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REVIEW ARTICLE

Jonathan R.T. Lakey
Philip W. Burridge
A.M. James Shapiro

Technical aspects of islet preparation and transplantation

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J.R.T. Lakey · P.W. Burridge
Surgical–Medical Research Institute,
1074 Dentistry/Pharmacy Centre,
Edmonton, Alberta, T6G 2N8, Canada
E-mail: jonathan.lakey@ualberta.ca
Tel.: +1-780-492-3074
Fax: +1-780-492-6335

J.R.T. Lakey (✉) · P.W. Burridge
Clinical Islet Transplant Program,
University of Alberta, Edmonton,
Alberta, Canada

J.R.T. Lakey · A.M.J. Shapiro
Department of Surgery,
Faculty of Medicine,
University of Alberta,
Alberta, Edmonton, Canada

Abstract The introduction of insulin therapy for the management of diabetes mellitus is arguably the greatest milestone in the history of modern medicine. β-cell replacement therapy is the only treatment that reestablishes and maintains long-term physiological normoglycemia. Until recently, successful clinical outcomes of pancreas transplantation for patients with long-standing diabetes were much superior to that of islet transplantation. Significant advances in islet isolation and purification technology, the development of more specific and less diabetogenic immunosuppressants and the prophylactic administration of antiviral agents have rekindled a worldwide interest in islet transplantation. This chapter will review the rationale of islet transplantation and the development of islet isolation and purification. The challenges facing clinical islet transplantation in the twenty-first century will also be introduced.

Keywords Islet · Pancreas · Diabetes · Complications · Isolation · Collagenase · Immunosuppression

Introduction

Diabetes mellitus is a clinical disorder of intermediary metabolism characterized by hyperglycemia and glucosuria due to the inadequate secretion and/or utilization of insulin. Defects in lipid and protein metabolism are also present. Insulin-dependent diabetes mellitus (IDDM) is caused by the progressive destruction of the β-cells in the islets of Langerhans [1]. The loss of greater than 90% of the β-cell mass, which is triggered by unknown environmental factors and mediated by a cell-selective autoimmune reactivity, condemns genetically predisposed individuals to a lifelong dependence on insulin therapy [2].

The Diabetes Control and Complications Trial (DCCT) demonstrated unequivocally that early tight glycemic control lowered but did not normalize glycated hemoglobin (HbA_{1c}) and significantly delayed the progression of microvascular complications [3, 4]. Similar evidence was also provided by the United Kingdom Prospective Diabetes Study (UKPDS) Group of type 2 diabetics [5]. Intensive glycemic control (3 or more insulin injections per day or the use of an insulin pump) is accomplished by frequent self-monitoring of capillary blood glucose using skin-puncture sampling and analysis with a portable glucose monitor. The penalty for this optimal metabolic control was a three-fold increase in severe hypoglycemia (despite 4 or more tests per day),

