Islet Cell Transplantation

14

Antonio Bruni, Michael McCall and A.M. James Shapiro

Diabetes mellitus currently affects more than 200 million people worldwide, with projections of it affecting 5% of the world population by 2025 [1]. Type 1 diabetes mellitus (T1DM), the most severe form of this disease, represents approximately 10% of all cases of diabetes and is characterized by a progressive autoimmune disorder resulting in the destruction of insulin-producing β-cells within the pancreatic islets of Langerhans. Due to progressive chronic micro- and macrovascular complications, T1DM is a major source of morbidity and mortality. Whilst T1DM has become a manageable condition, largely owing to the availability of exogenous insulin following the discovery of insulin by Banting, Best, Collip and McLeod [2, 3], many patients still develop a multitude of chronic complications of diabetes including nephropathy, neuropathy, retinopathy, peripheral vascular disease and coronary artery disease. Although the etiology of these complications is multifactorial, the Diabetes Control and Complications Trial (DCCT) clearly demonstrated that their development can be reduced by tight glycemic control. This was achieved in the trial by the use of intensive rather than conventional insulin therapy [4–6]. However, one of the downsides of intensive insulin was a substantial increase in life-threatening hypoglycemia [5].

Safer and innovative means to tighten glycemic control have been developed, including the use of insulin pumps, dynamic continuous glucose monitoring, and closed loop systems. These have achieved improved glycemic control, reduced hypoglycemic risk, and moderate improved protection from secondary diabetic complications. However, while these technological advances offer patients improved benefit, they continue to fall short as a definitive, robust cure for diabetes. Indeed, these treatments all involve the use of exogenous insulin and work by better 'controlling' diabetes rather than by 'reversing' it. An alternative approach is to attempt to actually restore the destroyed beta (β)-cell mass by transplanting the insulin-producing tissue, either in the form of a whole vascularized pancreas transplant, or by a cellular islet transplant.

First attempted in 1966, whole pancreas transplantation initially showed poor clinical outcomes [7]. However, following considerable advances in surgical technique, immunosuppressive strategies, and postoperative management, the results have improved drastically, with 80–85% of patients remaining insulin independent for at least a year following this procedure. However, it remains a major intra-abdominal surgical procedure, with significant morbidity

A. Bruni · M. McCall
5-040 Li Ka Shing Health Research, 112 Street and 87 Avenue, Edmonton, AB T6G 2E1, Canada
e-mail: bruni1@ualberta.ca

M. McCall
e-mail: mmccall@ualberta.ca

A.M. James Shapiro (✉)
2000 College Plaza, 8215 112 Street, Edmonton, AB T6G 2C8, Canada
e-mail: amjs@islet.ca

D.J. Ledbetter and P.R.V. Johnson (eds.), *Endocrine Surgery in Children*,
DOI 10.1007/978-3-662-54256-9_14

and a mortality rate of 4–7% in leading centers around the world. In addition, as the pancreas only comprises 2% endocrine tissue, it could be argued that patients with T1DM receiving a whole pancreas graft are receiving 98% of pancreatic tissue that they do not require! It is therefore, very unlikely that this treatment will become a routine treatment for children with T1DM.

Conversely, pancreatic islet cell transplantation is a minimally invasive procedure, involving only the implantation of the pancreatic endocrine component. In addition, as a cellular transplant, islet transplants have the real potential to be immunomodified or immunoisolated at the time of transplantation, meaning that in the future it may be possible to undertake an islet transplant without the need for immunosuppression. This, combined with the simplicity of the procedure, mean that this therapy has real potential for future use in children.

In this chapter, we provide a historical perspective of islet transplantation; outline the challenges of donor selection; provide an overview of human islet isolation; discuss the different aspects of the islet transplant procedure, including some of the challenges; a review of the current outcomes of clinical islet transplantation; and finally, discuss the potential use of islet transplantation in children.

Islet Transplantation: A Historical Perspective

The work of von Mering and Minkowski in 1889, was essential for first identifying the pivotal link between the pancreas and elevated blood glucose levels, when they showed that total pancreatectomy in dogs resulted in fatal diabetes [8]. Two decades later, Sharpey-Shafer's hypothesis provided insight into the link between diabetes and insulin, a key chemical found within pancreas. However, although the recognition of diabetes and the hypothesis about insulin were monumental, it was not until Banting, Best, Collip, and McLeod discovered and isolated insulin in 1922, that diabetes became a chronically manageable condition [2, 3]. The introduction of intensive blood glucose monitoring and the frequent daily administration of exogenous insulin improved things further, and therapeutic efforts shifted more from the acute phase of the disease to strategies to stabilize, reverse, or ideally prevent the chronic complications of the disease [9]. As highlighted above, however, focus has turned to strategies that enable true reversal of diabetes, and currently this can only be achieved by restoring β-cell mass through transplantation.

One of the most important advances that enabled clinical islet transplantation to be made possible was the ability to isolate sufficient numbers of human islets from a donor pancreas. The isolation of rodent islets was first accomplished by Lacy in the 1960s when a number of islets were isolated following the enzymatic digestion of rodent pancreases from obese animals with hypertrophic islets [10]. Further refinements by Lacy and Kostianovsky then established the technical ability to isolate hundreds of metabolically active and structurally intact islets from the pancreas of normal rats [11]. While several authors reported the correction of hyperglycemia in diabetic mice using varied islet doses and success rates via the intraperitoneal route, the work of Reckard and Barker in 1973 was the first to effectively cure diabetes in a chemically induced model of diabetes [12]. Yet despite these successes, the same methods of islet isolation and purification could not be applied to larger animals or humans, whose pancreases contain several million islets, and whose pancreatic structure differs greatly from rodents [11, 13].

Further refinements to the methods of islet isolation and purification for islet transplantation continued for decades (and still do continue), with improved success in isolating greater quantities with greater purity. Injection of collagenase into the pancreatic duct proved an effective method for successful islet isolation from large animals and humans [13, 14]. However, it was the development of the Ricordi digestion chamber in 1988, enabling a semi-automated process for human islet isolation, that was

instrumental in enabling sufficient isolation and purification of islets for clinical use [13, 15]. This method of islet isolation is still considered the universal 'gold standard', and has made clinical islet transplantation a reality [13].

With regard to islet transplantation itself, outcomes have progressed significantly since clinical islet transplants were first performed. This is partly due to improved islet manufacturing processes, but has also been greatly facilitated by the availability of more effective induction and maintenance immunosuppression to protect against both auto- and alloreactivity [16]. In subjects with poor glycemic control, islet-alone transplantation has recently become an accepted practice to stabilize frequent hypoglycemia or severe glycemic lability [17]. While Lacy's work established the liver as an ideal site for islet transplantation [18], further work by Najarian et al. in 1977 reported the first successful clinical islet transplant paired with the administration of azathioprine and corticosteroids [19]. In spite of these achievements, only 9% of the 267 islet transplant recipients since 1999 were insulin independent for >1 year [20]. It was not until 2000 that the 'Edmonton Protocol' reported insulin independence in seven consecutive T1DM patients over a median follow-up of 11.9 months with sustained C-peptide [16]. Patients had received at least two different islet transplants and a mean islet mass of 13,000 IEQ/kg. Perhaps most notably, patients received a steroid-free immunosuppressive regimen of anti-interleukin (IL)-2 receptor antagonist antibody therapy, daclizumab. These monumental results were pivotal in driving forward both interest and activity in clinical islet transplantation over the subsequent decade, which resulted in the expansion of islet transplantation programs in North America and abroad through remarkable inter-center collaboration.

