CHAPTER
Twenty Years of Clinical Islet Transplantation at the Diabetes Research Institute – University of Miami

A. Pileggi, C. Ricordi, N.S. Kenyon, T. Froud, D.A. Baidal, A. Kahn, G. Selvaggi, and R. Alejandro

Clinical Islet Transplant Program and Cell Transplant Center, Diabetes Research Institute, University of Miami - Leonard Miller School of Medicine, Miami, Florida

Intensive insulin therapy with the goal of maintaining blood glucose concentrations close to the normal range can delay or prevent the onset of diabetes complications in patients with type 1 diabetes mellitus (T1DM)(1). This goal is difficult to achieve in the majority of patients and is associated with a three-fold increase in severe hypoglycemia. Transplantation of pancreatic islets in patients with T1DM can restore β-cell function and provide a more physiological glycemic control than exogenous insulin administration (2).

Steady progress in the field of β-cell replacement has been observed in recent years (2, 3), starting from the seminal work by Lacy, et al (4, 5) in the late 1960s demonstrating reversal of hyperglycemia by islet transplantation in diabetic rodents. Numerous challenges have been overcome, while many still remain unsolved (2, 3). Technological advances in organ procurement and preservation techniques together with improvements in pancreatic islet isolation, purification and culture have allowed for the increasing success rate of islet transplantation worldwide. Implementation of safer transplantation procedures has also contributed to the increasing numbers of transplants performed in the recent years. A total of 493 allogeneic islet transplants (including historical cases) and 240 autologous grafts from 51 centers (18 North American, 30 European, and 6 elsewhere) were published by the International Islet Transplant Registry in 2001 (6). In light of the increasing number of centers performing islet transplantation, the National Institute of Health sponsored a North American Collaborative Islet Transplant Registry (CITR) that was established in 2001 (7, 8).

It has been twenty years since the first islet transplant was performed by the Clinical Islet Transplant Program (CITP) at the Diabetes Research Institute / University of Miami School of Medicine. One hundred-one islet infusions have been performed in 73 patients during this period. Here we present an overview of the activity of our CITP and of the progress in the islet transplantation field in recent years.

METHODS

Human Cell Processing Facility. The Diabetes Research Institute - University of Miami School of Medicine was the first academic research center to house a cur-
rent Good Manufacturing Practice (cGMP) facility dedicated exclusively to human islet cell isolation. This facility operates following cGMP and Food and Drug Administration regulations to ensure the highest standards of quality, purity, potency and safety for medical products destined for human use (47, 48). In addition to the isolation of human islets for research or clinical trials, the cGMP facility is also utilized for manufacturing human bone marrow cells (BMC). The Cell Processing and Distribution Center of our cGMP facility currently provides human islets, as well as other cells, to more than 50 collaborating scientists worldwide. The cGMP facility includes clean room suites, and various laboratory support areas over approximately 16,000 square feet. The access to the facility is restricted to authorized cGMP personnel only. The clean room suites meet ISO Class 7 (ISO 14644) requirements with HEPA-filtered air conditioning. Procedures pertaining to required dirty/clean flow patterns and directions are enforced by standard operating procedures, training, management and facility design. All procedures, processes and equipment are periodically validated, and testing ensures that they operate according to the pre-established performance specifications.


Organ harvesting is performed using standard techniques by the surgical team (either local or distant), generally consisting of the removal of the gland along with duodenum and spleen (49). The pancreas is collected and stored in University of Wisconsin (UW) solution (DuPont-Pharma; Wilmington, Delaware) on ice and shipped to our processing facility within 12 hours. In the 2000’s, the use of oxygen carrier moieties (Perfluorodecalin; FluoroMEd, L.P.) was introduced in order to reduce ischemic injury during preservation (50, 51). Briefly, the gland is immersed in preservation solution and maintained at the interface between oxygenated perfluorocarbon and UW (the two-layer method; TLM), as described by Kuroda, et al (52), and transported to the cell processing laboratory within 24 hours. The TLM is currently utilized in most of the pancreata obtained at our center, allowing us to improve islet yields even from marginal donors (51), and therefore improving the utilization of islet preparations processed for transplantation.

Islet Isolation. Islets are obtained using the automated method (53) consisting of a mechanically-enhanced enzymatic digestion of the pancreas. Briefly, the pancreatic gland is dissected-free from surrounding tissue. After dividing the pancreas through its neck, retrograde injection of dissociation buffer in the pancreatic duct is performed. Since the 1990’s, we use the enzyme blend Liberase HI® (Boehringer Mannheim-Roche) characterized by reduced endotoxin contamination and higher reproducibility, when compared to other collagenase blends; this has significantly contributed to improved islet yields from human pancreata (54-57).

After distension, the pancreatic gland is divided into 9-11 pieces and placed into the dissociation chamber. The chamber (Fig. 1A) consists of two portions divided
the islets from the non-endocrine pancreatic tissue is performed using a computerized COBE centrifuge (H) by sedimentation on continuous or discontinuous gradients (I). The purified fractions are collected and assessed for purity and counted (J-K) before being cultured (L). All procedures are performed under cGMP standard following SOP and maintaining documentation (M).
by a mesh: the lower cylindrical portion contains the pancreatic tissue and marbles and has two inlets on its base; the upper portion is conical with an outlet at its apex. Digestion of the pancreatic tissue is obtained by the combination of enzymatic activity at 37°C and the mechanical action of the marbles in the chamber that is shaken throughout the dissociation phase. A system of tubes connected to the chamber and a peristaltic pump allow the circulation of the enzyme from the base of the chamber through the mesh and toward its apex (Fig. 1B). The hydrostatic pressure applied contributes to the removal of small pancreatic fragments from the chamber as they can be pushed through the mesh, preventing excessive damage from mechanical injury. Numerous improvements have been introduced in the dissociation chamber over the years, with the more recent transparent disposable models allowing for real-time monitoring of the digestion process (Fig. 1A-C), and the use of motorized mechanical arms to better standardize the procedure (Fig. 1C). The digestion circuit allows collecting samples and assessing islet integrity to decide when to stop the enzymatic digestion. This is obtained by collecting the tissue into cold media supplemented with serum, while continuing with the mechanical digestion until all tissue has been obtained from the chamber. The pancreatic digest is then concentrated by centrifugation (Fig. 1D), and prepared for the subsequent purification step. Several variables may contribute to the outcome of islet isolation. We are currently utilizing a continuous multi-parametric monitoring system (Fig. 1E) to assess selected physiological parameters (e.g., temperature, pH, pCO₂, etc.) during the critical phases of isolation and culture in order to identify and correct variations that could negatively influence the overall outcome of the procedure (58).