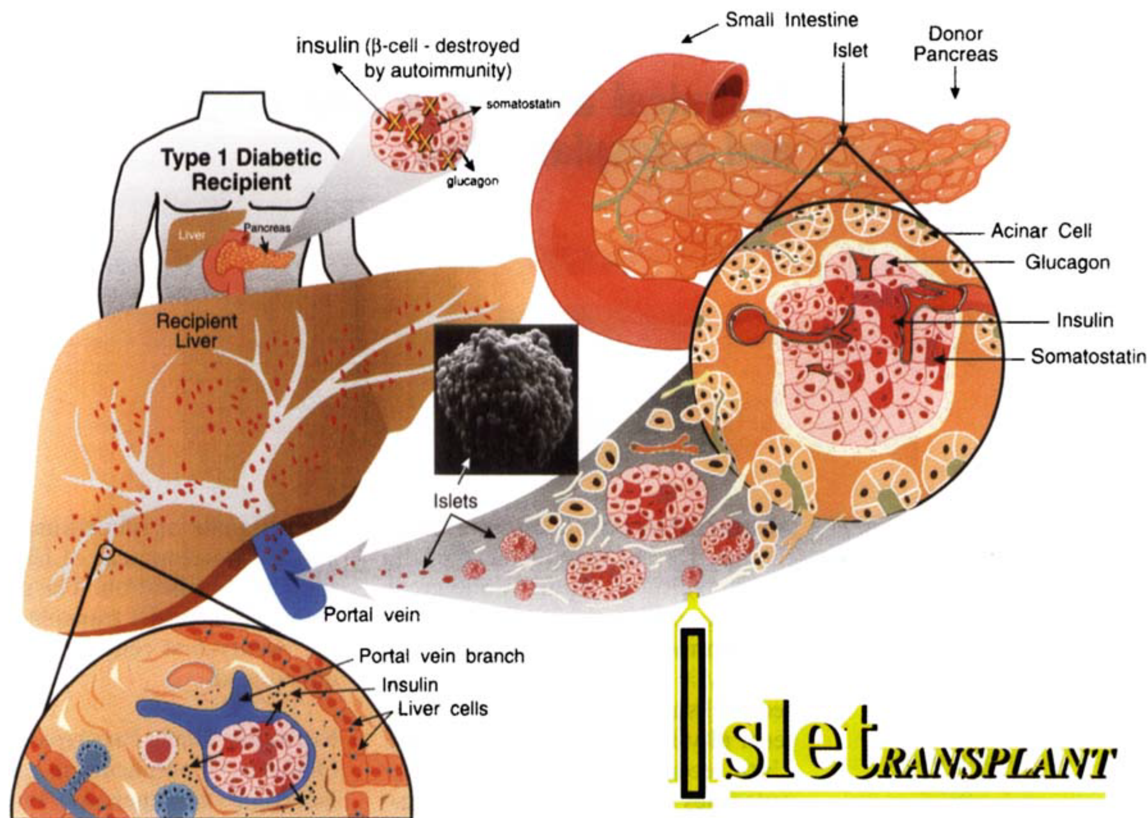

Fig. 1 Islet transplant

thereby prompting researchers to find better methods to restore physiological, moment-by-moment control of blood glucose.

Transplantation of insulin-producing tissue is the only treatment that consistently restores normoglycemia and maintains long-term glucose homeostasis [6]. Simultaneous pancreas and kidney transplantation (SPK) for end-stage renal failure is the standard therapy for carefully selected patients with longstanding diabetes [7]. Near-perfect glycemic control and the elimination of diurnal variation in blood glucose can prevent, stabilize and even reverse some secondary complications of diabetes [8, 9]. Although pancreas transplantation is associated with insulin independence in > 80% of patients, it is nonetheless a complicated procedure with significant peri-operative morbidity and mortality. On the other hand, islet transplantation (Fig. 1) with its reduced antigen load, technical simplicity and low morbidity has the potential to restore glucose homeostasis and prevent long-term complications.

Historical background

In 1889 von Mering and Minkowski discovered the vital link between the pancreas and diabetes when they observed hyperglycemia and glucosuria in a pancreatectomized dog [10]. The first clinical attempt to transplant the pancreas was performed 5 years later at the Bristol Royal Infirmary in England. Williams buried three pieces of freshly slaughtered sheep's pancreas, "each about the size of a Brazil nut," under the skin of a 13-year old boy dying of diabetic ketoacidosis [11]. Although there was temporary improvement in glucosuria before his death three days later, the xenograft was destined to fail without immunosuppression. This was a most remarkable feat when one realizes that the existence of the immune system had yet to be discovered and that modern anti-rejection therapy would not become a reality for more than 60 years. The concept was not new, however, for Minkowski had successfully transplanted autologous pancreatic fragments in a pancreatectomized dog a year earlier [12].

In 1902, Ssobolew proposed transplanting only the endocrine tissue, but this approach would be ignored for more than half a century [13]. In 1916 Pybus of Newcastle-on-Tyne reported a modest reduction in glucose excretion in one of two diabetics implanted with fragments of human cadaveric pancreas [14]. Four years later at the University of Toronto, Banting conceived the idea of 'isletin' (from the Latin for 'island' and later known as 'insulin') after reading Moses Barron's treatise

'The Relation of the Islets of Langerhans to Diabetes with Special Reference to Cases of Pancreatic Lithiasis' [15]. He abandoned his original idea of transplanting the pancreas for the treatment of diabetes and concentrated his efforts on recovering the "internal secretions" [22]. Independent pioneering studies by Paulesco of Romania [16] and others [17, 18, 19, 20, 21] would culminate in 1922 when Banting and Best reported the first successful reversal of hyperglycemia in a gravely ill 14 year-old boy treated with bovine pancreatic extract [23]. Further studies by Banting, Best, Collip and Macleod quickly lead to the introduction of insulin into clinical practice [23, 24]. By 1923 Connaught Laboratories and Eli Lilly were mass-producing unlimited quantities of purified insulin, thereby transforming diabetes from a disease with a virtual death sentence following the onset of ketoacidosis to that of a chronic incurable illness with significant morbidity and premature death [25].

Interest in pancreas transplantation was revived in the 1930s when it became obvious that insulin therapy did not prevent the progression of chronic complications (renal failure, blindness, heart disease, neuropathy and atherosclerosis) [26, 27, 28, 29, 30]. Although it was apparent early in the 20th century that the islets of Langerhans were responsible for regulating carbohydrate metabolism through the synthesis and release of insulin, glucagon and other humoral agents, almost 100 years would pass before it was possible to produce sufficient quantities of high quality human islets for experimental and clinical islet transplantation.

Islet transplantation

Activity in clinical islet transplantation can be subdivided into five categories: (1) islet autografts in patients undergoing total or near-total pancreatectomy, (2) islet allografts in patients after total pancreatectomy, (3) islet allografts in type 1 diabetic patients, (4) fetal islet allografts or xenografts in type 1 diabetics, and (5) islet allografts in type 2 diabetics. Success can be defined in terms of patient survival, graft survival (C-peptide >0.5 ng/ml), attainment of insulin independence, effect upon glycemic control (glycosylated $HbA_{,C}$ $<8\%$), overall quality of life, and impact upon secondary diabetic complications.