The success of the Edmonton Group ignited widespread enthusiasm. However, the initial waning of complete insulin independence by 3–5 years post-transplantation raised further concerns that islet transplantation could not permanently ameliorate the diabetic state in patients. Strategies to improve islet transplantation outcomes have continued over subsequent years, including but not limited to, improved donor selection, optimized techniques for organ donation and preservation and immunosuppression regimens. Undoubtedly, such refinements will enable this treatment to fulfill its potential in the coming decades and expand its therapeutic benefit to patients afflicted with this debilitating illness.

Donor Selection and Donor Availability

The number of patients that can receive an islet transplant is largely limited by the availability of donor pancreases. In addition, optimal donor selection is an important factor influencing successful islet transplant outcome. Several donor-related variables that may contribute to islet isolation outcomes have been demonstrated through single-center retrospective studies. Variables include donor age, cause of death, body mass index (BMI), cold ischemia time, length of hospitalization, use of vasopressors, and blood glucose levels [21–28]. In spite of the observation that a larger pancreas contains a larger β-cell mass, pancreas weight does not appear to correlate directly with successful islet yield [27, 29]. In a study analyzing data from 345 deceased donors, it was determined that although BMI correlates with pancreas weight, body surface area is a better predictor of pancreas weight than BMI [29]. Several other groups have indicated that BMI itself positively affects islet yield [30], leading many to consider BMI as an important donor predictor islet isolation outcome [25–27]. This view, however, has led to the misconception that an obese donor is a good candidate for successful transplantation, whereas it is important to distinguish between factors that lead to a high islet yield, and those that correlate with optimal islet physiology.

With regard to pancreas allocation, in most countries to date, 'optimal' pancreases are still prioritized for whole organ transplantation before they are offered to islets. A review by Berney and Johnson concluded that transplanted islet mass does not unequivocally correlate with islet graft

function (also see below), further arguing that based on this premise, donor selection criteria for islet transplantation and hence allocation rules (pancreas for whole organ or islet transplant) may need to be redefined [31]. A joint whole pancreas and islet donor allocation system for whole pancreas and islet transplantation has now been introduced in the United Kingdom.

O'Gorman et al. developed a scoring system based on donor characteristics that can predict islet isolation outcomes and has been an instrumental tool in assessing whether a pancreas should be processed for islet isolation [32]. Though this tool has been sufficient for determining which organs are optimal for islet isolation in terms of islet yields and crude islet function, it does not predict optimal islet transplant outcome. Similarly, other published studies investigating optimal donor factors for islet isolation have not taken transplant outcome into consideration [21, 22, 24, 25, 26, 27, 28]. Lakey et al. retrospectively reviewed human islet isolation preparations and studied the effects of donor age on islet yield and function (insulin secretory capabilities) [25]. Their study suggested that older donors (51–65 years old) are more likely to produce a transplantable yield of islets when compared with their younger donor counterparts (83% compared to 37% in 19–28 year old donors). However, the secretory capabilities of these islets were significantly reduced. Other studies have confirmed these results and shown higher rates of diabetes reversal in immunodeficient diabetic mice receiving human islet grafts from younger donors [23]. While this would point to younger donors as 'ideal' for islet transplantation, one must also realize that digestion of a young pancreas is also technically more difficult and hence islet yields lower. The more 'fibrous' nature of the pancreatic matrix within young donors considerably reduces the success rate and yield of islets. More recently, groups from Minnesota and San Francisco in the US have modified islet isolation protocols for optimized success with the younger human pancreas.

Prolonged cold ischemic time for the pancreas during shipment from the donor center to the isolation center can be injurious to human islet isolation (both islet yield and function), with times exceeding 6–8 h being less optimal than locally procured donors [24]. While pancreatic transport in University of Wisconsin (UW) solution was standard previously, most transplant programs (at least in Canada and in parts of the US) have switched to histidine-tryptophan-ketoglutarate (HTK) solution, which may be less optimal for pancreas storage [33]. The two-layer oxygenated UW–perfluorodecalin method for pancreas transportation initially looked promising, but with increased use, it appeared to add little protection to the islets prior to isolation, and has therefore been abandoned by most programs at the present time [34].

Human Islet Isolation

One of the most important prerequisites of successful clinical islet transplantation is an optimized islet isolation process. Unquestionably, human islet isolation requires considerable skill and involves a significant financial cost, especially now that the regulation of human islet isolation has become so stringent around the world. Whereas in the 1990s, human islet isolation was performed in modified research laboratories, this now has to be conducted in ultraclean Good Manufacturing Practice (GMP) facilities that meet strict national governmental oversight. This has led over recent years to the development of a number of 'hub and spoke' clinical islet transplant networks, in which islet isolation is only performed in one or two 'clinical-grade' isolation facilities that provide quality islets for a network of different islet transplant centers. Successful networks include the GRAGIL Network in Switzerland and France [35], the NORDIC network in Scandinavia, and the UK Islet Transplant Consortium (UKITC) in Britain. Clinical islets have also been successfully shipped between centers in the US [36]. This approach allows resources and expertise to be centralized, making the clinical islet isolation process much more cost-effective.

The process of islet isolation starts at the time of cadaveric organ donation with the need for meticulous surgery and minimal handling of the pancreas during procurement. This is combined with rapid cooling of the lesser sac at the time of aortic cross-clamp placement and arterial flushing. Once procured, the pancreas is then placed in a cold storage solution and transported to the islet isolation facility.

The challenge of islet isolation is to produce high yields of 'in tact' islets of optimal viability, purity, and function. Human islet isolation involves three steps, namely pancreas digestion, density gradient purification, and islet culture.

Pancreas Digestion

The pancreas is digested by a combination of physical and enzymatic dissociation. This stage is intended to liberate islets from the surrounding pancreatic exocrine matrix, producing a pancreatic digest in which both islets and exocrine sit. This digestion stage is performed within a Ricordi chamber (Fig. 14.1) and uses commercially available collagenase enzyme produced from the bacteria Clostridum histolyticum. An efficient enzymatic digestion is critical for successful islet isolation, and this is achieved by a careful balance of enzyme composition and duration of

