 2001).

An average human pancreas contains ~1 million islets (Gray et al. 1995) and a very good isolation process obtains ~500,000 islets with a viability of >80%, though most isolations give lower yields (Agrawal et al. 2008; Bucher et al. 2005; Kaddis et al. 2010; Mahler et al. 1999; O'Connell et al. 2006; Ricordi et al. 1988; Shapiro et al. 2000, 2006; Toso et al. 2002). Islets are usually cultured after isolation prior to transplantation, to allow various metabolic and viability tests. This also allows non-viable islets and remaining exocrine tissue to die prior to transplantation, increasing purity and decreasing the mass of transplanted tissue without adversely affecting outcomes. On average, approximately 20% of islets are lost during overnight culture (Kin et al. 2008). Interestingly, Noguchi and et al. investigated the effect of different culture temperatures and reported that islet morphology after 4°C preservation was similar to and islet diameter after 22 or 37°C was smaller than that of fresh islets. In addition, islet yield significantly decreased at higher temperatures (22 and 37°C) and level of post-transplantation normoglycaemia achievement was significantly higher in the 4°C preservation group compared to islets cultured at 22 and 37°C (Noguchi et al. 2010) (Fig. 1).

Factors in the Early Post-Transplantation Period

Compared to whole-organ transplants, islet transplantation is a relatively non-invasive procedure. In most recipients it is a radiological procedure involving portal vein cannulation, although some centres choose to use mini-laparotomy. In either case, islets are infused into the portal vein, under low pressure, frequently gravity alone. Due to the relatively low invasiveness of the procedure, patients are normally discharged from hospital within a week, usually within 48 h.

Immunosuppressive Medication

Allogeneic islet recipients require immunosuppression to prevent graft rejection. The medications have potential side effects, which are covered in many papers and reviews (Froud et al. 2005; Gruessner 1997; Pirsch et al. 1997; Przepiorka et al. 2000; Shapiro et al. 2000; Webster et al. 2006) and will not be covered here. Of relevance to islet transplant outcomes, the immunosuppressive regimens, since the landmark Edmonton protocol, have all been corticosteroid free (Shapiro et al. 2000) since they are toxic to β-cell function. However, it is has become appreciated that the combination of tacrolimus and sirolimus, while a major improvement over corticosteroids, may still inhibit β-cell engraftment, survival, function and proliferation (Bussiere et al. 2006; Redmon et al. 1996; Rostambeigi et al. 2011; Zhang et al. 2006). In addition, the orally administered drugs achieve relatively high levels in the portal circulation, thus exposing the grafted islets to potentially toxic concentrations (Shapiro et al. 2005).

Instant Blood-Mediated Inflammatory Reaction

It is estimated that after transplantation, a large proportion of islets are lost within a week (Jirak et al. 2009). Post-transplant islet loss can be immediate (within minutes) or delayed. Immediate islet loss occurs if there is an IBMIR (Bennet et al. 2000; Johansson et al. 2005; Moberg et al. 2002; Ozmen et al. 2002). IBMIR is characterised by very rapid activation of coagulation and complement systems and platelet consumption. One of the major triggers is islet

release of tissue factor (Johansson et al. 2005; Moberg et al. 2002). IBMIR can be ameliorated or avoided by transplantation of unstressed islets, as these elaborate lower levels of tissue factor. Furthermore, treatment of patients with heparin, dextran, factor VIIa inhibitors and thrombin inhibitors has been used (Bennet et al. 1999, 2000; Cabric et al. 2007; Johansson et al. 2005, 2006; Ozmen et al. 2002; van der Windt et al. 2007), though patients may be at high risk for bleeding following transplantation. One option to circumvent this problem is to coat the islets with anticoagulant factors, such as heparin (Cabric et al. 2007), thrombomodulin (Stabler et al. 2007) or fibrinolytic urokinase (Teramura and Iwata 2008).

Delayed Immune Attack

In addition to IBMIR, less acute immune attack also occurs (Eisenbarth and Stegall 1996; Roep et al. 1999; Stegall et al. 1996) and numerous different immunosuppressive regimens are being currently tested. Anti-LFA-1 (leucocyte functional antigen-1) antibodies had promising results from the islet transplant view, but were withdrawn due to toxicity (Posselt et al. 2010). Promising preliminary results for alemtuzumab (anti-CD52) induction with sirolimus–tacrolimus followed by sirolimus–mycophenolic acid have been reported for three patients (Froud et al. 2008). Hering et al. (2004) cultured isolated islets to give time to achieve recipient preconditioning with good results for single donor transplants. In their subsequently published study they added induction with ATG, daclizumab and etanercept, and maintenance with sirolimus, low-dose tacrolimus and mycophenolate mofetil and achieved insulin independence in 100% of eight patients, with maintenance at 1 year of 62.5% (Hering et al. 2005). However, longer term follow-up of these patients does not appear to have been reported.