Early efforts of islet transplantation

The first reports of successful islet transplantation in diabetic rats were published in 1973 [31, 32]. Four years later Najarian et al. at the University of Minnesota performed the first clinical islet allotransplants in seven insulin-dependent diabetics undercover of azathioprine and corticosteroid therapy [33]. Many researchers naively believed that islet transplantation would replace vascularized pancreas transplantation, which at that time was associated with dismal morbidity and mortality rates [34]. However, while initial attempts appeared to be safe, dispersed pancreatic tissue implanted in the peritoneal cavity or embolized into the liver via the portal vein was largely ineffective. None of the patients achieved insulin independence but some were able to reduce insulin requirements for limited periods. In 1978 Largiader et al. of Zurich reported the first C-peptide negative type 1 diabetic to achieve sustained insulin independence at one year after simultaneous kidney transplant and intrasplenic infusion of non-purified tissue from a single donor [35]. While many different sites have been tried for human islet transplantation, the optimal site appears to be the liver. Attempts to embolize human islets to the spleen have resulted in significant life-threatening complications of splenic infarction, rupture and gastric perforation [36].

Islet autografts in type 1 diabetes mellitus

The first islet autotransplant was performed by Najarian et al. at the University of Minnesota in 1977 [37]. In 1992 Pyzdrowski et al. reported a small well-documented series in which all recipients became insulin independent after islet autotransplantation [38]. Liver biopsies confirmed the presence of functional intrahepatic islets that stained positive for insulin, glucagon and somatostatin but not pancreatic polypeptide. Intrahepatic insulin and glucagon secretion in response to arginine stimulation was detected on hepatic vein catheterization. During the last 25 years, more than 240 autotransplants have been performed worldwide [39]. Most patients had undergone total or near-total pancreatectomy for intractable pain and failure to thrive secondary to small duct chronic pancreatitis. Oberholzer et al. have extended the indication for islet autotransplantation to include extensive ($>80\%$) pancreatectomy for benign tumors of the pancreas [40].

Most centers use non-purified pancreatic homogenates for autotransplantation. Grafts scarred by chronic pancreatitis usually yield low tissue volumes, typically <7–10 ml. Consequently, any further purification of an already marginal islet mass can render the exercise futile. While complications of portal vein thrombosis, disseminated intravascular coagulopathy and fatality have been reported following islet autotransplantation, the risks have been minimized in recent years by systemic heparinization and better characterization of the dispersed grafts [41, 42].

An analysis of only the well-documented cases reported to the International Islet Transplant Registry (IITR) as of December 31, 2000 indicated that 64% of patients with islet autografts were insulin independent for at least one week, and 47% were able to maintain

insulin independence beyond one year. The longest period of insulin independence follow-up after autotransplantation is > 13 years [43]. The best predictor of insulin independence in the autograft setting is the number of islets transplanted, with a transplant mass > 300,000 IE associated with an insulin independence rate of 71% at two years post-transplant [44]. Farney et al. reported a series of 29 intrahepatic autografts with a maximum follow-up > 12 years. About 21% of patients lost graft function between 3 and 24 months when a median of 148,000 islets was transplanted. There were no late graft failures beyond 2 years if a median of 384,500 islets were transplanted [45]. These studies established beyond all doubt that insulin independence following islet transplantation was possible.

Islet allografts in type 1 diabetes mellitus

Of the 237 adult islet allotransplants reported to the IITR as of December 31, 2000, < 12% of recipients were insulin-free at one-year post-transplant, although 41% of grafts remained C-peptide positive [39]. The longest period of insulin independence follow-up after allotransplantation is > 70 months. Most recipients were treated with a regimen of either anti-lymphocyte globulin (ALG) or anti-thymocyte globulin (ATG) in combination with other anti-rejection agents i.e. cyclosporine, azathioprine, glucocorticoids. The majority of these grafts were combined islet-kidney transplants, since it was felt to be inappropriate to initiate immunosuppression in islet-alone recipients who otherwise would not have required therapy to sustain a kidney or liver graft. An islet mass > 6,000 IE per kilogram recipient body weight is generally required to achieve insulin independence [39]. At least 16,000 IE are required to reduce overall insulin requirements by one unit. (unpublished data)

These results were in sharp contrast to the remarkable success of islet autotransplantation. There were however, two notable exceptions. In 1990 Tzakis et al. at the University of Pittsburgh reported a series of nine non-diabetic patients undergoing abdominal exenteration with multi-visceral resection for primary or secondary hepato-biliary malignancies followed by simultaneous islet and cluster transplantation of liver, kidney and bowel [46]. In 1992 Ricordi et al. completed a series of 22 cluster-islet allotransplants. The islets were isolated from a single multi-visceral donor pancreas in most cases and implanted in the liver via the portal vein after reperfusion. More than 50% of recipients in each of these studies were able to achieve and maintain insulin independence before succumbing to recurrent metastatic disease [47]. These studies provided a unique opportunity to transplant islet allografts in the absence of an autoimmune background, which no doubt contributed to the preservation of the functional reserve of these grafts [46, 47]. Other major factors contributing to the success of the cluster-islet transplant included the embolization of partially purified islet preparations and the use of steroid-free immunosuppression (high-dose tacrolimus monotherapy).