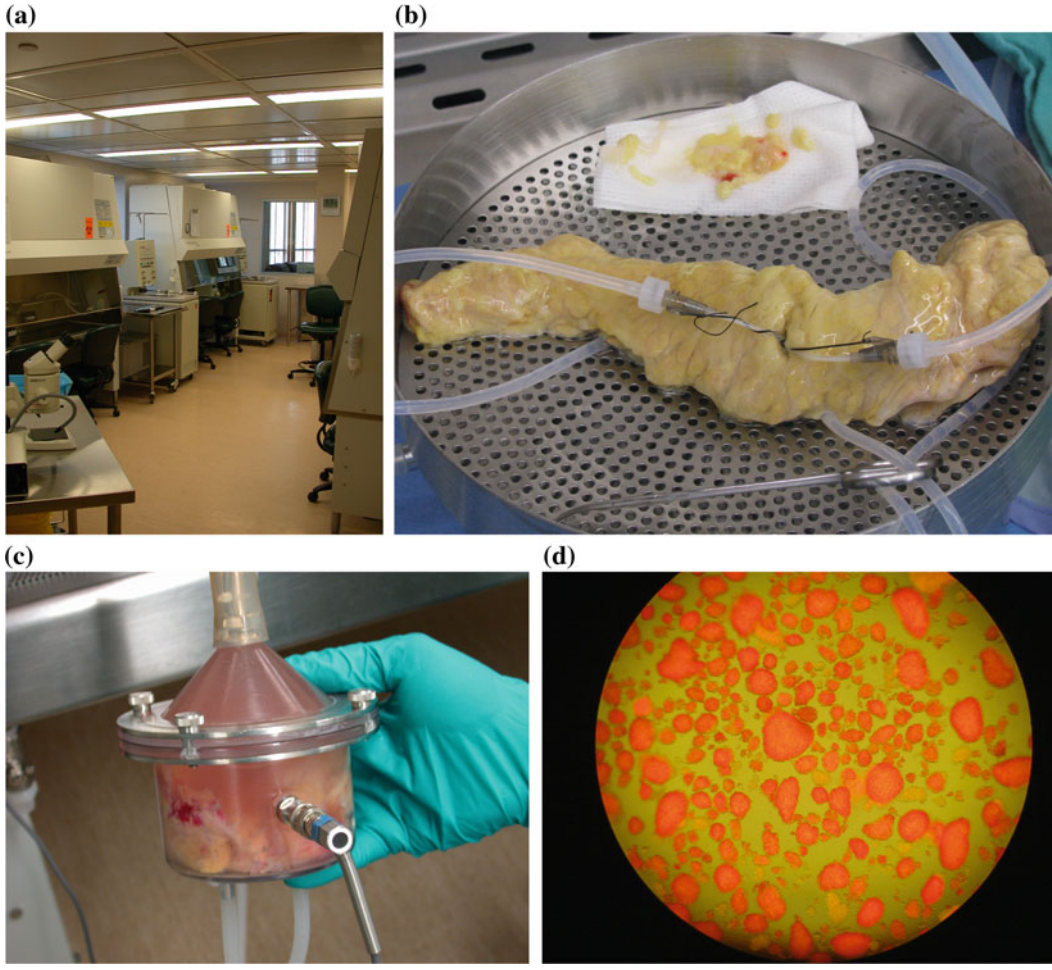

Fig. 14.1 Human islet isolation. **a** Human islet isolation occurs in a specialized laboratory. **b** The pancreatic duct is cannulated after the pancreas is trimmed. **c** The sectioned pancreas is loaded into the Ricordi chamber for digestion. **d** Isolated islets are stained with dithizone and counted

collagenase digestion [37]. Suboptimal collagenase composition, or insufficient collagenase exposure, leads to incomplete liberation of islets from the exocrine tissue resulting in low islet yields of decreased purity. However, overactive collagenase or increased duration of collagenase exposure, can lead to islet fragmentation. Thus, one of the greatest challenges for islet transplant programs has for many years been the 'batch-to-batch' variability and non-reproducibility of collagenase preparations [38, 39].

Density Gradient Purification

It was discovered a number of years ago that islets infused into the portal vein with large amounts of accompanying exocrine tissue can lead to portal vein thrombosis which can be fatal [40]. The pancreatic digest is therefore, purified by density gradient centrifugation, enabling 'pure' islets to be retrieved. Islet purification takes advantage of the fact that islets and exocrine tissue have different buoyant densities. When placed in specially developed media of known density and spun on a centrifuge, tissue will migrate to a layer corresponding to their own density, with the less dense islets settling at a higher density than exocrine tissue. The introduction of the COBE 2991 machine by the Leicester Group, originally utilized for blood component separation, has become one of the key factors in achieving sufficient numbers of purified islets for clinical use [41].

Islet Culture

While the islets used in the original Edmonton Protocol were transplanted immediately after isolation, culturing islets post-isolation is believed to be important for their recovery from isolation-induced damage and enables islets to be carefully assessed before infusion. It also enables immunomodulatory therapy to be started in the recipient and potentially reduces the immunogenicity of the graft. A culture period also greatly enhances the practicality of an islet transplant in terms of planning for the patient's admission and the availability of the radiology suite. However, this may be at the cost of impaired revascularization subsequent to transplant, due to the loss of intra-islet endothelial cells during this culture period. Therefore, essential components of the culture conditions for human islet preparations are sufficient oxygen and nutrient supply. During the culture stage, the maintenance of the tridimensional islet cluster and preventing islet mass loss should be accomplished during the culturing phase. To date, sufficient investigation of optimal culture conditions has occurred, yet in spite of this, protocols have yet to be standardized and culture conditions may vary between islet isolation centers [42]. Other considerations like media composition, seeding density, and incubation temperature play a significant role in maintaining viability and recovery [42].

During the 24–48 h of islet culture, 10–20% of the islet mass is 'lost' due to islet disaggregation and islet death. However, these 'lost' islets would probably not have survived in the recipient, and a period of islet culture enables more extensive evaluation of the graft pre-transplantation.

The Islet Transplant Procedure

Pre-transplant Preparation

Once a patient has been placed on the waiting list for islet transplantation, they await a suitable donor pancreas for islet isolation and subsequent transplantation. Once the islet yield of the matched pancreas has been confirmed to be sufficient for transplanting into that recipient (usually ≥ 5000 islet equivalents (IEQ) per kg, where an IEQ is an islet count that has been adjusted to standardize the islet diameter to 150 μm), the patient is admitted to the transplant centre to begin immunosuppressive induction therapy. Prior to the introduction of the Edmonton Protocol in the late 1990s, corticosteroids were a mainstay component of the immunosuppressive protocol. It is widely known, however, that these

medications are themselves diabetogenic and are particularly damaging to newly transplanted islets. The Edmonton Protocol provided a steroid-free approach, employing sirolimus and low-dose tacrolimus maintenance therapy after the potent induction agent daclizumab, an anti-CD25 (IL-2R) monoclonal antibody [16]. Since the removal of daclizumab from the market after patent expiry, other T-cell depletional agents have been used including thymoglobulin (antithymocyte globulin), basiliximab (Il-2R antibody) and, more recently, some of the best results have been used with the anti-CD52 alemtuzumab. At some centers, sirolimus is being replaced from post-transplant immunosuppressive protocols by the better tolerated mycophenolate mofetil.

Many centers are employing adjuvant regimens to enhance outcomes. The blockade of tumor necrosis factor α (TNFα) as a means to prevent its inflammatory attack on islets post-transplantation has been used in the form of Etanercept [43, 44] and Infliximab [45]. Exenatide (a GLP-1 agonist) has found use in patients with graft dysfunction, promoting insulin secretion and improving islet function [46, 47]. In fact, the combination of Etanercept and Exenatide may enhance islet engraftment when given in combination [48]. All patients are given broad-spectrum prophylactic antibiotics shortly before the procedure.