While immune attack clearly plays a major role in graft loss, auto-antibodies did not correlate with loss of function and there was no evidence for HLA sensitisation in the multicentre study (Shapiro et al. 2006). Follow-up studies using more sensitive techniques have suggested that presence of allo-immunity and islet-reactive T cells predict poorer outcomes (Campbell et al. 2007a, b; Roep 2008; Roep et al. 1999). Other studies were not powered to make definitive conclusions, although there was a suggestion of recurrent immunity accompanying sub-therapeutic immunosuppressive levels (Hering et al. 2005).

Hypoxia

After being trapped in the small portal vessels of the liver, islets must obtain oxygen and nutrients from the host by diffusion. In addition, the portal circulation is a naturally low oxygen tension environment. Thus, after transplantation, islets will suffer from relative or severe hypoxia. As full revascularisation of islets can take days to weeks, hypoxia is a serious contributor to islet loss in the early post-transplant period (Brissova and Powers 2008; Korsgren et al. 2008; Morini et al. 2007). And even in established transplanted islets, oxygen tension and blood perfusion rate remain lower compared to native pancreas islets (Brissova and Powers 2008; Carlsson et al. 1998, 2001; Carlsson and Palm 2002; Mattsson et al. 2002).

In the clinical setting, islets with a diameter of $\sim$100 to 150 μm are the most common size used for transplantation. They provide the largest proportion of islet volume per donor pancreas and β-cell mass per transplant. Many of the smaller islets are lost in the isolation process. However, recent studies have shown that due to lack of optimal oxygen diffusion these large islets have poor oxygen utilisation and poor survival, due to core cell death (Janette Williams et al. 2010; MacGregor et al. 2006). In addition, large islets actually secrete less insulin per volume compared to smaller islets, indicating a loss of β-cell functionality (MacGregor et al. 2006; Williams et al. 2009). And as oxygen cannot diffuse to the core of large islets, so does glucose not reach the core cells until the islets are re-vascularised. Gently breaking up large islets into smaller, more porous segments, improved islet survival in vitro but did not improve insulin secretion in response to glucose (MacGregor et al. 2006). Overall, studies suggest that smaller islets perform better after transplantation, both for human and rodent islets (Lehmann et al. 2007; MacGregor et al. 2006).

Re-oxygenation of islets following transplantation is a key issue to survival and successful transplant outcome. Studies have shown that treating isolated islets with pro-angiogenic growth factors such as VEGF and HGF can markedly enhance angiogenesis following islet transplantation and thus improve islet survival in vivo (Fiaschi-Taesch et al. 2007; Golocheikine et al. 2010; Lai et al. 2005; Lammert et al. 2003; Zhang et al. 2004).

Alternate Transplant Sites

Many investigators have suggested that the liver is not the optimal site for transplantation, but there is no consensus for another site as yet. Liver, kidney capsule, spleen, peritoneal cavity, omentum, muscle and pancreas have all been considered as potential sites of islet infusion. Transplanting islets into their native environment i.e. the pancreas, provides superior outcomes to intraportal transplants in animals (Lau et al. 2007), but because of the risk of pancreatitis and other complications, this site, while intellectually attractive, is not likely to be a viable option in humans. Christoffersson et al. (2010) transplanted islets into striated muscle tissue and reported that in contrast to islets transplanted into the liver, vessel densities and blood

flow of islets transplanted into muscle were similar to those of native islets of the intact pancreas. Islets were functional both in the mouse model as well as in human subjects who received islet auto-transplantation. Islets grafted into muscle were found to have triple the number of blood vessels and a sixfold higher oxygen tension compared to corresponding islets transplanted at the renal sub-capsular site (Svensson et al. 2010).