By the late 1990s, controlled pancreas distension with low-endotoxin Liberase (Boehringer Mannheim, Indianapolis, IN), the introduction of the Ricordi chamber, and the COBE continuous purification system contributed significantly to the manufacture of high-yield islet preparations suitable for clinical transplantation [48, 49, 50, 51]. Studies from Milan and Giessen reported that almost 50% of recipients treated with cyclosporine, glucocorticoid and mycophenolate mofetil (MMF)-based regimens were insulin-free at one-year post-transplant [52, 53].

The IITR data clearly demonstrated that many patients were unable to achieve or maintain insulin independence because: (1) the islet implant mass was subtherapeutic (< 6,000 IE/kg), (2) a high proportion of the islets failed to engraft, (3) the islets were damaged by direct, local toxic effects of the immunosuppressants, and (4) ineffective immunosuppression failed to prevent acute or chronic rejection, or the recurrence of autoimmune diabetes [39, 54, 55, 56]. About 20–50% of the implanted islet mass can be destroyed by apoptosis and other non-immune inflammatory pathways, including rapid non-specific blood-mediated platelet binding and activation [57, 58].

A major breakthrough in clinical islet transplantation was reported in the July 27th 2000 issue of the New England Journal of Medicine. Shapiro et al. introduced the "Edmonton Protocol," a glucocorticoid-free immunosuppression regimen combined with the titrated delivery of an optimal islet engraftment mass [58]. The novel cocktail of daclizumab (anti-interleukin-2 receptor antibody), low-dose tacrolimus and sirolimus counteracts the dual forces of autoimmune recurrence and allograft rejection after islet transplantation [59, 61]. Consequently, the one-year rate of insulin independence in seven consecutive patients who had received sequential islet-only grafts rose dramatically to 100% [58]. This trial demonstrated for the first time in the history of clinical islet transplantation that long-term islet function and insulin independence could be achieved with results comparable to that of pancreas transplantation. Immediate graft processing and expeditious transplantation further optimized islet function by limiting prolonged cold ischemia (< 20 min), avoiding culture and cryopreservation, and eliminating exposure to xenoproteins (fetal calf serum). Subsequent followup of the initial and expanded cohort treated with the Edmonton protocol indicated that insulin independence could be maintained,

and that the therapy was generally safe and well tolerated [39, 40].

Evolution of methods of islet isolation

The adult human pancreas weighs about 50 grams and contains about 1 million islets, constituting 1–4% of the mass of the pancreas [20, 62]. Modern islet research began in 1911 when Bensley handpicked guinea pig islets for morphological study from pancreatic tissue stained with neutral red [63]. In 1964 Hellerström meticulously micro-dissected islets from the pancreas of obese hyperglycemic mice for biochemical and physiological study [64]. The first major development in islet isolation occurred three years later when Moskalewski introduced a mechanical and enzymatic method of dispersing guinea pig pancreatic tissue with collagenase, a fermentation product derived from Clostridium histolyticum [65]. Although the enzyme produced widespread destruction, it did permit complete separation of the islets from the surrounding exocrine tissue. In 1967 Lacy and Kostianovsky substantially modified Moskalewski's technique to isolate rat islets [66]. Their method involved distending the pancreas with a balanced salt solution delivered via the pancreatic duct, chopping the gland into small fragments, and mechanically agitating the tissue with bacterial collagenase enzymes at 37°C. Intralobular distension prior to mincing and enzyme digestion allowed uniform distribution of the collagenase throughout the parenchyma, which, in turn, resulted in acinar disruption, breakdown of the interstitial matrix, and enhanced islet separation. Efforts to improve tissue digestion and increase islet yields, however, would be hampered by crude bacterial enzyme preparations and technical obstacles, thus hindering islet transplantation research for almost 30 years.