The Transplant Procedure

Today, most clinical islet transplants are performed using percutaneous intrahepatic islet infusion via the portal vein under radiological control. However, a few centers still prefer the surgical mesenteric cannulation approach for additional safety.

Portal infusion offers a minimally invasive procedure, accomplished without need for surgery or general anaesthesia, with the ability to regulate glycemic levels through portal insulin delivery [49]. Once at the confluence of the portal vein, islets are infused aseptically under gravity while the portal venous pressure is monitored. Heparin (70 units/kg) is included in the islet preparation to minimize the chance of portal vein thrombosis. While catheter tract bleeding is a known complication, multiple approaches can be used to prevent this including D-STAT [50], coils and gelfoam or microfibrillary collagen (Avitene®) paste. However, although rare, this percutaneous approach still has some potential procedural risks, including portal thrombosis and bleeding [51].

The surgical approach to portal venous access is less commonly used, though it may be necessary if the percutaneous approach cannot be utilized (e.g., large right-sided liver hemangioma). In this instance, a mesenteric vein is cannulated, utilizing complete surgical control to prevent bleeding. However, this approach has the inherent risks of laparotomy/laparoscopy including bleeding, infection, adhesion formation, and wound breakdown/incisional hernia (especially if on sirolimus).

The liver is the currently the preferred transplant site for a number of reasons. It is easily accessible percutaneously, it has high a vascularity supplying sufficient oxygen and nutrients during the revascularization period, and it contains a sinusoidal structure that enables islets to become trapped and engrafted. Having islets placed within the liver also ensures a physiological release of insulin into the portal vein, although compared with the native pancreas, it suffers from a low oxygen content. Moreover, a significant amount of intraportal islet mass is lost immediately post-transplant due to innate immune pathways involving platelet and complement activation.

To date, numerous alternative islet transplantation sites have been proposed and tested, both experimentally and in some cases clinically. These include the kidney subcapsule, the spleen, pancreas, omentum, gastrointestinal wall, immune privileged sites such as the eye and testis/ovary, and the subcutaneous spaces. Though some alternative sites offer advantageous results in experimental models, their feasibility and translation into clinical settings have been limited. Undoubtedly, ongoing experimental and clinical investigation is required to elucidate an optimal islet transplant site with efforts aimed to

improve islet engraftment, long-term insulin independence, and transplant outcomes from single donors.

Post-transplant Care

To minimize stress on the newly transplanted islets, tight glycemic control is maintained using an insulin/glucose sliding scale. It is known that islets engraft more readily if they are able to do so in a euglycemic environment [52]. However, apoptotic islets will release insulin, making the patient susceptible to hypoglycemia. To prevent portal vein thrombosis and combat IBMIR (instant blood mediated inflammatory reaction), unfractionated heparin is infused in the postoperative period. Ultrasonography is routinely performed at day one and one week post-transplant to rule out intraperitoneal hemorrhage and ensure patency of the portal vein. In addition to immunosuppressive drugs, patients are discharged home 1–2 days later on a range of post-transplant medications.

Complications

Procedure-Related Complications

Portal vein thrombosis and major hepatic bleeding account for two of the most serious complications associated with the percutaneous approach to islet transplantation [53]. Portal vein is extremely uncommon now, especially as we now transplant purer islet preparations and use heparin in the preparation and also systemically in the patient. The incidence of bleeding from the catheter tract was not uncommon in the early incidences of clinical islet transplantation [53]. Many of these events have all but been eliminated through methods to reduce the catheter tract. The clinical islet transplantation site in Edmonton currently utilizes Avitene® paste to seal and abate the catheter tract. Although segmental vein thrombosis can occur (5% in the above-mentioned series), main portal vein thrombosis is extremely rare. This risk is largely reduced through the administration of unfractionated heparin (70 units/kg) in the islet preparation, as well as through instituting systemic anticoagulation, post-procedure.

Immunosuppression-Related Complications

Corticosteroids used to form the backbone of immunosuppression regimes in early clinical islet transplantation settings and were found to be quite toxic to islets. The success of the 'Edmonton Protocol' is attributed to the immunosuppression scheme that utilized the combination of sirolimus, low-dose tacrolimus and daclizumab in an effort to prevent the deleterious effects of calcineurin inhibitors and steroids [54]. In spite of these refinements, most patients returned to modest amounts of insulin despite the elimination of recurrent hypoglycemia by 5 years post-transplant, clearly indicating room for improvement [55]. Moreover, β-cell survival and function are also compromised due to the proximity of the transplanted islets to high concentrations of these drugs in the hepatoportal circulation [56, 57].

Due to the multiple pathways known to contribute to β-cell attrition and the alloresponse to foreign antigens, it is unlikely that a monotherapy will optimize clinical islet transplantation outcomes and lead to single donor recipients [55]. The implementation of highly potent and selective biological agents for the initiation and maintenance of immunosuppression has made significant progress in reducing the frequency of acute rejection, prolonging graft survival and minimizing the complications of these therapeutic schemes [58, 59]. The University of Minnesota reported improvements to single donor success rates as a result of combining anti-inflammatory biologics to maintenance immunosuppression [43, 60]. In addition, peri-transplant insulin and heparin administration greatly increased the success rate of single donor islet transplants from 10 to 40% [61].

Furthermore, the blockade of tumor necrosis factor alpha with etanercept has also enhanced single donor islet transplant outcomes [43, 61, 62, 63, 64].

The successful establishment of an immunosuppressive regimen that promotes self-tolerance is critical for the long-term success of clinical islet transplantation. A tolerizing regimen that utilizes biologics and techniques that selectively target donor-reactive T cells while expanding populations of regulatory T cells, in an 'islet friendly' manner will undoubtedly lead to the definitive cure of T1DM.

Islet transplant recipients often have some degree of renal impairment at the time of transplantation, which can be exacerbated by calcineurin inhibitors. This is true even with the low doses used currently, which can be compounded with the use of sirolimus [65, 66]. Consequently, renal function of islet transplant recipients must be monitored diligently. In addition, islet recipients are prone to the more generalized immunosuppressive complications including leucopenia, mouth ulcers, infections, and malignancy.

Clinical Islet Transplantation Outcomes

Since the inception of the Edmonton Protocol, over 750 islet transplants have been performed in over 30 International transplant centers around the world. Unquestionably, the concept of islet transplantation has evolved in a number of countries from an experimental procedure to one that recognized standard clinical therapy.

To date, 677 allogeneic islet transplants have been reported to the Collaborative Islet Transplant Registry (CITR). Of these, 44% were insulin independent at three years post-transplant in the 'new era' of islet transplants (2007–2010), as compared to 27% of clinical islet transplant recipients in 1999–2002 [67, 68] (Fig. 14.2a). Moreover, marked improvements in clinical islet transplantation have been observed from 2007 to 2010, as evidenced by retained C-peptide levels, reduction in HbA1c levels and reduced islet reinfusion rates [68]. Shifts in immunosuppression strategies can account for these success rates, though improvements to islet engraftment and subsequent survival are paramount to achieving durable insulin independence.