Co-Transplantation: Improving Islet Survival in the Early Post-Transplant Period

Co-transplantation of islets with other cell types that offer support or protection has been under investigation in a number of centres. Co-transplantation with endothelial cells improved transplant outcomes in rodents (Johansson et al. 2008). Bone marrow-derived mesenchymal stem cells (MSCs), which are known to produce the pro-angiogenic factor VEGF, show improvement of islet graft morphology and function when co-transplanted with islets, most likely by promoting revascularisation of islets (Ito et al. 2010; Johansson et al. 2005). Others found that MSCs protected the islets from hypoxia/reoxygenation induced injury by decreasing the apoptotic cell ratio and increasing hypoxia and oxidative stress-related genes (Lu et al. 2010). Another recent paper described the use of adipose tissue-derived stem cells (ADSCs) as they have angiogenic potential and anti-inflammatory properties. Significant revascularisation (larger number of von Willebrand factor-positive vascular cells) and marked inhibition of inflammatory cell infiltration, including CD4 and CD8 T cells and macrophages, were noted in the islets co-transplanted with ADSCs compared to islet-alone transplants. Similar to MSCs, ADSCs promoted islet graft survival and insulin function of the graft and reduced the islet mass required for reversal of diabetes (Ohmura et al. 2010). Sertoli cells (SCs), normally found in the testes, have been used in co-transplantation studies with promising results. SCs protect sperm cells from immune damage and have been shown to self-protect when transplanted into allogeneic and xenogeneic environments (Emerich et al. 2003). In murine T1D models, SCs protect co-grafted allogeneic and xenogeneic islets from immune destruction (Suarez-Pinzon et al. 2000; Yang et al. 2002). Infused SCs following transplantation also offer protection and prolong islet survival by reducing peripheral blood lymphocyte and cytokine levels (Li et al. 2010).

Co-transplantation appears to have major potential as a therapeutic strategy for improving tolerance post-transplant and could potentially lead to a reduction in the number of islets needed for transplantation and use of immunosuppressive medication.

Concluding Remarks

It is thought that in the week following transplantation, many transplanted islets are lost to IBMIR and hypoxia, both leading to β-cell apoptosis and necrosis. If 30–70% of the original islet mass is transplanted, and only 25% of transplanted islets are lost in the first week, then 22–52% of the original islet mass would remain. Short-term islet loss is often much greater than 25% (Ryan et al. 2001; Toso et al. 2002).

In people with normal glucose tolerance at baseline, decreasing β-cell mass by removal of 50% of the pancreas causes glucose intolerance at 1 year in 25–43% (Kendall et al. 1990; Seaquist et al. 1996), and larger resections cause glucose intolerance more commonly: 80% plus pancreatectomy causes glucose intolerance in 73–100% of patients (Slezak and Andersen 2001). Thus, islet yield and short-term post-transplant survival remain major issues which contribute to longer term graft exhaustion and failure.

Major strides in islet transplantation have been made in recent years, with recipients transplanted since 2005 retaining insulin independence significantly longer than those transplanted between 1999 and 2004 (CITR 2009). However, transplant outcomes are not yet comparable to whole pancreas transplants. Islet survival is affected by numerous complex factors during the isolation process, immediately post-transplant and long-term in the host. Research continues to determine optimal immunosuppressive pre-conditioning, ongoing immunosuppression and to improve islet engraftment. Reconsideration of the islet transplant site seems to be gaining momentum. An additional challenge is posed by centre to centre variability in both results and protocols. There is difficulty in conducting adequately sized trials: in order to achieve sufficient power for statistical comparison multiple centres are usually needed.

Strategies currently proven to improve islet yield include good donor and recipient selection, meticulous attention to surgical retrieval, thorough perfusion, cold preservation and transport, islet isolation with experienced operators, and islet culture to allow optimal immunosuppressive pre-conditioning of the recipient patient. Islet transplantation remains an exciting area of intense research which has future promise for the cure of T1D.

References

(1998) Effect of intensive insulin therapy on residual beta-cell function in patients with type 1 diabetes in the Diabetes Control and Complications Trial a randomized controlled trial. The