These preliminary experiments lead the way for transplantation studies in diabetic rodents. In 1970 Younoszai et al. demonstrated some amelioration of hyperglycemia in rats intraperitoneally implanted with islet allografts [67]. Two years later Ballinger and Lacy showed sustained improvement (but not complete correction) of hyperglycemia of inbred Lewis rats implanted with 400–600 islets into the peritoneal cavity or thigh muscle [68]. Graft excision worsened the blood glucose and histological examination of the recovered islets revealed degranulated β-cells, indicating a high degree of metabolic stress. Rechard and Barker were the first to successfully correct streptozotocin (STZ)-induced hyperglycemia in rats by transplanting 800–1,200 isotologous islets into the peritoneal cavity [31]. Kemp et al. found that intrahepatic embolization of 400–600 rodent islets resulted in complete reversal of diabetes within 24 h, whereas a similar intraperitoneal or subcutaneous islet load was inadequate [32]. The liver was thus recognized to be the most effective environment for islet implantation in the rodent model. It has the benefits of high vascularity, proximity to islet-specific nutrients and growth factors, and physiological first-pass insulin delivery to the liver. Animal studies have shown that islets embolized to the liver undergo a process of angiogenesis and neovascularization to form a rich microvascular network and to re-establish a nutritional blood supply [69, 70]. In the mouse model, host arterial vessels pierce the islet and branch into capillaries within the center of the graft to create a 'core-to-mantle' circulation that optimizes intercellular beta-to-alpha/delta sensing and signaling for precise insulin and glucagon release [71]. Although each site has its own merits based on technical simplicity and/or the capacity to induce immune tolerance, transportal embolization is the method of choice in clinical islet transplantation [72, 73, 74, 75].

Having demonstrated that islet transplantation could cure diabetes in rodents, investigators then went about ways to isolate and purify human islets. Extrapolation of rodent islet isolation and purification techniques to large animals and humans has been problematic. Because the canine pancreas resembles its human counterpart in density and fibrous composition, the dog has become the traditional preclinical model for the development and testing of islet isolation and transplantation techniques and immunosuppression protocols. Mirkovitch et al. were the first to reverse diabetes in pancreatectomized dogs by intrasplenic autotransplantation of partially digested pancreatic tissue [76]. Warnock et al. subsequently demonstrated that canine islet autografts prepared by enzymatic digestion and mechanical dispersion could reverse hyperglycemia [77]. Griffin et al. further showed that as many as three recipients could be normalized with intrasplenic implantation of unpurified canine pancreatic tissue from a single-donor graft [78].

Several methods of dissociating pancreatic tissue have been attempted including tissue maceration, counter-rotational blades, and Velcro [79, 80, 81]. However, the shear forces created by these methods resulted in excessive islet fragmentation. Gray et al. described a less traumatic method whereby human islets could be separated from the undigested fibrous capsule by gently teasing the gland apart, shaking the tissue with forceps, and then passing the partially collagenase-digested tissue through a series of different-sized needles until the islets were free from the exocrine tissue [82].

Ductal collagenase delivery, whether by direct injection [83, 84, 85] or continuous perfusion, [86, 87] cleaves the connective tissue matrix more readily than any method previously described, although inadvertent islet enzyme penetration still produces significant islet destruction [87]. Nonetheless, it was possible to successfully isolate islets from dog, [88] pig, [89] monkey, [90] and human pancreata [27]. Using an automated

recirculating perfusion apparatus based on technology originally described by Horaguchi and Merrell [83], Lakey et al. demonstrated that retrograde intraductal Liberase delivery produced superior islet recovery and islet survival when compared to syringe loading [91].

The next major advancement in islet isolation technology was in 1988 when Ricordi et al. introduced a tissue dissociation chamber [89]. Briefly, the collagenase-distended pancreas was placed inside a stainless steel chamber containing glass marbles (or more recently, stainless steel balls) and a 500 μm mesh screen, and mechanically dissociated by gentle agitation. This approach minimized trauma to the islets by collecting the islets as they were liberated from the digestion chamber. Sequential tissue samples were evaluated to determine the endpoint before the islets were fragmented by overdigestion. Today, the modified 'Ricordi chamber' is the universal device for isolating large animal and human islets [91, 92, 93]. The Automated Cell Extraction System (ACES) is based on concepts of the continuous digestion device (CDD) originally described by Ricordi. This computerized system made it possible to standardize and control the isolation process using a single-use disposable tubing set [94]. By controlling the perfusion pressure, collagenase temperature and rate of enzyme delivery, this system enhanced islet recovery.

A major obstacle to successful human and canine pancreatic dissociation has been the low enzymatic activity of the bacterial collagenase preparations. The introduction of Liberase-HI and Liberase-CI for human and canine islet isolation, respectively (Boehringer Mannheim, Indianapolis, IN), has helped to eliminate some of the lot-to-lot and intra-lot variability of enzyme effectiveness and the need for pre-isolation screening. These highly purified, low-endotoxin enzyme blends contain collagenase I and II and thermolysine. The latter is thought to enhance the degradation of all the major components of the extracellular matrix (ECM) [95, 96]. Liberase digestion consistently yields large numbers of islets without compromising functional viability and has become the 'gold standard' for islet isolation [97, 98, 99, 100].