Islet Purification. After the digestion phase, the pancreatic slurry is purified on density gradients. Numerous modifications of the purification process have been introduced during the years. In the late 1980’s, based on the promising results reported by Lake, et al (59), we introduced the use of the COBE computerized cell separation system (60) that allows for more efficient and time-effective purification of large volumes of pancreatic tissue (Fig. 1H-I). Separation of islet clusters from non-endocrine tissue was initially obtained by the means of discontinuous density gradients made in human serum albumin (60-62), and later in Ficoll dissolved in Euro-Collins preservation solution (Euro-Ficoll; Mediatech, Herndon, VA)(63). Since the late 1990’s, Ficoll-based continuous density gradients are utilized as first choice for the purification of all human islet preparations obtained at our center. Separate fractions are collected and assessed for purity (Fig. 1F-K); the fractions with lower exocrine contamination are generally pooled for transplantation, paying attention that overall tissue volumes do not exceed 10 mls. In order to maximize islet recovery, we have implemented an additional purification step on discontinuous Euro-Ficoll gradients that is performed on the less pure fractions obtained with the standard continuous step (Fig. 1I). This ‘rescue’ purification step has allowed us to increase the yields of human islet preparations and to meet the requirements for transplantation (>5,000 IEQ/kg of recipient’s body weight) in a higher number of preparations, therefore maximizing the overall utilization of pancreata processed at our facility (64).

Islet Assessment. Assessment of isolated islets is a prerequisite to evaluate the efficiency of the procedure and the quality of the final product before its release for transplantation. Product release criteria following islet isolation and culture include assessment of a number of variables to prevent the administration of adventitious agents to the recipient, and to ascertain that viable and potent islet cells are being transplanted (Table 1). In the 1980’s, we described the use of dithizone staining (Fig. 1G and K) that binds to the zinc of secretory granules conferring a characteristic red color to endocrine cells (65). This quick method is now the gold standard utilized for the assessment of islet integrity and of the purity of islet preparations (Fig. 1G and K)(65, 66). Islet enumeration allows assessing the islet yields after isolation and culture. Islets are counted and scored by size using an algorithm to calculate islet equivalents (IEQ; the ‘ideal’ spherical islet with diameter of 150µm)(67, 68). Routinely performed sterility tests should be negative and include cultures for aerobic and anaerobic microbes, mycoplasma, and fungi. Pyrogenicity is also assessed by measuring endotoxin concentrations in the reagents used for islet isolation and culture, which should be <5EU/kg. Assessment of islet viability is generally performed by the use of membrane dye exclusion protocols that allow for the discrimination of viable and dead cells in sampled islets (64, 69-71). Product release criteria require a viability ≥70% using such methods.

Potency tests are utilized to assess the ability of isolated islets to produce and secrete insulin in a regulated fashion in response to glucose stimulation (69, 72).
Sequential or parallel incubation of isolated islets in vitro in the presence of increasing concentrations of glucose results in insulin release that is quantified in the supernatants by enzyme-linked immunosorbent assay. The stimulation index of glucose-stimulated insulin over baseline is generally used as a surrogate endpoint to assess islet potency, and it is generally accepted that indices >1 are required in order to release an islet preparation for transplantation. Dynamic tests (i.e., perifusion) are also utilized to determine insulin release from islet aliquots in response to glucose challenge (64, 73). Transplantation of islets into chemically-induced diabetic immunodeficient mice was introduced in the late 1980's (74-76). This allows for the in vivo assessment of human islet preparations by measuring the ability of transplanted islets to reverse diabetes (64, 72, 74-76). By transplanting decreasing islet numbers in diabetic mice and comparing both (i) time to diabetes reversal and (ii) proportion of animals reversing diabetes, it is possible to determine differences in potency between islet preparations. Notably, although this in vivo assay is predictive of graft function, it requires relatively long periods of follow-up. This approach and the use of glucose tolerance tests in the transplanted animals allow assessing islet engraftment and may be of assistance toward the validation of quicker and more predictive in vitro tests for human islet quality (64, 77-81).

**Table 1. Product release criteria for human islet preparations for transplantation.**

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<thead>
<tr>
<th>Test</th>
<th>Method</th>
<th>Required outcome</th>
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<tbody>
<tr>
<td>Islet Yields</td>
<td>Count of IEQ on aliquots of the final preparation</td>
<td>≥5,000 IEQ/Kg recipient’s b.w.</td>
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<tr>
<td>Purity</td>
<td>Dithizone staining</td>
<td>≥30% final preparation</td>
</tr>
<tr>
<td>Islet Morphology</td>
<td>Dithizone staining</td>
<td>Preserved shape and granularity</td>
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<tr>
<td>Sterility</td>
<td>Aerobic/Anaerobic cultures</td>
<td>Negative</td>
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<td></td>
<td>Mycoplasma</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Fungal cultures</td>
<td>Negative</td>
</tr>
<tr>
<td>Pyrogenicity</td>
<td>Endotoxin</td>
<td>≤5 EU/kg</td>
</tr>
<tr>
<td>Viability</td>
<td>Membrane dye exclusion</td>
<td>≥70%</td>
</tr>
<tr>
<td>Potency</td>
<td>In vitro</td>
<td>Stimulation Index &gt;1</td>
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<tr>
<td></td>
<td>Glucose-stimulated insulin release</td>
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</table>

Shipping of Human Islets to Distant Transplant Centers. Since 2001 our Human Cell Processing facility has processed human pancreata procured from our partner CITP at the Baylor College in Houston, TX. After isolation, the islet preparations were shipped to Houston where they were transplanted (ITA) into patients with brittle T1DM using a modification of the Edmonton protocol (30, 31, 49, 82). Islets are assessed for product release criteria (quality, viability, potency and sterility) at our facility and at the remote transplant site prior to transplantation. Similar collaborations are being established with additional distant CITP’s.

Transplantation Techniques. Pancreatic islets are embolized into the recipient’s liver by cannulation of the portal vein. Most of the initial patients were transplanted by performing a laparotomy (SIK and IAK)(83, 84), an approach that is currently limited to a few cases at risk of bleeding. More recently, recipients of ITA and IAK are transplanted using a minimally-invasive interventional radiology approach consisting of percutaneous catheterization of the portal vein (62, 85), that can be performed under ultrasound (Fig. 2A) and fluoroscopic (Fig. 2B) guidance under conscious sedation (82, 86-89). To reduce the risk of thrombosis due to the islet infusion, heparin (35U/kg) is added to the islet preparation immediately before infusion. Portal pressure is constantly monitored during the transplant procedure, and in order to limit the risk of portal pressure increase consequent to islet implant (90, 91), islets are infused by gravity using a closed gravity transfusion bag system (Fig. 2C-E) utilized at our institution since 1990 (33, 86). D-Stat™ (Vascular Solutions, MN) is used for closure of the needle tract to prevent the occurrence of bleedings after transhepatic percutaneous access (Fig. 2F)(87).