In spite of marked improvements in clinical islet transplantation outcomes and substantial transplant activity in international islet transplant centers, few centers are currently active in North America. In the United States, islet transplantation is still classified as an experimental therapy, and as a result immensely lacks the available funds necessary to conduct and support large-scale clinical trials. In an effort to support the FDA biological license application mandate, two pivotal Phase III clinical trials are currently being conducted in specialized islet transplantation centers through the Clinical Islet Transplant (CIT) Consortium (CIT-06 and CIT-07, Clinical Trials.gov NCT00468117 and NCT00434811, respectively). Successful licensure will inevitably recognize islet transplantation as a clinical therapy, thus expanding its therapeutic applicability to patients with T1DM in the United States.

The University of Alberta's Clinical Islet Transplant Program remains an active site, and in 2013 alone, 66 islet transplants were conducted at the Edmonton site (Fig. 14.2b). The Edmonton Group reports that of over 200 patients transplanted with more than 400 intraportal islet preparations, 79% of recipients continue to show full or partial islet graft function [69]. Notably, the median duration of insulin independence is 34.6 and 11 months for subjects with full or partial graft function. Moreover, the duration of C-peptide is 53.3 and 70.4 months, respectively, for those same patients [70–72].

To date, the application of islet allotransplantation is only suitable for patients with unstable glycemic control that is life-threatening (e.g., hypoglycemia unawareness) and that cannot be corrected by standard conventional and intensive insulin therapies [17]. Patients who exhibit good glycemic control, as well as children, are not currently considered for islet allotransplantation, largely owing to the need for lifelong, chronic immunosuppression. In a recent trial by Ly et al.,

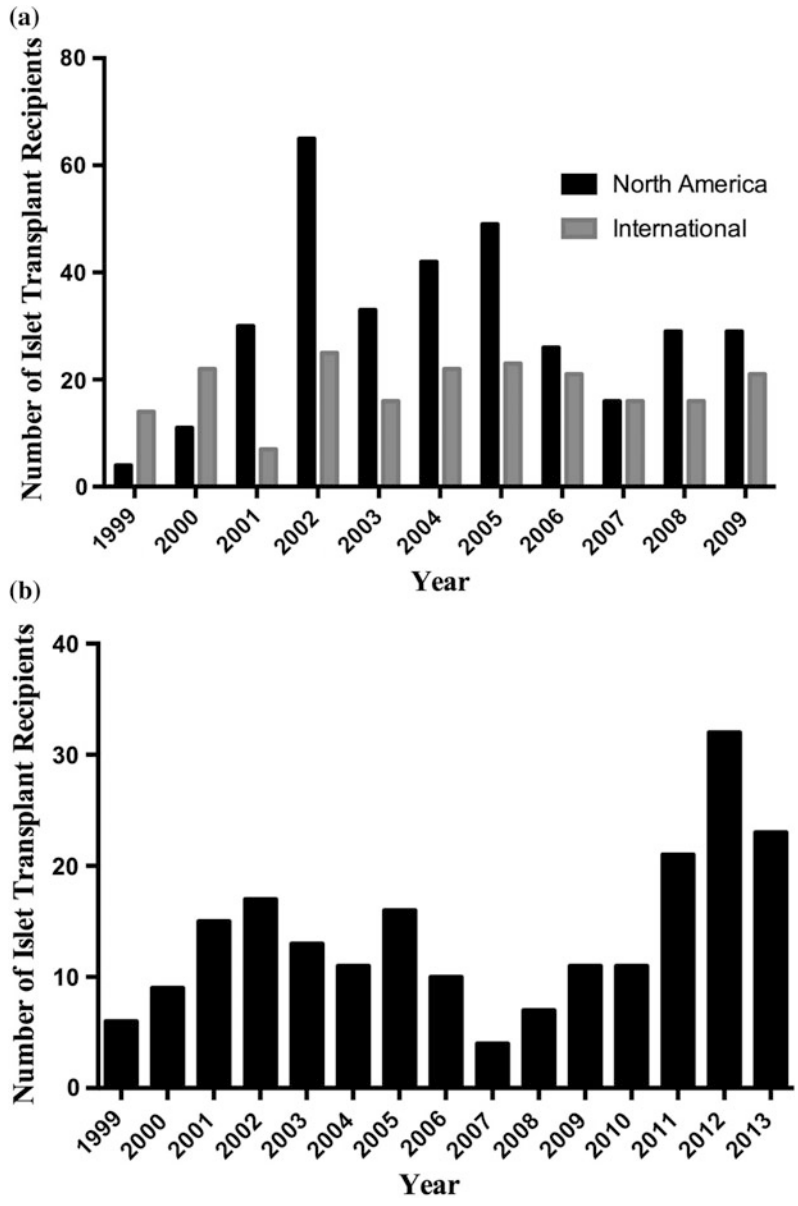

Fig. 14.2 Clinical islet transplant recipients per year. **a** Number of islet transplant recipients per year completed in North America and Internationally, registered by the CITR. **b** Number of islet transplant recipients completed by the Edmonton Clinical Islet Transplant Program

sensor-augmented pump therapy with automated insulin suspension reduced the rate of moderate and severe hypoglycemia, as well as impaired hypoglycemia awareness over a 6-month period in trial participants. Yet, when compared to the standard insulin pump control group, no change in glycosylated hemoglobin (HbA1C) was observed [72]. Conversely, in islet transplant recipients, HbA1C levels were corrected to levels that could predictably reverse the secondary consequences of diabetes [73]. Moreover, a one-way crossover study conducted by Thompson and colleagues demonstrated that clinical islet transplantation was more effective in reducing progression of diabetic retinopathy and nephropathy than intensive medical therapy [74]. In this therapeutic setting, the lifelong need for immunosuppressive therapy may be readily justified.

Indications and Patient Selection for Islet Transplantation

As noted, the current indications for islet allotransplantation do not include the pediatric population, though the potential applicability to children will be explored below (Table 14.1). Indeed, islet transplantation has been carried out in children, although most have been in the setting of total pancreatectomy and islet autotransplantation for hereditary pancreatitis. In this clinical setting the necessity for immunosuppression is not required.

Secondary to the procedure and consequences of the immunosuppressive therapies, there are a number of risks associated with islet transplantation. As such, adult patients selected for islet transplantation must have T1DM with life-threatening complications to justify these risks. Suitable patient populations include those with severe and recurrent hypoglycemic unawareness and/or those with unstable glucose control despite an optimized insulin regime (glycemic lability). The latter includes those requiring hospitalization for hypoglycemia or ketoacidosis. Those with advanced secondary complications of T1DM may also be considered.

In addition to thorough characterization of secondary complications, patient selection also involves the determination of metabolic status. Typically, patients are selected provided there is no endogenous insulin reserve, indicated by the absence of C-peptide. Patients with elevated BMI (>30 kg/m^2) or weight > 90 kg may be excluded since the transplanted islet tissue may not meet metabolic demand. The evaluation of hypoglycemia and glycemic lability is assessed through the HYPO score and Lability Index (LI), respectively, developed by Ryan et al. [75]. While the former is based on the frequency, severity and degree of unawareness of the hypoglycemia, the latter is calculated based on the change in glucose levels over time. Patients ranking in the 90th percentile for either score are given consideration for islet transplantation.