Endogenous proteases and their respective inhibitors of the donor pancreas have critical roles in the islet isolation process by their effects on collagenase proteolysis, digestion times, islet yield and functional viability. Endogenous pancreatic enzyme activity of the donor pancreas increases during the digestion phase. High trypsin levels are associated with poor islet yields and adverse viability and functional outcomes [101, 102, 103]. Trypsin is believed to act through the proteolysis of collagenase [100, 101, 102]. Pefabloc [4-(2-aminoethyl)-benzene sulfonyl fluoride, hydrochloride] (Roche Molecular Biochemicals, Mannheim, Germany), a broad-spectrum serine-protease inhibitor, has been used successfully to isolate pig and human islets [104, 105, 106]. We have previously shown that Pefabloc supplementation during the isolation phase can improve islet recovery from human pancreata with prolonged cold ischemia times [107]. There was no significant difference in the enzymatic activity digestion time with or without Pefabloc, suggesting that other proteases may be altering collagenase activity [108, 109, 110].

Human islet isolation outcomes remain highly variable despite considerable efforts to manufacture highly purified and standardized collagenase blends. Commercial collagenases are a complex blend of various collagenase isoenzymes, neutral protease, trypsin, clostripain, and several other hydrolytic enzymes [95, 111]. The heterogeneity of collagenase preparations and the immense variability between human donor pancreata continue to hamper a process that is inherently difficult to control [96]. A better understanding of the characteristics and specific activities of each component in the collagenase blends will allow more specific and selective cleavage of the islets from the surrounding extracellular matrix (ECM). The optimal combination of enzymes necessary to maximize the isolation of large numbers of high-quality islets has yet to be determined. The slightest amount of hydration of the Liberase during storage can reduce enzyme function [111]. This hydration activates the proteases, which then degrade the higher molecular weight collagenases, resulting in poor yields, adverse viability and functional outcomes. We are currently evaluating the extent of degradation of collagenase that occurs during storage.

Evolution of methods of islet purification

The inability to produce consistent highly purified human islet preparations has hindered the development of islet transplantation as a realistic treatment option for patients with insulin-dependent diabetes mellitus [58, 60]. Although purification is not essential, there are several advantages to transplanting highly purified islet preparations: (1) improved engraftment, (2) increased safety, (3) reduced graft immunogenicity, and (4) immunomodulation procedures will likely require purified preparations [112, 113, 114, 115, 116, 117, 118, 119]. Gores et al. have suggested that until specific tolerance protocols are a reality, more effort should be directed at modifying the host's immune response while using impure preparations to maximize islet yield [119].

Crude or partially purified pancreatic homogenates have been used to maximize islet engraftment mass [120, 121, 122, 123, 124, 125, 126]. Early attempts to transplant human islets were disappointing although insulin independence had been achieved within the autotransplant setting [18]. Despite overwhelming success in animal models, implantation of unpurified human pancreatic preparations (which may contain greater than

90% exocrine tissue) has been plagued with serious complications: wedge splenic infarction, splenic capsular tear, bleeding esophageal varicies, disseminated intravascular coagulation (DIC), systemic hypertension, portal vein thrombosis and the sequelae of portal hypertension, hepatic infarction, liver failure and even death [125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135]. This increase in portal pressure is believed to be the direct result of embolization of large volumes of unpurified tissue into the liver. Thrombotic complications are believed to be secondary to the thromboplastins released from the digested exocrine tissue. The aforementioned studies demonstrated that allotransplantation of dispersed human pancreatic tissue was unsafe, suggesting that some form of purification was necessary to improve islet engraftment and reduce graft immunogenicity. Mehigan et al. found that the addition of heparin and aprotinin (Trasylol) to the tissue preparation at the time of transplantation could ameliorate the risk of DIC [42]. We have demonstrated that highly purified islet preparations, small packed cell volumes (PCV) < 10 ml (preferably < 5 ml), graded low-dose heparinization and careful monitoring of portal pressure during islet infusion reduces the risk of portal vein thrombosis and its sequelae [132, 135]. The introduction of low-endotoxin Liberase may also be critical in minimizing the acute risk of physiological perturbations associated with infusion of non-purified islet preparations [97].

Attempts to purify islets with nylon mesh sieves, sedimentation at unit gravity, centrifugal elutriation and isokinetic gradient centrifugation [136, 137, 138, 139, 140] have been unsuccessful due to the minimal size difference (average diameter about 150 μm) between the islets and exocrine tissue.

The most common method of islet purification is density gradient centrifugation [141]. Density-dependent elutriation or isopynic separation of tissue separates individual cells as they migrate and settle within the density gradient that is equal to their own density. Lacy and Kostianovsky were able to separate rodent islets from digested exocrine tissue by differential density elutriation using discontinuous sucrose gradients although the islets were unresponsive to hyperglycemic challenge in vitro [66, 142]. This observation was more likely the result of hyperosmolar injury from cellular and islet dehydration rather than dissociation-induced trauma. The replacement of sucrose with Ficoll, a high molecular weight polymer of sucrose (40 kD), permitted the recovery of functionally viable islets [143, 144]. When Ficoll powder is dissolved in Euro-Collins (EC) solution (Euro-Ficoll), hypertonic exposure of the exocrine tissue reduces cell swelling and enhances the islet-exocrine density differential, thereby improving islet recovery [145]. Other continuous and noncontinuous density gradients have been tested with varying degrees of success: bovine serum albumin (BSA), dextran, hypaque-Ficoll, metrizamide, percoll and sodium diatrizoate [146, 147, 148, 149, 150, 151, 152].