Bone Marrow Cell Isolation. Since the early 1990’s, the Human Cell Processing cGMP Facility performs isolation of BMC that are utilized in numerous clinical trials aiming at the induction of hematopoietic chimerism in combination with solid organ and cellular grafts (42, 92-115). The vertebral column (VC) is harvested from the same multi-organ cadaveric donor of the islets...
Vertebral bone marrow cells (VBMC) are obtained by subjecting bone fragments to gentle shaking in media followed by filtration through stainless steel meshes, in order to separate bone chips from free VBMC (104-107, 117-124). Assessment of VBMC yields is performed using a cell counter (Coulter) to obtain a nucleated cell count, and trypan blue exclusion is employed to determine viability (125-127). In selected protocols, enrichment of CD34+ stem cells is obtained by positive selection using magnetic beads (128). Whole bone marrow cells or CD34+ BMC are cryopreserved into a transfusion bag and stored in liquid nitrogen until use (104-107, 129). Just before infusion, the bag containing the donor marrow inoculum is thawed and infused intravenously in the recipient (42, 44-46, 130, 131).

Selection of Study Subjects. Our study subjects come predominantly from the diabetes clinics at the University of Miami, referrals from local and outside physicians, and self-referred patients from all over the United States. In the current islet transplantation trials, only individuals with T1DM, with documented absence of C-peptide response to mixed meal challenge (<0.3 ng/ml), who fulfill the selection criteria were selected as candidates for islet transplantation. Patients with advanced macroangiopathy, including symptomatic ischemic and non-ischemic heart disease and patients that are unable to follow the rigorous requirements for follow-up, including those who did not pass a preliminary psychological evaluation were not considered for inclusion. Allogeneic islet transplantation has also been performed in selected patients with T1DM due to diseases of the exocrine pancreas (insulin-requiring diabetes mellitus; IRDM) and liver cirrhosis that received orthotopic allogeneic liver (24, 25) or in patients with ESRD and T2DM.

Treatments. Different therapeutic protocols have been utilized over the years in patients with T1DM that were dictated by the availability of new reagents and the promising results reported in solid organ transplantation trials. Most of the early trials included a limited number of patients and were aimed at assessing both safety and efficacy (Phase I-II) of allogeneic islet transplantation in the treatment of patients with T1DM. These clinical trials have contributed substantially to the acquisition of critical experience in the management of recipients of islet grafts and of immunosuppression, allowing for the steady progress in this field of investigation. We have established a multidisciplinary team composed of endocrinologists, transplant surgeons, immunologists, and basic scientists that are involved in our center’s clinical research efforts. Early trials included only IAK and SIK recipients for whom immunosuppression was implemented to sustain kidney function. In 1998 we started our clinical ITA trials in patients with brittle diabetes, for whom the risks associated with the transplant procedure and immunosuppression were justified by the frequency of life-threatening hypoglycemic events (3, 132, 133). Two major categories of clinical trials are being pursued at our center: chronic immunosuppression (Table 2) and protocols that attempt to induce tolerance (Table 3). The latter protocols include the use of bone marrow cells (BMC), either as whole BMC or fractioned CD34+ cells obtained from the same islet donor to induce hematopoietic chimerism (134), and are designed to wean immunosuppression at selected time points after transplantation (42, 44-46, 130).

Assessment of Graft Function. Graft function is monitored using finger-stick blood glucose (FBG), mean amplitude of glycemic excursions (MAGE), basal C-peptide, glycosylated hemoglobin (HbA1c), and fructosamine. We have recently implemented the use of a continuous subcutaneous glucose monitoring system (18, 83, 84, 135-137) for a more accurate assessment of glycemic control throughout the day after transplantation. Stimulation testing includes mixed meal tolerance test (MMTT), intravenous glucose tolerance test (IVGTT), and arginine stimulation test. An oral glucose
Table 2. Clinical islet transplantation trials – Chronic immunosuppression protocols.

<table>
<thead>
<tr>
<th>Period</th>
<th>Type of transplant</th>
<th>No.</th>
<th>Induction</th>
<th>Immunosuppression</th>
<th>Indication</th>
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<tr>
<td>1985-89</td>
<td>IAK</td>
<td>4</td>
<td>None</td>
<td>Cyclosporine</td>
<td>T1DM</td>
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<td></td>
<td>SIK</td>
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<td>Azathioprine</td>
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<tr>
<td>1990-93</td>
<td>IAK</td>
<td>7</td>
<td>OKT3</td>
<td>Cyclosporine</td>
<td>T1DM</td>
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<td>Azathioprine</td>
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<td>1995</td>
<td>SIL</td>
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<td>None</td>
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<td>T1DM</td>
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<td>1998</td>
<td>SIK</td>
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<td>2001-03</td>
<td>ITA</td>
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ALG: Anti-Lymphocyte Globulins; ATG: Anti-Thymo-Globulin; AZA: Azathioprine; BMC: Bone Marrow Cells; IAK: Islet After Kidney; IRDM: Insulin Requiring Diabetes Mellitus; ITA: Islet Transplantation Alone; MMF: mycophenolate mofetil; SLI: Simultaneous Islet and Liver transplantation; T1DM: Type 1 Diabetes Mellitus.

Table 3. Clinical islet transplantation trials – Immune tolerance induction protocols.

<table>
<thead>
<tr>
<th>Period</th>
<th>Type of transplant</th>
<th>No.</th>
<th>Induction</th>
<th>Immunosuppression</th>
<th>Indication</th>
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<tbody>
<tr>
<td>1994-97</td>
<td>SIL + BMC</td>
<td>5</td>
<td>None</td>
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<td>SIL</td>
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<td>1994-96</td>
<td>IAK + BMC</td>
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<td>ALG (n=3)</td>
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<td>T1DM</td>
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<tr>
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<td>SIK + BMC</td>
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<td>OKT3 (n=5)</td>
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<td>1996-1998</td>
<td>SIK + BMC</td>
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<tr>
<td>1998-99</td>
<td>ITA + CD34+ BMC</td>
<td>5</td>
<td>Daclizumab</td>
<td>Tacrolimus</td>
<td>T1DM</td>
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<td>ATG</td>
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<td>Cyclosporine</td>
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<tr>
<td>2000</td>
<td>ITA + CD34+ BMC</td>
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<td>Sirolimus</td>
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tolerance test (OGTT) is also performed yearly after transplantation on those patients who are insulin independent (62).

**Clinical Trials**

Since 1985, a total of 101 allogeneic islet transplants have been performed in 73 patients at the CITP of the Diabetes Research Institute–University of Miami (Tables 2-3).

**Type 1 Diabetes Mellitus - Protocols Requiring Permanent Immunosuppression**

**1985-1989.** In our first clinical trial, patients received single donor allogeneic islet infusions as either IAK (n=3) or SIK (n=1) and were treated with cyclosporine (CyA; Neoral, Novartis; 8-12 mg/kg; target trough levels 100-300 ng/ml) and methylprednisolone (1.0 mg/kg tapered within one week to maintenance 0.1-0.2 mg/kg). Patients under azathioprine (AZA; Imuran, Prometheus Lab.; 2.0 mg/kg) for previous kidney transplant were converted to CyA before islet implant. In this trial, cadaveric

![Figure 3. Islet allograft function (measurable C-peptide) in patients with T1DM recipients per era.](image)

The outcome of islet transplantation protocols requiring chronic immunosuppression has steadily improved (A). In the first trial (1985-89, ○), early graft loss was observed possibly because the patients received a small islet mass and therapeutic levels of the immunosuppression were not always achieved. One of the patients received sequential islet infusions and maintained measurable C-peptide for 13.3 months, but lost graft function following modification of the immunosuppression treatment. In the 1990-93 trial (□), graft function was observed in all patients, and two of them had measurable C-peptide for over 12 and 14 years, respectively. Introduction of daclizumab (1998, △) resulted in sustained graft function (2.5 years) in a recipient of SIK with a marginal islet graft. Introduction of a steroid-free immunosuppression regimen with daclizumab and infliximab in ITA recipients dramatically improved graft function (2001-03, ◊).