Patients selected for islet transplantation undergo a full cardiac assessment and should have no evidence of uncontrolled hypertension, absence of myocardial infarction in the preceding six months and left ventricular ejection fraction >30%. Since immunosuppressive therapy (specifically tacrolimus and sirolimus) may exacerbate renal failure, a glomerular filtration rate of >80 ml/min/1.73 m^2 and no evidence of macroscopic proteinuria is preferred. All recipients are also screened for any evidence of early neoplasias.

Pediatric Islet Allotransplantation—A Real Possibility?

In the history of solid organ transplantation, it did not take long for life-saving transplants and the need for chronic immunosuppression in adults to be translated to the pediatric population. Some of the earliest successful liver transplants were carried out in children, and today end-stage renal failure in children is optimally managed with renal transplantation and chronic immunosuppression. The risks associated with immunosuppression (increased rates of infection and

Table 14.1 Indications and exclusions for islet allotransplantation

Indications for islet transplantation	Exclusions for islet transplantation
• Type 1 diabetes for >5 years • Above 18 years old • Negative stimulated C-peptide (<0.3 ng/ml) • Despite adequate insulin therapy: – Hypoglycemic unawareness[a] – Glycemic lability[b] – Composite score >75th percentile	• Uncontrolled hypertension • Severe cardiac disease • Macroalbuminuria • Glomerular filtration rate <80 ml/min/1.73 m^2 • Potential inability to comply with imunosuppression

[a]Clark Score >4, HYPO score > 90th percentile
[b]Lability index >90th

malignancy, renal impairment and specific drug-related side effects) are well defined in the pediatric and adult populations. Whole pancreas transplantation has rarely been applied to children, largely owing to the risk associated with surgical intervention of such magnitude, as well as the life-threatening complications. Consequently, such a procedure would be difficult to justify in a child with T1DM without other life-threatening complications. It is often questioned when islet transplantation would be a suitable therapy for children. If the need for lifelong immunosuppression could be negated through the induction of tolerance, then islet transplantation in children could adequately be justified. Conversely, with today's standard of care treatment, the lifelong commitment to immunosuppression represents a challenging balance against the known, unmitigated complications associated with T1DM.

Alarmingly, the incidence of both T1DM and type 2 diabetes mellitus (obesity-related diabetes) is progressively rising in children globally. To mitigate the long-term complications associated with diabetes, tight glycemic control must be maintained, though this is not without inherent risks. Notably, in children, there are increased risks of fatal hypoglycemia, behavioral and cognitive impairment and the masking of future episodes of hypoglycemia [4, 76, 77].

There are numerous obstacles that are associated with the optimal care of this age group, including accuracy of blood glucose monitors, family commitment and the compliance of the patient as they reach adolescence [78]. Children occasionally face life-threatening, asymptomatic nocturnal hypoglycemia despite adequate exogenous insulin therapy [78]. Challenges arise in adequately identifying these risks at an appropriate time and improving insulin management while ensuring the prevention of a fatal hypoglycemic episode. Unquestionably, islet transplantation will likely become a therapeutic strategy in children with unstable and recalcitrant forms of T1DM. As inroads continue to be made in the safety of the procedure itself, as well as improvements to the side effects associated with acute and chronic immunosuppression therapies. Moreover, effective control of the autoimmune process in type 1 diabetes will be essential if these approaches are to move forward successfully.

In the adult population, islet transplantation has proven effective in preventing hypoglycemic events and enabling insulin independence. The requirement for chronic, long-term immunosuppression, paired with their potential side effects, limits its use in this population. As newer, less toxic immunosuppressive regimens and the potential for both steroid and calcinerin inhibitor-free protocols are developed, islet transplantation may be a possible therapy in very select groups of children. These include:

- Children suffering from recurrent, severe hypoglycemic events despite diet and insulin alterations.
- Children who develop secondary complications of diabetes, especially retinopathy and nephropathy, likely leading to severe deficits in adulthood.
- Those children already on immunosuppression for a previous solid organ allograft.

From a practical perspective, the first and third groups would derive the most benefit in light of the risks associated with islet transplantation and immunosuppression. However, the optimization of diet and insulin therapy would need to be considered in order for islet transplantation to be implemented. For islet transplantation to be widely implemented in children, a number of key questions will need to be answered, including [78]:

- Will the recipient outgrow the islet mass or will the islet mass expand over time as the patient develops?
- Will islets from one donor be sufficient to promote insulin independence?
- Will adolescent patients be able to comply with maintenance immunotherapy after successful islet transplantation?
- How should immunosuppressive regimens be tailored in female patients who wish to conceive in early adulthood?

Elucidating answers to these questions are critical for the application of clinical islet transplantation in children afflicted with T1DM.

Concluding Remarks

Undoubtedly, improvements in human islet isolation, the introduction of the 'Edmonton Protocol', and more recent developments in anti-inflammatory and immunosuppressive strategies have played a major role in improving the results and activity of clinical islet transplantation. Islet transplantation cannot currently be defined as a cure for T1DM, though this therapeutic treatment can offer an improved quality of life in recipients, evidenced by remarkable stability of glycemic control and correction of HbA1C. Such clinical outcomes provide an increasing number of patients with sustained periods of complete independence from insulin. Prevention of life-threatening hypoglycemia is a major advance that can often not be sustained by optimized exogenous insulin therapy. Continued, concerted efforts are still required to further establish islet transplantation as a suitable treatment modality for all patients afflicted with T1DM. The applicability of whole organ allotransplantation in children emphasizes the ongoing need to establish less toxic immunosuppression regimes aimed to improve all lives of those afflicted with T1DM. This need calls for a continued rapid drive to transition islet transplantation as a treatment for some, to a therapy for all.

References

1. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995–2025: prevalence, numerical estimates, and projections. Diabetes Care. 1998;21(9):1414–31.
2. Polonsky KS. The past 200 years in diabetes. New Engl J Med. 2012;367(14):1332–40.
3. Banting FG, Best CH, Collip JB, Campbell WR, Fletcher AA. Pancreatic extracts in the treatment of diabetes mellitus. Can Med Assoc J. 1922;12(3):141–6.
4. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. New Engl J Med. 1993;329(14):977–86.
5. Keen H. The diabetes control and complications trial (DCCT). Health Trends. 1994;26(2):41–3.
6. Nathan DM, Lachin J, Cleary P, Orchard T, Brillon DJ, Backlund JY, et al. Intensive diabetes therapy and carotid intima-media thickness in type 1 diabetes mellitus. New Engl J Med. 2003;348(23):2294–303.
7. Kelly WD, Lillehei RC, Merkel FK, Idezuki Y, Goetz FC. Allotransplantation of the pancreas and duodenum along with the kidney in diabetic nephropathy. Surgery. 1967;61(6):827–37.
8. Brogard JM, Vetter T, Blickle JF. Discovery of pancreatic diabetes in Strasbourg. Diabete Metabol. 1992;18(2):104–14.
9. Agarwal A, Brayman KL. Update on islet cell transplantation for type 1 diabetes. Semin Intervent Radiol. 2012;29(2):90–8.
10. Lacy PE, Kostianovsky M. Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes. 1967;16(1):35–9.
11. Misler S. The isolated pancreatic islet as a micro-organ and its transplantation to cure diabetes: celebrating the legacy of Paul Lacy. Islets. 2010;2(4):210–24.
12. Reckard CR, Ziegler MM, Barker CF. Physiological and immunological consequences of transplanting isolated pancreatic islets. Surgery. 1973;74(1):91–9.
13. Shapiro AM. A historical perspective on experimental and clinical islet transplantation. Informa Health Care. 2007;1.
14. Lakey JR, Warnock GL, Shapiro AM, Korbutt GS, Ao Z, Kneteman NM, et al. Intraductal collagenase delivery into the human pancreas using syringe loading or controlled perfusion. Cell Transplant. 1999;8(3):285–92.
15. Ricordi C, Lacy PE, Scharp DW. Automated islet isolation from human pancreas. Diabetes. 1989;38(Suppl 1):140–2.
16. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. New Engl J Med. 2000;343(4):230–8.
17. Ryan EA, Bigam D, Shapiro AM. Current indications for pancreas or islet transplant. Diabetes Obes Metab. 2006;8(1):1–7.
18. Scharp DW, Kemp CB, Knight MJ, Ballinger WF, Lacy PE. The use of ficoll in the preparation of viable islets of langerhans from the rat pancreas. Transplantation. 1973;16(6):686–9.
19. Najarian JS, Sutherland DE, Matas AJ, Steffes MW, Simmons RL, Goetz FC. Human islet transplantation: a preliminary report. Transpl Proc. 1977;9(1):233–6.