Diabetes Control and Complications Trial Research Group. Ann Intern Med 128:517–523
Agrawal A, Gurusamy K, Powis S et al (2008) A meta-analysis of the impact of the two-layer method of preservation on human pancreatic islet transplantation. Cell Transplant 17:1315–1322
Atkinson MA, Eisenbarth GS (2001) Type 1 diabetes: new perspectives on disease pathogenesis and treatment. Lancet 358:221–229
Baertschiger RM, Berney T, Morel P (2008) Organ preservation in pancreas and islet transplantation. Curr Opin Organ Transplant 13:59–66
Ballinger WF, Lacy PE (1972) Transplantation of intact pancreatic islets in rats. Surgery 72:175–186
Banting FG, Best CH (2007) The internal secretion of the pancreas. 1922. Indian J Med Res 125:251–266
Banting FG, Best CH, Collip JB et al (2007) Pancreatic extracts in the treatment of diabetes mellitus. 1922. Indian J Med Res 125:141–146
Bennet W, Sundberg B, Groth CG et al (1999) Incompatibility between human blood and isolated islets of Langerhans: a finding with implications for clinical intraportal islet transplantation? Diabetes 48:1907–1914
Bennet W, Groth CG, Larsson R et al (2000) Isolated human islets trigger an instant blood mediated inflammatory reaction: implications for intraportal islet transplantation as a treatment for patients with type 1 diabetes. Ups J Med Sci 105:125–133
Bliss M (1993) Rewriting medical history: Charles Best and the Banting and Best myth. J Hist Med Allied Sci 48:253–374
Brandhorst D, Hering BJ, Brandhorst H et al (1994) Influence of donor data and organ procurement on human islet isolation. Transplant Proc 26:592–593
Brandhorst H, Brandhorst D, Hering BJ et al (1995) Body mass index of pancreatic donors: a decisive factor for human islet isolation. Exp Clin Endocrinol Diabetes 103(suppl 2):23–26
Brissova M, Powers AC (2008) Revascularization of transplanted islets. Diabetes 57:2269–2271
Brown LJ, Scott RS, Moir CL (2001) All-cause mortality in the Canterbury (New Zealand) insulin-treated Diabetic Registry population. Diabetes Care 24:56–63
Bucher P, Mathe Z, Morel P et al (2005) Assessment of a novel two-component enzyme preparation for human islet isolation and transplantation. Transplantation 79:91–97
Bussiere C, Lakey J, Shapiro A et al (2006) The impact of the mTOR inhibitor sirolimus on the proliferation and function of pancreatic islets and ductal cells. Diabetologia 49:2341–2349
Caballero-Corbalan J, Eich T, Lundgren T et al (2007) No beneficial effect of two-layer storage compared with UW-storage on human islet isolation and transplantation. Transplantation 84:864–869
Cabric S, Sanchez J, Lundgren T et al (2007) Islet surface heparinization prevents the instant blood-mediated inflammatory reaction in islet transplantation. Diabetes 56:2008–2015
Campbell PM, Salam A, Ryan EA et al (2007a) Pretransplant HLA antibodies are associated with reduced graft survival after clinical islet transplantation. Am J Transplant 7:1242–1248
Campbell PM, Senior PA, Salam A et al (2007b) High risk of sensitization after failed islet transplantation. Am J Transplant 7:2311–2317
Carlsson PO, Palm F (2002) Oxygen tension in isolated transplanted rat islets and in islets of rat whole-pancreas transplants. Transpl Int 15:581–585
Carlsson PO, Liss P, Andersson A et al (1998) Measurements of oxygen tension in native and transplanted rat pancreatic islets. Diabetes 47:1027–1032
Carlsson PO, Palm F, Andersson A et al (2001) Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site. Diabetes 50:489–495
Christoffersson G, Henriksnas J, Johansson L et al (2010) Clinical and experimental pancreatic islet transplantation to striated muscle: establishment of a vascular system similar to that in native islets. Diabetes 59:2569–2578
CITR (2009) 2009 Scientific Summary of the Collaborative Islet Transplant Registry (CITR). http://www.CITRegistry.org
Daneman D (2006) Type 1 diabetes. Lancet 367:847–858
de Gruyl J, Westbroek DL, Macdicken I et al (1977) Cryoprecipitated plasma perfusion preservation and cold storage preservation of duct-ligated pancreatic allografts. Br J Surg 64:490–493
Eisenbarth GS, Stegall M (1996) Islet and pancreatic transplantation—autoimmunity and alloimmunity. N Engl J Med 335:888
Emerich DF, Hemendinger R, Halberstadt CR (2003) The testicular-derived Sertoli cell: cellular immunoscience to enable transplantation. Cell Transplant 12:335–349
Fiaschi-Taesch N, Stewart AF, Garcia-Ocana A (2007) Improving islet transplantation by gene delivery of hepatocyte growth factor (HGF) and its downstream target, protein kinase B (PKB)/Akt. Cell Biochem Biophys 48:191–199
Florack G, Sutherland DE, Heil J et al (1983) Preservation of canine segmental pancreatic autografts: cold storage versus pulsatile machine perfusion. J Surg Res 34:493–504
Froud T, Ricordi C, Baidal DA et al (2005) Islet transplantation in type 1 diabetes mellitus using cultured islets and steroid-free immunosuppression: Miami experience. Am J Transplant 5:2037–2046
Froud T, Baidal DA, Faradji R et al (2008) Islet transplantation with alemtuzumab induction and calcineurin-free maintenance immunosuppression results in improved short- and long-term outcomes. Transplantation 86:1695–1701
Golocheikine A, Tiriveedhi V, Angaswamy N et al (2010) Cooperative signaling for angiogenesis and neovascularization by VEGF and HGF following islet transplantation. Transplantation 90:725–731
Gores P, Najarian J, Stephanian E et al (1993) Insulin independence in type I diabetes after transplantation of unpurified islets from single donor with 15-deoxyspergualin. Lancet 341:19–21
Gray H, Williams PL, Bannister LH (1995) Gray's anatomy: the anatomical basis of medicine and surgery. Churchill-Livingstone, New York
Gruessner RW (1997) Tacrolimus in pancreas transplantation: a multicenter analysis. Tacrolimus Pancreas Transplant Study Group. Clin Transplant 11:299–312
Grundfest-Broniatowski S, Novick A (1986) Pancreas transplantation—1985. Transplant Proc 18:31–39
Hering BJ, Kandaswamy R, Harmon JV et al (2004) Transplantation of cultured islets from two-layer preserved pancreases in type 1 diabetes with anti-CD3 antibody. Am J Transplant 4:390–401
Hering BJ, Kandaswamy R, Ansite JD et al (2005) Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. JAMA 293:830–835
Hubert T, Gmyr V, Arnalsteen L et al (2007) Influence of preservation solution on human islet isolation outcome. Transplantation 83:270–276
Ito T, Itakura S, Todorov I et al (2010) Mesenchymal stem cell and islet co-transplantation promotes graft revascularization and function. Transplantation 89:1438–1445
Janette Williams S, Huang HH, Kover K et al (2010) Reduction of diffusion barriers in isolated rat islets improves survival, but not insulin secretion or transplantation outcome. Organogenesis 6:115–124
Jirak D, Kriz J, Strzelecki M et al (2009) Monitoring the survival of islet transplants by MRI using a novel technique for their automated detection and quantification. MAGMA 22:257–265
Johansson H, Lukinius A, Moberg L et al (2005) Tissue factor produced by the endocrine cells of the islets of Langerhans is