Trials aiming at the induction of hematopoietic chimerism and donor-specific unresponsiveness were performed (B). No primary nonfunction was observed in the first trial that included the use of donor-derived whole BMC, calcineurin inhibitors and induction with ALG or OKT3 (1994-96, ■), but islet graft function was limited. In the first ITA trial, daclizumab induction with conventional levels of tacrolimus and MMF with CD34+ BMC were given (1988-99a, ●): one patient had primary nonfunction, while one other had sustained graft function until immunosuppression was discontinued. The use of CyA and MMF plus induction with ATG (1998-99b, ▲) showed only transient graft function. The use of the Edmonton style immunosuppression with infliximab and CD34+ BMC (2000, ○) resulted in measurable graft function in all patients. Two patients lost their graft due to sub-therapeutic sirolimus levels, while 4 patients had sustained graft function at one year, despite having received a single donor graft. However, discontinuation of immunosuppression resulted in graft loss within 3 months.
donor pancreata were either immersed in Ringer’s Lactate solution at 4°C for up to 6 hours, or preserved by hypothermic pulsatile perfusion with cryoprecipitated silica-treated human plasma for up to 12 hours prior to isolation (85, 138, 139). Islets were isolated by intraductal instillation of collagenase solution and mechanical dispersion by passages of the digest through a series of graded needles (62). Purification was obtained by repeated gravity sedimentation yielding final preparations comprising 20-40% islets in a volume of 5-6 mls. Prior to transplantation, isolated islets were cultured for ≤48 hours and pre-treated in vitro with anti-MHC class II monoclonal antibody in order to reduce immunogenicity. In this trial the percutaneous transhepatic catheterization technique for islet implant was utilized for the first time (62, 85). After transplantation, all patients showed measurable graft function assessed by basal and stimulated C-peptide levels (Fig. 3A).

One patient received two sequential islet infusions. The first graft led to measurable function with a 30% reduction in insulin requirements and decreased HbA1c (although not reaching normal ranges) from pre-transplant levels. A second islet infusion was performed 6 months after the first, and led to normalization of HbA1c levels, while insulin requirements remained stable. Graft function in this patient was sustained for 13.3 months from the first islet infusion. Three weeks after the second infusion, conversion from CyA to AZA was performed due to diagnosis of breast adenocarcinoma. Decreased basal and stimulated C-peptide were observed after the change in therapy, although insulin requirements remained lower than pre-transplant until C-peptide was undetectable.

One recipient of SIK showed basal C-peptide levels rising after transplant (0.64 pmol/ml at 3 weeks) although with blunted responses to Sustacal. This patient experienced two kidney rejection episodes (serum creatinine >1.5 mg/dl) 3 weeks after transplantation possibly related to inadequate trough levels of CyA that were treated with external irradiation of the kidney and two cycles of high doses of methylprednisolone (1,000 mg/day for 3 days). While serum creatinine improved after treatment, basal C-peptide declined after the first and second cycle of steroids (0.38 and 0.05 pmol/ml, respectively) and it was undetectable by 7 weeks post-transplant.

The other two patients lost function after 2 and 4 weeks from transplantation, respectively. It is noteworthy that therapeutic levels of cyclosporine were inconsistently obtained in these patients, a factor that may have contributed to the relative early loss of graft function in this trial. Subsequent to the loss of islet function, all patients returned to their pre-transplant insulin requirements. Survival of the kidney grafts persisted in all patients, despite loss of islet function.

1990-1993. A subsequent trial included eight patients (IAK, n=7; SIK, n=1) receiving allogeneic islets obtained from multiple donors to provide a large endocrine cells mass (infusion of 9,092-21,185 IEQ/kg, isolated from a total of 2-5 donor pancreata). Treatment consisted of induction with OKT3 antibody (Orthoclone OKT3, Orthobiotech) and maintenance immunosuppression with CyA, methylprednisolone, and AZA. Measurable C-peptide was observed after transplantation in all patients. Insulin independence was achieved in two patients for a period of 36 and 38 days, respectively. Six patients had measurable basal C-peptide for 1 (n=2), 4, 5, 11.8, and 25.2 months, respectively, while two patients had sustained graft function for >12 and >14 years, respectively (Fig. 3A).

One of the patients with longest graft function maintained good glycemic control with normalization of HbA1c (Fig. 4) and significantly reduced insulin requirements, when compared to pre-transplant doses. Remarkably, this patient did not experience any hypoglycemic episodes for >12 years, regardless of the need for exogenous insulin, while 3-4 severe hypoglycemic episodes a year were common prior to islet transplantation (18, 140). In spite of persistent basal C-peptide, the patient developed ESRD 10.9 years after receiving IAK. Immunosuppression was discontinued and dialysis initiated. The patient developed intra-cerebral hemorrhage and died at 12.5 years of follow-up.

The other patient with long-term function showed normalization of HbA1c paralleled by sustained basal C-peptide for the first two years after islet infusion (1). Although C-peptide was detectable for several years after IAK, the patient developed insulin resistance and difficulty to maintain HbA1c within the levels suggested by the DCCT (Fig. 4C)(18, 83, 84, 140). Detectable C-peptide with sustained kidney graft function was present for >14 years from IAK in this patient. Deterioration of the general health status at the long follow-up was noted. The patient died 14.4 years after islet transplantation of an unknown cause.

1998. In April 1998 we performed a SIK transplant in a 59-year old male patient with T1DM and ESRD. This
was the first islet transplantation trial to include an induction therapy with the anti-interleukin (IL)-2 receptor antibody daclizumab (1mg/kg; Zenapax, Roche). Maintenance immunosuppression included tacrolimus (Prograf, Fujisawa), mycophenolate mofetil (MMF; Cellcept, Roche) and methylprednisolone. The patient received both islet and kidney from the same allogeneic cadaveric donor. Measurable islet graft function was observed for up to 2.5 years (Fig. 3A), while the kidney graft continued functioning longer. Notably, the recipient had a BMI of 34 and received a suboptimal islet graft mass (2,464 IEQ/kg).