20. Brendel M HB, Shulz A, Bretzel R. International islet transplant registry report. 1999.1–20.
21. Benhamou PY, Watt PC, Mullen Y, Ingles S, Watanabe Y, Nomura Y, et al. Human islet isolation in 104 consecutive cases. Factors affecting isolation success. Transplantation. 1994;57(12):1804–10.
22. Goto M, Eich TM, Felldin M, Foss A, Kallen R, Salmela K, et al. Refinement of the automated method for human islet isolation and presentation of a closed system for in vitro islet culture. Transplantation. 2004;78(9):1367–75.
23. Ihm SH, Matsumoto I, Sawada T, Nakano M, Zhang HJ, Ansite JD, et al. Effect of donor age on function of isolated human islets. Diabetes. 2006;55 (5):1361–8.
24. Lakey JR, Rajotte RV, Warnock GL, Kneteman NM. Human pancreas preservation prior to islet isolation. Cold ischemic tolerance. Transplantation. 1995;59 (5):689–94.
25. Lakey JR, Warnock GL, Rajotte RV, Suarez-Alamazor ME, Ao Z, Shapiro AM, et al. Variables in organ donors that affect the recovery of human islets of Langerhans. Transplantation. 1996;61(7): 1047–53.
26. Matsumoto I, Sawada T, Nakano M, Sakai T, Liu B, Ansite JD, et al. Improvement in islet yield from obese donors for human islet transplants. Transplantation. 2004;78(6):880–5.
27. Nano R, Clissi B, Melzi R, Calori G, Maffi P, Antonioli B, et al. Islet isolation for allotransplantation: variables associated with successful islet yield and graft function. Diabetologia. 2005;48(5):906–12.
28. Zeng Y, Torre MA, Karrison T, Thistlethwaite JR. The correlation between donor characteristics and the success of human islet isolation. Transplantation. 1994;57(6):954–8.
29. Kin T, Murdoch TB, Shapiro AM, Lakey JR. Estimation of pancreas weight from donor variables. Cell Transplant. 2006;15(2):181–5.
30. Brandhorst H, Brandhorst D, Hering BJ, Federlin K, Bretzel RG. Body mass index of pancreatic donors: a decisive factor for human islet isolation. Exp Clin Endocrinol Diabetes. 1995;103(Suppl 2):23–6.
31. Berney T, Johnson PR. Donor pancreata: evolving approaches to organ allocation for whole pancreas versus islet transplantation. Transplantation. 2010; 90(3):238–43.
32. O'Gorman D, Kin T, Murdoch T, Richer B, McGhee-Wilson D, Ryan E, et al. The standardization of pancreatic donors for islet isolation. Transpl Proc. 2005;37(2):1309–10.
33. de Boer J, De Meester J, Smits JM, Groenewoud AF, Bok A, van der Velde O, et al. Eurotransplant randomized multicenter kidney graft preservation study comparing HTK with UW and Euro-Collins. Transpl Int. 1999;12(6):447–53.
34. Hering BJ, Matsumoto I, Sawada T, Nakano M, Sakai T, Kandaswamy R, et al. Impact of two-layer pancreas preservation on islet isolation and transplantation. Transplantation. 2002;74(12):1813–6.
35. Benhamou PY, Oberholzer J, Toso C, Kessler L, Penfornis A, Bayle F, et al. Human islet transplantation network for the treatment of Type I diabetes: first data from the Swiss-French GRAGIL consortium (1999–2000). Groupe de Recherche Rhin Rhjne Alpes Geneve pour la transplantation d'Ilots de Langerhans. Diabetologia. 2001;44(7):859–64.
36. Goss JA, Schock AP, Brunicardi FC, Goodpastor SE, Garber AJ, Soltes G, et al. Achievement of insulin independence in three consecutive type-1 diabetic patients via pancreatic islet transplantation using islets isolated at a remote islet isolation center. Transplantation. 2002;74(12):1761–6.
37. Wolters GH, Vos-Scheperkeuter GH, van Deijnen JH, van Schilfgaarde R. An analysis of the role of collagenase and protease in the enzymatic dissociation of the rat pancreas for islet isolation. Diabetologia. 1992;35(8):735–42.
38. Johnson PR, White SA, London NJ. Collagenase and human islet isolation. Cell Transplant. 1966;15 (4):437–52.
39. Wang X, Meloche M, Verchere CB, Ou D, Mui A, Warnock GL. Improving islet engraftment by gene therapy. J Transplant. 2011;2011:594851.
40. Mehigan DG, Bell WR, Zuidema GD, Eggleston JC, Cameron JL. Disseminated intravascular coagulation and portal hypertension following pancreatic islet autotransplantation. Ann Surg. 1980;191(3): 287–93.
41. Lake SP, Bassett PD, Larkins A, Revell J, Walczak K, Chamberlain J, et al. Large-scale purification of human islets utilizing discontinuous albumin gradient on IBM 2991 cell separator. Diabetes. 1989;38(Suppl 1):143–5.
42. Ichii HPA, Khan A, Fraker C, Ricordi C. Culture and transportation of human islets between centers. Islet transplantation and beta cell replacement therapy. New York: Informa healthcare; 2007. p. 251.
43. Hering BJ, Kandaswamy R, Ansite JD, Eckman PM, Nakano M, Sawada T, et al. Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. JAMA. 2005;293(7):830–5.
44. Gangemi A, Salehi P, Hatipoglu B, Martellotto J, Barbaro B, Kuechle JB, et al. Islet transplantation for brittle type 1 diabetes: the UIC protocol. Am J Transplant. 2008;8(6):1250–61.
45. Froud T, Ricordi C, Baidal DA, Hafiz MM, Ponte G, Cure P, et al. Islet transplantation in type 1 diabetes mellitus using cultured islets and steroid-free immunosuppression: Miami experience. Am J Transplant. 2005;5(8):2037–46.
46. Froud T, Faradji RN, Pileggi A, Messinger S, Baidal DA, Ponte GM, et al. The use of exenatide in islet transplant recipients with chronic allograft dysfunction: safety, efficacy, and metabolic effects. Transplantation. 2008;86(1):36–45.