associated with a negative outcome of clinical islet transplantation. Diabetes 54:1755–1762
Johansson H, Goto M, Siegbahn A et al (2006) Low molecular weight dextran sulfate: a strong candidate drug to block IBMIR in clinical islet transplantation. Am J Transplant 6:305–312
Johansson U, Rasmusson I, Niclou SP et al (2008) Formation of composite endothelial cell-mesenchymal stem cell islets: a novel approach to promote islet revascularization. Diabetes 57:2393–2401
Johnson PR, White SA, London NJ (1996) Collagenase and human islet isolation. Cell Transplant 5:437–452
Jung HS, Choi SH, Kim SJ et al (2007) A better yield of islet cell mass from living pancreatic donors compared with cadaveric donors. Clin Transplant 21:738–743
Kaddis JS, Danobeitia JS, Niland JC et al (2010) Multicenter analysis of novel and established variables associated with successful human islet isolation outcomes. Am J Transplant 10:646–656
Kahn CR, Weir GC, King GL et al (2006) Joslin's Diabetes Mellitus, 14th edn. Lippincott Williams & Wilkins, Boston
Kemp CB, Knight MJ, Scharp DW et al (1973) Effect of transplantation site on the results of pancreatic islet isografts in diabetic rats. Diabetologia 9:486–491
Kendall DM, Sutherland DE, Najarian JS et al (1990) Effects of hemipancreatectomy on insulin secretion and glucose tolerance in healthy humans. N Engl J Med 322:898–903
Kenmochi T, Maruyama M, Saigo K et al (2008) Successful islet transplantation from the pancreata of non-heart-beating donors. Transplant Proc 40:2568–2570
Kin T, Senior P, O'Gorman D et al (2008) Risk factors for islet loss during culture prior to transplantation. Transpl Int 21:1029–1035
Kinasiewicz A, Fiedor P (2003) Amylase levels in preservation solutions as a marker of exocrine tissue injury and as a prognostic factor for pancreatic islet isolation. Transplant Proc 35:2345–2346
Kloppel G, Lohr M, Habich K et al (1985) Islet pathology and the pathogenesis of type 1 and type 2 diabetes mellitus revisited. Surv Synth Pathol Res 4:110–125
Korsgren O, Lundgren T, Felldin M et al (2008) Optimising islet engraftment is critical for successful clinical islet transplantation. Diabetologia 51:227–232
Kuhtreiber WM, Ho LT, Kamireddy A et al (2010) Islet isolation from human pancreas with extended cold ischemia time. Transplant Proc 42:2027–2031
Lacy PE, Kostianovsky M (1967) Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes 16:35–39
Lai Y, Schneider D, Kidszun A et al (2005) Vascular endothelial growth factor increases functional [beta]-cell mass by improvement of angiogenesis of isolated human and murine pancreatic islets. Transplantation 79:1530–1536
Lake SP, Bassett PD, Larkins A et al (1989) Large-scale purification of human islets utilizing discontinuous albumin gradient on IBM 2991 cell separator. Diabetes 38(suppl 1):143–145
Lakey J, Warnock G, Rajotte R et al (1995) Factors in cadaveric donors that affect recovery of human islets of Langerhans. Transplant Proc 27:3265
Lakey JR, Warnock GL, Rajotte RV et al (1996) Variables in organ donors that affect the recovery of human islets of Langerhans. Transplantation 61:1047–1053
Lammert E, Gu G, McLaughlin M et al (2003) Role of VEGF-A in vascularization of pancreatic islets. Curr Biol 13:1070–1074
Langer R (2010) Islet transplantation: lessons learned since the Edmonton breakthrough. Transplant Proc 42:1421–1424
Lau J, Mattsson G, Carlsson C et al (2007) Implantation site-dependent dysfunction of transplanted pancreatic islets. Diabetes 56:1544–1550