**2001-2005.** In light of the promising results of the Edmonton trial (141) and our own preliminary data with the Edmonton-style immunosuppression combined with BMC (see below), we initiated a clinical ITA trial in patients with brittle T1DM. At variance to the Edmonton protocol, isolated islets were cultured in CMRL-based supplemented medium (142-145) for <72 hours prior to transplant (Fig. 4). Induction therapy included a 5-dose course of daclizumab (1mg/kg) given biweekly from the day of transplant. Our protocol also included monthly (for the first year) and bimonthly (for the second year) administrations of daclizumab in 12 of the 16 patients. In addition, the anti-Tumor Necrosis Factor (TNF)-α monoclonal antibody infliximab (Remicade, Centocor Inc.) was introduced in this study in order to reduce the level of inflammation generated early after intra-hepatic implant and to favor islet engraftment (146-150). The recipients were therefore randomized to receive a single dose of infliximab: five patients received 5 mg/kg, and three 10 mg/kg given 2 hours prior to their first islet infusion. Maintenance therapy consisted of tacrolimus (target trough level 4-6ng/ml), and sirolimus (Rapamune, Wyeth Pharmaceuticals Inc.; target trough level 12-15 ng/ml for three months and 10-12ng/ml thereafter). Similar to the Edmonton protocol, sequential islet infusions were performed in order to achieve insulin independence (146-150).

Sixteen patients (7 males, 9 females; 41±10 year old; diabetes duration 27±13; BMI 25±1.7) were enrolled in the study. All patients showed a substantial reduction of exogenous insulin requirements and improved glycemic control after receiving the first islet graft (Fig. 5A). Two patients did not receive additional islet infusions due to adverse events (patient 1: aspiration pneumonia; patient 11: parvovirus infection). These patients were discontinued from immunosuppression and withdrawn from the study. Insulin independence was achieved in all 14 patients that remained in the study following a single (n=1)
or two (n=13) islet infusions (Fig. 5A-B). Insulin independence was maintained in 79% (11/14) of the recipients at one year follow-up (Fig. 5B), while patients that required reintroduction of exogenous insulin (at lower doses than baseline) showed sustained graft function (measurable basal and stimulated C-peptide, and normal HbA1c), with no hypoglycemia (Fig. 3A)(27, 28, 151). Assessment of cytotoxic lymphocyte gene expression in patients that required reintroduction of insulin showed a clear elevation (particularly of granzyme B) 25-203 days before hyperglycemia onset (152). Additionally, antidonor mixed lymphocyte reactions showed sensitization in patients who experienced partial graft loss (152). No cytomegalovirus infections or reactivation were observed even in the case of donor/recipient mismatch (153).

2002-2004. Our institution is part of the multi-center clinical trial sponsored by the Immune Tolerance Network (NIH – National Institute of Diabetes and Digestive and Kidney Diseases, NIDDK) to reproduce the Edmonton Protocol (27, 28, 151). Five patients were enrolled at our site to receive intra-hepatic allogeneic islets (ITA), induction with daclizumab, and maintenance immunosuppression based on sirolimus and tacrolimus. Sequential islet infusions were performed to achieve insulin independence in these patients. This ITA trial has shown that the Edmonton protocol could be successfully reproduced (27, 28, 151). Notably, centers with the longest experience in islet isolation and transplantation reported higher success rates than younger, inexperienced centers, highlighting the importance of acquiring the critical experience in islet cell processing, transplantation, and patient management in order to achieve consistently successful outcomes (154).

2003-2005. In 2003 we started an IAK transplantation trial in patients with T1DM consisting of sequential infusions of cultured allogeneic islets and an immunosuppressive protocol similar to the one described above for ITA (3, 61, 103, 126, 132, 133, 155-175): induction treatment with daclizumab with infliximab, sirolimus, and tacrolimus. Two patients also received maintenance steroids. All patients bearing a kidney graft and previously receiving different immunosuppressive protocols were
converted to sirolimus and tacrolimus prior to or at the time of islet transplantation. Seven IAK transplants using this protocol have been performed to date. Similar to what was observed in the ITA trial, all patients showed improved glycemic control with reduced insulin requirements after islet infusion. Insulin independence was achieved after a single infusion in two patients, after two sequential islet infusions in four patients, and one patient is currently waiting for a second infusion. Creatinine clearance remained stable in all patients, with the longest follow-up of over two years. Our preliminary data suggests that the Edmonton style of immunosuppression may be a viable option for patients with T1DM recipients of kidney grafts.

**Type 1 Diabetes - Tolerance-Induction Protocols**

The need for chronic immunosuppression in recipients of allogeneic tissues represents one of the major hurdles to the widespread use of organ and cellular transplantation. Most of the drugs currently utilized to prevent allograft rejection are not selective enough and are associated with untoward side effects (e.g., infections, organ toxicity, and neoplasm)(176) that limit their use to selected categories of patients in which the risk is justified. Definition of therapeutic protocols able to induce permanent acceptance of allogeneic grafts is a highly desirable goal pursued by the transplant community. Increasing evidence from experimental transplantation suggests that indefinite survival of allogeneic tissues including pancreatic islets can be obtained under selected treatments in animal models (92-94, 177). Furthermore, clinical evidence of indefinite graft survival in recipients of solid organ allografts associated with measurable donor leukocytes (microchimerism)(45, 46, 94-129, 132-134, 178-199) has opened the way to a number of clinical trials aiming at the induction of hematopoietic chimerism to favor acceptance of donor-specific tissues by the administration of BMC. Our Center has been involved in several clinical trials of solid organ (multi-visceral, liver, intestine, kidney, and heart) and islet transplantation with BMC (3, 132, 133). Induction of tolerance in recipients of islet allograft represents an appealing therapeutic approach (200-204). Notably, calcineurin-inhibitors and steroids are toxic to islet cells (3, 132, 133), and their chronic use may lead to loss of islet graft function. Induction of hematopoietic chimerism in patients with T1DM may also be beneficial to restore self tolerance and treat the underlying autoimmunity (3, 144, 145). Based on these premises, we have performed a number of trials in which donor-specific, non-fractioned or CD34+ BMC were infused in the recipients aiming at the induction of hematopoietic chimerism and acceptance of islet grafts.

**1994-1996.** The first trial including donor-specific BMC infusion was started in 1994 and included a total of eight patients. The protocol consisted of SIK (n=7) or IAK (n=1) transplantation. Islet grafts (14,800±7,152 IEQ/kg) were infused on day 0 (2 patients received islets from 2 donors), and non-fractioned donor-specific BMC (1x10⁹/kg) was infused on days 5 and 11. Immunosuppression consisted of an induction with either OKT3 (n=5) or ALG (n=3) and maintenance with triple immunosuppression (tacrolimus, methylprednisolone and AZA or MMF). Chimerism levels were detected by PCR-flow assay (97) in peripheral blood (7.1±1.4% at one month, and ~1% at 6 and 12 months). Loss of graft function was observed at a mean time of 142±53 days after implantation (Fig. 3B). One patient rejected both kidney and islet grafts after treatment with interferon (IFN)-α due to diagnosis of acute hepatitis C virus infection and died of complications secondary to the viral infection. Only the recipient of IAK had a kidney rejection episode (creatinine 2.5 mg/dl) 3 months after islet implant (4 years after kidney transplant), while all other patients maintained kidney function in the absence of rejection episodes. Six patients developed cytotoxic anti-donor MHC antibodies, but their presence did not correlate with loss of kidney function.