In 1989 Lake et al. developed a method for the large-scale purification of human islets suitable for safe transplantation [153]. Originally designed to process bone marrow and to remove the cryoprotectant from banked blood, the COBE 2991 cell processor (COBE BCT, Lakewood, CO) permitted rapid, large volume (600 ml) Ficoll gradient processing of a single pancreas within a sterile, self-contained disposable system. Unfortunately, this method still produces significant β-cell stress, as demonstrated by zymogen degranulation and loss of insulin content [153, 154].

Double staining with fluorescein diacetate and propidium iodide (FDA/PI) is the current international standard to determine islet viability. Preliminary data from our laboratory using the SytoGreen/ethidium bromide (SG/EB) technique suggests that FDA/PI staining could over-estimate islet viability. (unpublished data)

Donor variables affecting islet isolation

Despite significant advances in collagenase quality, intraductal enzyme delivery, and automated tissue dissociation, islet isolation is difficult, expensive, labor-intensive and time-consuming. Even in the best of preparations, the process recovers only about 20 to 50% of the potential islet mass [155]. Donor factors affecting the success of islet isolation have been studied extensively [114, 156]. While donor factors can only be influenced by rigorous donor selection [age > 20 years, high body mass index (BMI), minimal elevated blood glucose (< 10 mmol/l), no cardiac arrest or severe hypotension], surgical team expertise, procurement technique and minimal cold ischemia time (< 20 min) have a major impact on the outcome of islet isolation and insulin independence post-transplantation [114].

Although minor modifications have been made to the automated process, it has been difficult to determine the effect that each modification has on the viability and function of the final product. The inability to identify specific processing parameters that may be predictive of insulin independence following transplantation, the lack of sensitive standardized assays and the inability to maintain normoglycemia following single-donor transplantation remain major obstacles [155, 156].

Matrix-degrading metalloproteinases (MMPs), also known as matrixins, and tissue inhibitors of metalloproteinases (TIMPs) play major roles in ECM catabolism during metamorphosis, development, wound healing and tissue resorption [157]. The proteolytic activity of MMPs is precisely regulated by their endogenous TIMPS [157, 158]. Disruption of this balance may result in diseases associated with uncontrolled proteolysis

of connective tissue matrices such as arthritis, atherosclerosis, tumor growth and metastasis. Preliminary data from our laboratory has shown that there is a significant and positive correlation of TIMP-1, -2, -3, and -4 expression with increased cold storage time before islet isolation. (Unpublished data) This increase in TIMP expression correlates with previous observations that cold storage times have a significant and negative impact on the successful recovery of functionally viable islets [159, 160]. TIMP expression did not correlate with donor age, BMI, gender or pancreas weight. We have identified TIMPs as putative targets to modify pancreatic islets. Current investigations in our laboratory are directed to localizing TIMPs in the donor pancreas and defining the relationship between TIMP mRNA and protein expression and donor variables.

Novel methods of islet purification

The purification of islets with magnetic microspheres coated with islet or cytotoxic anti-acinar monoclonal antibodies (MAbs) is a unique concept that has the potential for large-scale purification [161, 162]. Photothermolysis of specifically targeted acinar tissue permits the recovery of functionally viable islets [163, 164]. Selective destruction of exocrine tissue by antibody-mediated radiosensitization is based on the premise that islets are less radiosensitive than exocrine tissue [165]. Another approach exploits the ten-fold osmotic permeability difference between the exocrine and endocrine tissues [166, 167]. A 30-second exposure of the pancreatic digest to a hypotonic solution selectively lyses the exocrine tissue without damaging the islets. Other methods not specifically discussed herein include cryopreservation, anti-acinar cytotoxic antibodies, tissue culture, florescence-activating cell sorting and cell sorting by simple filtration [168, 169, 170, 171, 172].

Pancreas procurement and preservation

Current multiorgan recovery techniques and transportation of the donor pancreas over long distances often result in more than 12 h of cold storage. Efforts to deliver the pancreas to a centralized islet laboratory within an optimal 8-hour window can involve challenging logistics and often requires expensive chartered air service [173]. Strict donor selection criteria and the need for short ischemia times also limit the availability of suitable cadaveric pancreata for islet transplantation. The pancreas is the most difficult solid organ to procure for transplantation [174]. The method of procurement has a major impact on the subsequent success of the recovery and purification of functionally viable islets [175, 176]. Most reports of human pancreas procurement describe methods for the combined removal of the pancreas and liver [174, 175, 176, 177, 178, 179, 180, 181, 182]. Until recently, the harvesting of the pancreas specifically for islet transplantation has not been addressed [176].