Lehmann R, Zuellig RA, Kugelmeier P et al (2007) Superiority of small islets in human islet transplantation. Diabetes 56:594–603
Li Y, Xue WJ, Tian XH et al (2010) Study on systemic immune tolerance induction in rat islet transplantation by intravenous infusion of Sertoli cells. Transplantation 89:1430–1437
Lillehei RC, Idezuki Y, Kelly WD et al (1969a) Transplantation of the intestine and pancreas. Transplant Proc 1:230–238
Lillehei RC, Idezuki Y, Uchida H et al (1969b) Pancreatic allotransplantation in the dog and in man. Br J Surg 56:699
Lu Y, Jin X, Chen Y et al (2010) Mesenchymal stem cells protect islets from hypoxia/reoxygenation-induced injury. Cell Biochem Funct 28:637–643
MacGregor RR, Williams SJ, Tong PY et al (2006) Small rat islets are superior to large islets in in vitro function and in transplantation outcomes. Am J Physiol Endocrinol Metab 290:E771–E779
Mahler R, Franke F, Hering B et al (1999) Evidence for a significant correlation of donor pancreas morphology and the yield of isolated purified human islets. J Mol Med 77:87–89
Manrique A, Jimenez C, Herrero ML et al (2006) Pancreas preservation with the University of Wisconsin versus Celsior solutions. Transplant Proc 38:2582–2584
Matsumoto S, Okitsu T, Iwanaga Y et al (2005) Insulin independence of unstable diabetic patient after single living donor islet transplantation. Transplant Proc 37:3427–3429
Mattsson G, Jansson L, Carlsson PO (2002) Decreased vascular density in mouse pancreatic islets after transplantation. Diabetes 51:1362–1366
Moberg L, Johansson H, Lukinius A et al (2002) Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. Lancet 360:2039–2045
Morini S, Brown ML, Cicalese L et al (2007) Revascularization and remodelling of pancreatic islets grafted under the kidney capsule. J Anat 210:565–577
Nano R, Clissi B, Melzi R et al (2005) Islet isolation for allotransplantation: variables associated with successful islet yield and graft function. Diabetologia 48:906–912
Nathan DM, Cleary PA, Backlund JY et al (2005) Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med 353:2643–2653
Niclauss N, Bosco D, Morel P et al (2011) Influence of donor age on islet isolation and transplantation outcome. Transplantation 15:360–366
Noguchi H, Ueda M, Hayashi S et al (2008) Ductal injection of preservation solution increases islet yields in islet isolation and improves islet graft function. Cell Transplant 17:69–81
Noguchi H, Naziruddin B, Jackson A et al (2010) Low-temperature preservation of isolated islets is superior to conventional islet culture before islet transplantation. Transplantation 89:47–54
O'Connell PJ, Hawthorne WJ, Holmes-Walker DJ et al (2006) Clinical islet transplantation in type 1 diabetes mellitus: results of Australia's first trial. Med J Aust 184:221–225
Ohmura Y, Tanemura M, Kawaguchi N et al (2010) Combined transplantation of pancreatic islets and adipose tissue-derived stem cells enhances the survival and insulin function of islet grafts in diabetic mice. Transplantation 90:1366–1373
Ozmen L, Ekdahl KN, Elgue G et al (2002) Inhibition of thrombin abrogates the instant blood-mediated inflammatory reaction triggered by isolated human islets. Diabetes 51:1779–1784
Pileggi A, Ribeiro MM, Hogan AR et al (2009) Effects of pancreas cold ischemia on islet function and quality. Transplant Proc 41:1808–1809
Pirsch JD, Miller J, Deierhoi MH et al (1997) A comparison of tacrolimus (Fk506) and cyclosporine for immunosuppression after cadaveric renal transplantation 1. Transplantation 63:977–983