**1998.** The first ITA trial at our Center was started in September 1998. Transplantation of allogeneic islets on day 0 and infusion of donor-derived CD34+ BMC on days 5 and 11, was performed in patients with T1DM (n=5).

Three patients received induction therapy with daclizumab (biweekly for the first 5 doses and monthly for the first year) and maintenance therapy with tacrolimus, MMF, and methylprednisolone (Fig. 3B). One patient received 6,922 IEQ/kg and achieved stable graft function for over one year with measurable C-peptide (0.63±0.14 at one year vs. 0.2 ng/ml pre-transplant), ~40% reduction of insulin requirements and improved HbA1c levels (8.1±0.6% pre-transplant vs. 6.9±0.6%) at one year. Chimerism levels in this patient were 7.7% at one month, 3.6% at 6 months, and <1% at one year. Weaning from immunosuppression as per protocol in this patient resulted in loss of graft function. The second patient in this group received 10,536 IEQ/kg and showed
good function (C-peptide: 0.72±0.21ng/ml), but did not tolerate immunosuppressive drugs leading to graft loss 45 days after transplantation. The third patient showed no measurable C-peptide (i.e., primary non-function or graft failure) after transplantation of 5,774 IEQ/kg. Primary non function was associated with high basal serum TNF-a levels, which may have contributed to the failure to engraft (42, 44-46, 130, 131).

Two patients received induction with thymoglobulin (rabbit anti-human thymocyte globulin, ATG), and maintenance with CyA and MMF. One patient received 10,669 IEQ/kg and showed normalization of HbA1c to 6.4% and good islet graft function after transplantation (C-peptide: 0.92±0.26) for approximately 130 days, when loss of graft function occurred, possibly related to lack of therapeutic trough levels of the immunosuppressive drugs. Another patient in this group received 7,981 IEQ/kg and showed rapid reduction of insulin requirements and good graft function (C-peptide: 0.52±0.46). Following the second BMC infusion the patient developed serum sickness syndrome secondary to ALG treatment. Hospitalization and discontinuation of immunosuppression was required, which resulted in complete recovery from symptoms but loss of graft function by day 21.

Microchimerism was detected in all patients in this study in both treatment groups. No episodes of graft versus host disease were observed. All patients returned to pre-transplant insulin requirements after discontinuing immunosuppression.

2000. In 2000 an ITA trial with Edmonton style immunosuppression and donor-specific BMC was started. Patients with brittle T1DM and hypoglycemia unawareness received a single intra-hepatic islet infusion on day 0, followed by two intravenous donor-specific CD34+ BMC inoculum on days 5 and 11 post-transplant (n=5). One patient received a single infusion of non-fractioned BMC. Induction treatment consisted of daclizumab and a single dose of the anti-tumor necrosis factor (TNF)-α monoclonal antibody infliximab, followed by tacrolimus and sirolimus maintenance. Endpoints of the study included (i) induction of hematopoietic chimerism that was assessed at monthly intervals, and (ii) acceptance of donor-specific islets after discontinuation of immunosuppressive drugs that were gradually tapered in the patients with a functional islet graft at one year. All patients showed measurable islet graft function after implant (8,629±2,102 IEQ/kg)(Fig. 3B). Two patients lost islet graft function at 141 and 143 days post-transplantation, respectively, possibly due to the sub-therapeutic sirolimus trough levels achieved. Four patients (9,029±2,589 IEQ/kg) maintained sustained islet graft function for up to one year, showing maximal mean C-peptide levels at 3 months (1.06±0.23ng/ml), and 68±26% reduction in insulin requirements from baseline. Transient insulin independence was achieved in three patients (5, 17 and 50 days, respectively). These four patients with sustained graft function at one year underwent weaning from immunosuppression (Fig. 3B), as per protocol. Only a transient hematopoietic chimerism was observed in these patients in peripheral blood, dropping from 4.6±0.5% at one month after ITA (n=6) to 0.14±0.05% at one year. After discontinuation of immunosuppression, all four patients invariably lost islet graft function with a mean time of 95±25 days (range, 74-131).

Allogeneic Islet Transplantation for Insulin Requiring Diabetes

1994-1997. A trial of simultaneous allogeneic islet and liver (SIL) transplantation was performed in eight patients with IRDM consequent to hepatic cirrhosis (42, 44-46, 130, 131). Five out of eight patients also received donor-specific BMC. Immunosuppression consisted of tacrolimus and steroids. A significant reduction of insulin requirements was observed in most patients with normalization of HbA1c and insulin independence in three patients. One patient resumed insulin treatment 5 months after transplant, while the other 2 remained insulin independent for over 17 months (42, 44). Three patients experienced mild liver rejection episodes that resolved after treatment with MMF and steroids. The proportion of rejection episodes observed was comparable to that of patients receiving orthotopic liver grafts and BMC without islets. Importantly, islet transplantation did not have any negative effects on liver function in this series. Furthermore, the results of this pilot trial demonstrated the safety of intra-hepatic islet cell transplantation in recipients of orthotopic liver for cirrhosis, and that inclusion of pancreatic islets can improve metabolic control and reduce insulin requirements in patients with liver cirrhosis (42, 44).

1996-1998. A pilot trial of SIK transplantation in patients with T2DM has been performed at our center in 3 patients. The characteristics of this category of patients with residual C-peptide pose some concerns regarding enrollment in islet transplantation trials, and care should be paid to the recipient body weight, age, and
insulin resistance in order to assess the potential benefits of an islet graft (205).

DISCUSSION

Unprecedented results have been reported in the field of islet transplantation in recent years, which are the fruit of a steep learning curve and steady progress (Fig. 6). Each clinical trial performed at our center since 1985 has been instrumental to acquire critical knowledge and to help to design subsequent protocols (Fig. 3).

Our first clinical trial of allogeneic islet transplantation into patients with T1DM showed that competent islets could be obtained from human pancreata that had been preserved in cold slush or hypothermic pulsatile perfusion ex vivo and demonstrated for the first time the feasibility and safety of the percutaneous tranhepatic catheterization of the portal vein for islet infusion without the need for general anesthesia (62, 85). The islet mass transplanted may have contributed to the partial success observed in this series, possibly consequent to relative inefficiency of isolation and purification procedures, and to the lack of standardized methods for the assessment of islet quality and numbers. Indeed, the use of sequential infusions, similar to what observed in recent trials (26, 29-31), resulted beneficial in one patient and lead to substantial improvement in glycemic control as long as therapeutic levels of the immunosuppressive drugs were maintained.

In the subsequent trial, islets were isolated using the automated method (53), and each patient received islet preparations obtained from multiple donors in order to provide a sufficient islet mass (18, 84). In this protocol, induction therapy with the T-cell depleting OKT3 antibody was given. The overall outcome in this trial was better than the previous one, as it permitted the achievement of sustained graft function (6 patients at 4 months, 4 patients at 11 months, 3 at 25 months, and two patients with measurable C-peptide for >14 years). The results observed in the 2 patients with extended graft function demonstrated that therapies able to prevent rejection and (possibly) recurrence of autoimmunity in patients with T1DM may allow for indefinite survival and function of allogeneic islets in an ectopic site. An important message from our data is that the beneficial impact of restoring β-cell function can be achieved in patients with T1DM even when partial function of the implanted islets is obtained, as it resulted in sustained improved glycemic control and prevention of severe hypoglycemia despite the use of exogenous insulin (26).