47. Ghofaili KA, Fung M, Ao Z, Meloche M, Shapiro RJ, Warnock GL, et al. Effect of exenatide on beta cell function after islet transplantation in type 1 diabetes. Transplantation. 2007;83(1):24–8.
48. Faradji RN, Tharavanij T, Messinger S, Froud T, Pileggi A, Monroy K, et al. Long-term insulin independence and improvement in insulin secretion after supplemental islet infusion under exenatide and etanercept. Transplantation. 2008;86(12):1658–65.
49. Rajab A. Islet transplantation: alternative sites. Curr Diab Rep. 2010;10(5):332–7.
50. Froud T, Yrizarry JM, Alejandro R, Ricordi C. Use of D-STAT to prevent bleeding following percutaneous transhepatic intraportal islet transplantation. Cell Transplant. 2004;13(1):55–9.
51. Barshes NR, Lee TC, Goodpastor SE, Balkrishnan R, Schock AP, Mote A, et al. Transaminitis after pancreatic islet transplantation. J Am Coll Surg. 2005;200(3):353–61.
52. Biarnes M, Montolio M, Nacher V, Raurell M, Soler J, Montanya E. Beta-cell death and mass in syngeneically transplanted islets exposed to short- and long-term hyperglycemia. Diabetes. 2002;51(1):66–72.
53. Owen RJ, Ryan EA, O'Kelly K, Lakey JR, McCarthy MC, Paty BW, et al. Percutaneous transhepatic pancreatic islet cell transplantation in type 1 diabetes mellitus: radiologic aspects. Radiology. 2003;229(1):165–70.
54. Ryan EA, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, et al. Five-year follow-up after clinical islet transplantation. Diabetes. 2005;54(7):2060–9.
55. Gala-Lopez B, Pepper AR, Shapiro AM. Biologic agents in islet transplantation. Curr Diab Rep. 2013;13(5):713–22.
56. Bloom SR, Polak JM. Somatostatin. Br Med J. 1987;295(6593):288–90.
57. Korsgren O, Lundgren T, Felldin M, Foss A, Isaksson B, Permert J, et al. Optimising islet engraftment is critical for successful clinical islet transplantation. Diabetologia. 2008;51(2):227–32.
58. Shapiro AM. A historical perspective on experimental and clinical islet transplantation. In: Shapiro AM, Shaw JA, editors. Islet transplantation and beta cell replacement therapy. New York, London: Informa Healthcare; 2007.
59. Gabardi S, Martin ST, Roberts KL, Grafals M. Induction immunosuppressive therapies in renal transplantation. Am J Health-System Pharm: AJHP: Official J Am Soc Health-System Pharmacists. 2011;68(3):211–8.
60. Hering BJ. Repurification: rescue rather than routine remedy. Am J Transplant. 2005;5(1):1–2.
61. Koh A, Senior P, Salam A, Kin T, Imes S, Dinyari P, et al. Insulin-heparin infusions peritransplant substantially improve single-donor clinical islet transplant success. Transplantation. 2010;89(4):465–71.
62. Matsumoto S, Takita M, Chaussabel D, Noguchi H, Shimoda M, Sugimoto K, et al. Improving efficacy of clinical islet transplantation with iodixanol based islet purification, Thymoglobulin induction and blockage of IL-1-beta and TNF-alpha. Cell Transplant. 2011;20(10):1641–7.
63. Shapiro AM, Ricordi C. Unraveling the secrets of single donor success in islet transplantation. Am J Transplant. 2004;4(3):295–8.
64. Xenos ES, Farney AC, Widmer MB, Casanova D, Stevens RB, Blazar BR, et al. Effect of tumor necrosis factor alpha and of the soluble tumor necrosis factor receptor on insulin secretion of isolated islets of Langerhans. Transpl Proc. 1992;24(6):2863–4.
65. Senior PA, Paty BW, Cockfield SM, Ryan EA, Shapiro AM. Proteinuria developing after clinical islet transplantation resolves with sirolimus withdrawal and increased tacrolimus dosing. Am J Transplant. 2005;5(9):2318–23.
66. Kaplan B, Schold J, Srinivas T, Womer K, Foley DP, Patton P, et al. Effect of sirolimus withdrawal in patients with deteriorating renal function. Am J Transplant. 2004;4(10):1709–12.
67. The CITR Coordinating Center and Investigators. The collaborative islet transplant registry (CITR) 2011 seventh annual report. https://web.emmes.com/study/isl/reports/01062012_7thAnnualReport.pdf. Accessed 27 Jan 2017.
68. Barton FB, Rickels MR, Alejandro R, Hering BJ, Wease S, Naziruddin B, et al. Improvement in outcomes of clinical islet transplantation: 1999–2010. Diabetes Care. 2012;35(7):1436–45.
69. Senior PA, Kin T, Shapiro AMJ, Koh A. Islet transplantation at the University of Alberta: status update and review of progress over the last decade. Can J Diabetes. 2012;36:32–7.
70. Pepper AR, Gala-Lopez B, Ziff O, Shapiro AJ. Current status of clinical islet transplantation. World J Transplant. 2013;3(4):48–53.
71. Senior PAKT, Shapiro AMJ, Koh A. Islet transplantation at the University of Alberta: Status update and review of progress over the last decade. Can J Diabetes. 2012;36:32–7.
72. Ly TT, Nicholas JA, Retterath A, Lim EM, Davis EA, Jones TW. Effect of sensor-augmented insulin pump therapy and automated insulin suspension vs standard insulin pump therapy on hypoglycemia in patients with type 1 diabetes: a randomized clinical trial. JAMA. 2013;310(12):1240–7.
73. Shapiro AM. Strategies toward single-donor islets of Langerhans transplantation. Curr Opin Organ Transplant. 2011;16(6):627–31.
74. Thompson DM, Meloche M, Ao Z, Paty B, Keown P, Shapiro RJ, et al. Reduced progression of diabetic microvascular complications with islet cell transplantation compared with intensive medical therapy. Transplantation. 2011;91(3):373–8.

75. Ryan EA, Shandro T, Green K, Paty BW, Senior PA, Bigam D, et al. Assessment of the severity of hypoglycemia and glycemic lability in type 1 diabetic subjects undergoing islet transplantation. Diabetes. 2004;53(4):955–62.
76. Patrick AW, Campbell IW. Fatal hypoglycaemia in insulin-treated diabetes mellitus: clinical features and neuropathological changes. Diabet Med. 1990;7(4): 349–54.
77. Gonder-Frederick LA, Clarke WL, Cox DJ. The Emotional, Social, and Behavioral Implications of Insulin-Induced Hypoglycemia. Semin Clin Neuropsychiatry. 1997;2(1):57–65.
78. Hathout E, Lakey J, Shapiro J. Islet transplant: an option for childhood diabetes? Arch Dis Child. 2003;88(7):591–4.