Posselt AM, Bellin MD, Tavakol M et al (2010) Islet transplantation in type 1 diabetics using an immunosuppressive protocol based on the anti-LFA-1 antibody efalizumab. Am J Transplant 10:1870–1880

Przepiorka D, Kernan NA, Ippoliti C et al (2000) Daclizumab, a humanized anti-interleukin-2 receptor alpha chain antibody, for treatment of acute graft-versus-host disease. Blood 95:83–89

Reckard CR, Ziegler MM, Barker CF (1973) Physiological and immunological consequences of transplanting isolated pancreatic islets. Surgery 74:91–99

Redmon JB, Olson LK, Armstrong MB et al (1996) Effects of tacrolimus (FK506) on human insulin gene expression, insulin mRNA levels, and insulin secretion in HIT-T15 cells. J Clin Invest 98:2786–2793

Rhodes CJ, White MF (2002) Molecular insights into insulin action and secretion. Eur J Clin Invest 32(suppl 3):3–13

Ricordi C (1991) Quantitative and qualitative standards for islet isolation assessment in humans and large mammals. Pancreas 6:242–244

Ricordi C, Lacy PE, Finke EH et al (1988) Automated method for isolation of human pancreatic islets. Diabetes 37:413–420

Ricordi C, Gray DW, Hering BJ et al (1990) Islet isolation assessment in man and large animals. Acta Diabetol Lat 27:185–195

Ridgway D, Manas D, Shaw J et al (2010) Preservation of the donor pancreas for whole pancreas and islet transplantation. Clin Transplant 24:1–19

Robertson RP (2001) Pancreatic islet transplantation for diabetes: successes, limitations, and challenges for the future. Mol Genet Metab 74:200–205

Roep BO (2008) Immune markers of disease and therapeutic intervention in type 1 diabetes. Novartis Found Symp 292:159–171 (discussion 171–153, 202–153)

Roep BO, Stobbe I, Duinkerken G et al (1999) Auto- and alloimmune reactivity to human islet allografts transplanted into type 1 diabetic patients. Diabetes 48:484–490

Rostambeigi N, Lanza IR, Dzeja PP et al (2011) Unique cellular and mitochondrial defects mediate FK506-induced islet beta-cell dysfunction. Transplantation 91:615–623

Ryan EA, Lakey JR, Rajotte RV et al (2001) Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol. Diabetes 50:710–719

Sabek OM, Cowan P, Fraga DW et al (2006) The effect of donor factors on human islet yield and their in vivo function. Prog Transplant 16:350–354

Saito Y, Goto M, Maya K et al (2009) The influence of brain death on tissue factor expression in the pancreatic tissues and isolated islets in rats. Transplant Proc 41:307–310

Salehi P, Hansen MA, Avila JG et al (2006) Human islet isolation outcomes from pancreata preserved with Histidine-Tryptophan Ketoglutarate versus University of Wisconsin solution. Transplantation 82:983–985

Scharp DW, Lacy PE, Santiago JV et al (1990) Insulin independence after islet transplantation into type I diabetic patient. Diabetes 39:515–518