In 1998 we introduced the anti-IL-2R antibody daclizumab as induction therapy in combination with tacrolimus and MMF in a SIK recipient that showed improved metabolic control and measurable C-peptide for 2.5 years despite receiving a marginal islet mass. The results of this pilot trial demonstrated the promising properties of daclizumab as an inducing agent for islet cell transplantation, although the combination of maintenance therapy drugs including steroids and the marginal size of the graft may have limited the overall success.

While exploring protocols aiming at chronic immunosuppression in patients with T1DM recipients of islet allografts, we have been also strongly pursuing alternative avenues for the induction of indefinite graft survival based on the use of donor-derived BMC. Our first protocol showed that microchimerism levels could be obtained after infusion of donor BMC, but that the use of standard immunosuppression, dictated by the combination with a kidney graft, did not suffice to sustain long-term islet survival, while kidney function was maintained after islet rejection. In a subsequent trial, we performed ITA with CD34+ BMC using daclizumab or ALG as induction, and calcineurin inhibitors with MMF as maintenance therapy. Only one patient maintained a functional graft for over one year, and weaning from immunosuppression resulted in graft loss to rejection. The use of the immunosuppressive protocol in this trial appeared inadequate to favor islet graft survival and BMC engraftment.

The availability of more potent immunosuppressive drugs with potential immunomodulatory effects permissive to tolerance induction such as sirolimus (206-208), and the success of the Edmonton style of immunosuppression in ITA (26, 29-31) prompted us to test this approach with a protocol aiming at the induction of tolerance. Patients in this trial received a single donor ITA with donor-specific CD34+ BMC, induction with daclizumab and maintenance with sirolimus and tacrolimus. The use of the anti-TNF-α antibody infliximab was introduced in this trial based on the observation that primary non-function of grafted islets in a patient from a previous trial was associated with high serum levels of this pro-inflammatory cytokine. Interestingly, primary non-function was not observed in this series, and all grafts functioned after implantation. Graft function was lost within 5 months in 2 patients due to sub-therapeutic trough levels of sirolimus. Four patients showed graft function at one year after transplantation and were there-
fore weaned from immunosuppression. After discontinuation of the immunosuppressive drugs they showed graft function for an additional 3 months. The results obtained in this trial showed that improved and sustained metabolic control can be obtained with a single islet infusion using this protocol, although without achieving permanent graft survival. This trial also demonstrated the efficacy of sirolimus administration for the success of the treatment, and the importance of closely monitoring and maintaining therapeutic trough levels of this drug. Future trials using alternative drug combinations are currently being evaluated in order to improve the success of tolerance-inducing protocols for islet transplantation.

The Edmonton style of immunosuppression has allowed for the achievement of unprecedented success rates in ITA for patients with T1DM, with a high proportion of recipients achieving insulin independence at one year after sequential islet infusions, or maintaining good metabolic control with insulin requirements much lower than baseline. We have confirmed such results in our ITA series and extended it to patients with IAK grafts.

**Current Challenges and Future Directions**

A prerequisite for the achievement of insulin-independence after islet transplantation is that a sufficient islet mass is implanted (145), which is generally obtained by the infusions of more than one islet preparation (sequential or pooled). Improvements in organ harvesting and preservation techniques have contributed substantially to render available a higher number of human pancreata suitable for islet isolation and transplantation, although underutilization of cadaveric donor pancreata for transplantation has been recognized (2, 6).

It is noteworthy that, despite the improved efficiency of isolation procedures and the introduction of cGMP standards and rigorous quality controls for product release criteria, islet preparations with comparable quality and numbers may result in different outcomes after transplantation, with partial function and primary non-function being not an uncommon problem (81). Multiple factors may contribute to this phenomenon and to the requirement of high islet numbers observed in islet recipients that are not explained solely by immunological mechanisms (144). Islet cells are particularly vulnerable to hypoxia and oxidative stress to which they are exposed as

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**Figure 6. Steady progress leading to improved success rate of islet isolation and transplantation.**

Numerous technological improvements have been introduced at our center in the recent years that have contributed to the steadily increasing success rate of islet transplantation. Percutaneous transhepatic catheterization of the portal vein (65), utilized for the first time in 1985, allowed performing the transplant procedure without the need for general anesthesia and surgery. The automated method for islet isolation introduced in 1988 (86) utilizes a dissociation chamber that allows for the recovery of higher islet yields from donor pancreas. The use of dithizone, a dye that stains red the secretory granules in islet cells was introduced in 1988 for the assessment of islet preparations (purity, granularity, and enumeration)(59, 60). In 1990 we started using a close system (the ‘bag method’) for the infusion of islets by gravity, which allows for controlled rate of infusion and reduced portal pressure rise (54, 55). The introduction of the semiautomatic COBE system in 1990 has allowed improving the efficiency of purification of large volumes of tissue from human pancreata (51). The use of the new enzyme blend Liberase (1997) with reduced endotoxin contamination and more consistent characteristics has contributed to further increase islet yields (152). In 1998 the anti-IL-2 receptor antibody daclizumab was utilized at our center for the first time as induction therapy in a recipient of pancreatic islets. In 2000 we implemented the use of anti-TNFα antibody treatment of islet recipients to favor engraftment. In 2001 we showed that the use of the two layer method for pancreas preservation results in improved islet yields even from marginal donors (64) which may be of assistance to increase the donor pool. Detection of cytotoxic lymphocyte markers correlates with graft dysfunction in islet graft recipients (2004) and may be of assistance to implement anti-rejection treatments to preserve islet mass (81). Utilization of an additional purification step for the less pure fractions obtained after isolation allowed us to maximize the utilization of islet preparations for transplantation at our center (2005), as more viable and potent islets could be recovered to meet the requirements for transplantation. Development of more sensitive tests for the assessment of human islets and predictive of the quality and potency may be of assistance to identify suboptimal preparations that should not be transplanted.
early as the time of donor brain death (209). They are subjected to hypoxia and oxidative stress throughout organ procurement and preservation, islet isolation, purification and culture, possibly reducing the number of viable and/or functional islet cells at the time of transplantation (70, 210). We appreciate the intrinsic limitations of the current methods utilized for the assessment of islet quality and potency prior to transplantation that may not allow for the identification of suboptimal islet preparations that should not be transplanted (80, 81). There is an urgent need for the development of sensitive tests to assess the quality of donor pancreata and of the islet preparations. To this aim, several tests are currently under evaluation, including flow cytometry quantification of mitochondrial membrane potential in islet cell subsets (81, 211), measurement of oxygen consumption rates of islets under selected stimuli (212), signal transduction (80, 210), and quantification of cell subsets in the islet preparations (81, 213). In order to address these issues and improve the quality of human islets for transplantation, the National Center for Research Resources and NIDDK from the NIH, the Department of Health and Human Resources and the Juvenile Diabetes Research Foundation International have sponsored a consortium of 10 Islet Cell Resources (ICR) Centers established in 2002 that promote the interaction between islet processing centers (144, 145).

The liver is currently considered the site of choice for islet grafts. The availability of interventional radiology techniques to perform intra-portal islet infusion by a minimally-invasive, outpatient procedure has made possible repeated infusions without the need for open surgery (85, 86, 139). Non-specific inflammation generated in the hepatic microenvironment upon islet implant has been recognized as one of the major contributing factors to the loss of viable and functional islet mass (214, 215). Islets produce mediators of inflammation (CD40, cytokines and chemokines) (143, 216) and coagulation (e.g., tissue factor) (144, 217-223) that may lead to hepatic microenvironment and macrophage activation, endothelial cell dysfunction, and release of reactive oxygen species. All these factors may contribute to reduce islet engraftment (77, 219, 224-229).

Strategies that may be of assistance to reduce the needs for large islet numbers to reverse diabetes after transplantation include delivery of cytoprotective molecules to islet cells during all critical steps (before, during, and after isolation) (141, 221, 222, 230-239), and implementation of treatments targeting coagulation and non-specific inflammation during the peri-transplant period (27, 28, 240). Notably, prevention of early inflammation in the peri-transplant period may also be beneficial to reduce triggering of specific allogeneic and autoimmune responses that affect the overall survival of islet grafts (176). Alternative sites for islet implantation and bioartificial devices are currently under evaluation as they may provide better engraftment and easier access for biopsies (241).

The introduction of more effective immunosuppressive protocols has been a key component of the recent success of allogeneic islet transplantation for the treatment of T1DM. However, the long-term effects of such drugs have not yet been elucidated in the islet transplantation setting. The recipients of ITA have brittle T1DM and are otherwise in good general health and, therefore, a careful assessment of the risk/benefit ratio should be considered before enrollment in clinical trials. Increasing evidence coming from the recent ITA trials suggests that achievement of relatively high trough levels of sirolimus is a prerequisite for sustained islet graft survival (242) posing some concerns for lipid homeostasis and other possible untoward side effects of the use of this drug (176, 243, 244). The use of calcineurin-inhibitors in the ongoing clinical trials is problematic due to the well-known nephrotoxicity (203, 245, 246) that may be enhanced by their combination with sirolimus (160, 161, 247, 248). The hepatic site for implantation of islet grafts may expose the islets to high concentrations of drug metabolites (249) that may exacerbate their toxic effects to islet cells (3, 249, 250) and lead to gradual loss of function. Interestingly, preclinical allogeneic islet transplantation studies in pancreatectomized non-human primates have shown improved islet function with time using a non-diabetogenic therapy based on anti-CD154 antibody, suggesting that selective targeting of the immune response with non-toxic reagents is a desirable goal to pursue (3, 132, 133, 160, 161, 163, 250-253). As new reagents with higher selectivity for immune cells and lower toxicity are becoming available for clinical use, we anticipate improved results in the near future (3, 152, 254-256). The emphasis on this issue has prompted important initiatives to promote the study of novel immunosuppressive protocols for clinical islet transplantation including the Immune Tolerance Network (47, 48), and
the more recent Consortium of Islet Transplant Centers sponsored by the NIH, the NIDDK and the National Institute of Allergy and Infectious Diseases. In order to benefit the large number of patients with T1DM awaiting replacement of islet cells, the ultimate goal remains the induction of permanent acceptance of the grafted islets without the need for chronic immunosuppression. Previous clinical trials aiming at the induction of donor-specific tolerance by the use of BMC transplantation have failed so far to provide indefinite islet graft survival after discontinuation of immunosuppression, possibly due to the inadequacy of the drugs utilized in these protocols to favor stable hematopoietic chimerism. Very promising data are emerging from the translational research focusing on the definition of novel protocols for the induction of tolerance (to self and allogeic antigens) that justify a cautious optimism (257, 258).

Development of sensitive analytical tests for the post-transplant immune monitoring of our patients will provide a better understanding of the complex phenomena involved in the immune response to islet allografts in patients with T1DM (85). These tests are of paramount importance not only to help us recognize rejection and recurrence of autoimmunity early enough to intervene and prevent islet graft loss, but also to identify patients eligible for discontinuation of immunosuppression in protocols aiming at the induction of tolerance.

While future trials will allow us to define optimal protocols for islet grafts, the shortage of deceased donor pancreata available for islet transplantation will be a limiting factor to a wider application of this procedure in clinical practice. Introduction of better organ procurement and preservation techniques has been instrumental to improve the utilization of the available pancreata, and the use of marginal and non-heart beating donors has been also proposed to enlarge the donor pool. However, there is still a window of opportunity to improve the utilization of cadaveric pancreata for transplantation. The quality of the deceased donor pancreas influences the yields and potency of islets that can be recovered after processing. Unfortunately, availability of suboptimal pancreata to the CITP’s has limited substantially the success rate of islet cell processing (close to ~50% in experienced centers) with remarkable economic and resource burden. Implementation of fair organ allocation rules between whole pancreas and islet cell transplant programs will contribute to a better utilization of both deceased donor pancreata and resources. Alternative sources for insulin-producing tissue for transplantation (xenogeneic and stem cell-derived) are potential avenues to overcome the shortage of deceased donor pancreata in the years to come (53).

The FDA regulates islet transplantation under investigational new drug (IND) application toward a Biologics License Application for pancreatic islets. For this reason, specific infrastructures and dedicated personnel are required along with the implementation of cGMP and SOP to warrant high quality standard of cell manufacturing for transplantation. These requirements impose a remarkable economic burden to the CITP, and the elevated costs of establishing a functional new facility have contributed to the emerging concept of creating ‘regional’ Human Islet Cell Processing Facilities that provide support to remote CITP’s as a viable option to grant consistent quality of the final product and contain the costs (30, 31, 49, 82).

Monitoring of graft function is currently obtained by the means of metabolic tests and assessment of glycemic control, C-peptide and insulin secretion during metabolic challenge. There is no consensus in the islet transplant community on which test should represent the gold-standard for assessing islet mass and function, possibly because most of the proposed tests have been designed for other purposes. There is, therefore, an increasing need for the definition of metabolic tests specific for islet grafts that will be essential for a better assessment of graft performance.
SUMMARY

Transplantation of allogeneic pancreatic islets for the treatment of patients with Type 1 diabetes mellitus (T1DM) is now a reality. The steady progress that has allowed for the recent successful clinical trials worldwide follows a steep learning curve and the perseverance of the international islet transplantation community. The Clinical Islet Transplant Program at the Diabetes Research Institute – University of Miami has contributed to the progress in the field with a 20-year track record. It has been a long journey and despite the intermediate success, more work is needed in order to achieve the ultimate goal of a safe and long-lasting treatment for patients with T1DM.

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