Scharp DW, Lacy PE, Santiago JV et al (1991) Results of our first nine intraportal islet allografts in type 1, insulin-dependent diabetic patients. Transplantation 51:76–85

Scott W, O'Brien T, Ferrer-Fabrega J et al (2010a) Persufflation improves pancreas preservation when compared with the two-layer method. Transplant Proc 42:2016–2019

Scott W, Weegman B, Ferrer-Fabrega J et al (2010b) Pancreas oxygen persufflation increases ATP levels as shown by nuclear magnetic resonance. Transplant Proc 42:2011–2015

Seaquist ER, Kahn SE, Clark PM et al (1996) Hyperproinsulinemia is associated with increased beta cell demand after hemipancreatectomy in humans. J Clin Invest 97:455–460

Shapiro AM, Lakey JR, Ryan EA et al (2000) Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 343:230–238

Shapiro AM, Gallant HL, Hao EG et al (2005) The portal immunosuppressive storm: relevance to islet transplantation? Ther Drug Monit 27:35–37

Shapiro AM, Ricordi C, Hering BJ et al (2006) International trial of the Edmonton protocol for islet transplantation. N Engl J Med 355:1318–1330

Slezak LA, Andersen DK (2001) Pancreatic resection: effects on glucose metabolism. World J Surg 25:452–460

Stabler CL, Sun XL, Cui W et al (2007) Surface re-engineering of pancreatic islets with recombinant azido-thrombomodulin. Bioconjug Chem 18:1713–1715

Stegall MD, Lafferty KJ, Kam I et al (1996) Evidence of recurrent autoimmunity in human allogeneic islet transplantation. Transplantation 61:1272–1274

Suarez-Pinzon W, Korbutt GS, Power R et al (2000) Testicular Sertoli cells protect islet beta-cells from autoimmune destruction in NOD mice by a transforming growth factor-beta1-dependent mechanism. Diabetes 49:1810–1818

Svensson J, Lau J, Sandberg M et al (2010) High vascular density and oxygenation of pancreatic islets transplanted in clusters into striated muscle. Cell Transplant (in press)

Takita M, Matsumoto S, Noguchi H et al (2010) One hundred human pancreatic islet isolations at Baylor Research Institute. Proc (Bayl Univ Med Cent) 23:341–348

Teramura Y, Iwata H (2008) Islets surface modification prevents blood-mediated inflammatory responses. Bioconjug Chem 19:1389–1395

Thomas F, Wu J, Contreras JL et al (2001) A tripartite anoikis-like mechanism causes early isolated islet apoptosis. Surgery 130:333–338

Toso C, Oberholzer J, Ris F et al (2002) Factors affecting human islet of Langerhans isolation yields. Transplant Proc 34:826–827

van der Burg MP, Guicherit OR, Frolich M et al (1994) Cell preservation in University of Wisconsin solution during isolation of canine islets of Langerhans. Cell Transplant 3:315–324

van der Windt DJ, Bottino R, Casu A et al (2007) Rapid loss of intraportally transplanted islets: an overview of pathophysiology and preventive strategies. Xenotransplantation 14:288–297

Warnock GL, Cattral MS, Rajotte RV (1988) Normoglycemia after implantation of purified islet cells in dogs. Can J Surg 31:421–426

Webster AC, Lee VW, Chapman JR et al (2006) Target of rapamycin inhibitors (TOR-I; sirolimus and everolimus) for primary immunosuppression in kidney transplant recipients. Cochrane Database Syst Rev 19:CD004290

White SA, James RF, Swift SM et al (2001) Human islet cell transplantation—future prospects. Diabet Med 18:78–103

Williams SJ, Wang Q, Macgregor RR et al (2009) Adhesion of pancreatic beta cells to biopolymer films. Biopolymers 91:676–685

Wojtusciszyn A, Bosco D, Morel P et al (2005) A comparison of cold storage solutions for pancreas preservation prior to islet isolation. Transplant Proc 37:3396–3397

Yang H, Al-Jazaeri A, Wright JR Jr (2002) The immunoprotective effect of Sertoli cells coencapsulated with islet xenografts is not dependent upon Fas ligand expression. Cell Transplant 11:799–801

Zhang N, Richter A, Suriawinata J et al (2004) Elevated vascular endothelial growth factor production in islets improves islet graft vascularization. Diabetes 53:963–970

Zhang N, Su D, Qu S et al (2006) Sirolimus is associated with reduced islet engraftment and impaired beta-cell function. Diabetes 55:2429–2436