Whole pancreas or a segmental graft can either be resected en bloc with the liver as part of the multiorgan retrieval process or removed while the liver is perfused with University of Wisconsin (UW) solution [175]. The following principles are of paramount importance: (1) atraumatic handling of the pancreas, (2) rapid in situ cooling to minimize warm ischemia and stabilize endogenous enzyme activity prior to islet isolation, and (3) immediate transfer of the pancreas to the islet isolation laboratory to minimize cold ischemic injury. We have demonstrated that rapid mobilization of the spleen to the midline after cross-clamping the aorta and embedding the entire pancreas in iced saline-slush led to a doubling of islet yield and a significant improvement in islet viability [176]. Ideally, the pancreas should be removed en bloc with the spleen and a stapled cuff of proximal and distal duodenum. A damaged pancreatic capsule leads to enzyme leakage and loss of ductal integrity. A pancreas that distends poorly rarely liberates a sufficient number of islets for clinical transplantation [83, 84].

Whole pancreas preservation before islet isolation

Most studies on pancreas preservation are based on the whole pancreas transplant model. Early methods were empirically based on techniques established for the retrieval of cadaveric kidneys. Many of these techniques were unsuccessful because of the pancreas's propensity to injury prior to procurement and during harvesting [174]. There are four methods of pancreas preservation: hyperbaric preservation, simple cold storage, oxygenated perfluorocarbon-based preservation and machine preservation.

Hyperbaric preservation

In 1966 Manax et al. demonstrated that the combination of hypothermia and hyperbaria could preserve canine heart, lung, spleen, intestine and kidney in vitro for periods as long as 72 h [183]. Attempts to preserve canine pancreaticoduodenal and segmental allografts resulted in a progressive decline of insulin secretion over a 48-hour period, with irreversible organ damage occurring after 20 h. Grafts preserved for longer periods became hemorrhagic shortly after restoration of blood flow. Although hyperbaric storage at 4 atmospheres minimized tissue edema, this method proved to be cumbersome, difficult to standardize and impractical [184, 185].

Hypothermic preservation

Simple cold storage is the most common method of solid organ preservation. Hypothermia slows down cellular metabolism by minimizing the consumption of energy substrates and the production of metabolic end products and other toxins that would otherwise lead to cell death, tissue necrosis, and eventual organ failure [174]. Preservation media originally developed in the 1960s and 1970s for kidney are relatively ineffective for pancreas preservation [186]. Unlike the kidney, the pancreas is very susceptible to tissue and cell edema and the activation of endogenous digestive enzymes, which eventually leads to graft pancreatitis. University of Wisconsin (UW) solution, developed by Belzer and Southard in the late 1980s, addressed these concerns and quickly became the standard in situ flush and storage solution for kidney, liver, pancreas and heart [187]. Replacement of glucose with metabolically inert substrates, lactobionate and raffinose, reduces cell swelling by eliminating lactic acid production. The low concentration of permeable anions in the presence of a large molecular weight colloid, hydroxyethyl starch, provides oncotic support during in situ flushing. The free radical scavengers, glutathione and allopurinol, minimize intracellular toxicity while adenosine, a substrate for high energy phosphate production, maintains cell membrane integrity and prevents cold ischemic cell swelling by stabilizing the sodium-potassium pump.

UW solution has proven to be very effective in experimental and clinical pancreas preservation. Human pancreatic grafts can be preserved in UW solution for periods exceeding 24 h. Islets are very vulnerable to irreversible damage after prolonged ischemia [188, 189, 190, 191, 192]. Prolonged cold storage of human pancreas has a negative impact on the recovery of functionally viable islets. In fact, the failure of single-donor islet transplants to reverse hyperglycemia is most likely the result of ischemic injury encountered during cold storage [193].

Islets are isolated from cadaveric pancreas using intraductal enzyme loading, automated enzymatic and mechanical dissociation, and osmotic stabilization with cold UW solution prior to purification on continuous Ficoll gradients [58]. Clinical outcomes can be influenced by numerous factors prior to the donor's demise, during procurement and preservation on through the isolation and purification process, during culture, and subsequent transplantation. More specifically, some of these steps are critically affected by cold storage conditions, which in turn, can activate endogenous pancreatic enzyme activity and/or alter the densities of the exocrine and endocrine tissues. An intact ductal system is necessary for the full distension of the pancreas with collagenase [83, 94, 194, 195]. Several components of UW solution are known to inhibit collagenase activity [196, 197, 198]. Whether the inhibitory effect of intraductal UW solution can be overcome by adjustments in collagenase concentration remains to be evaluated. We have demonstrated that although in situ UW flushing at the time of procurement lead to longer digestion times, there was no significant effect on the recovery and function of human islets [199]. Technical simplicity, decreased operative time and increased safety compensate for the longer digestive phase in order to optimize islet recovery.

We have reported a progressive decline in human islet yield and viability with increasing storage times. The upper limit for cold storage before islet isolation was 16 h [199]. More importantly, cold storage beyond 8 h was associated with a significant reduction in islet yield and functional viability. Zeng et al. confirmed that cold storage beyond 8 h prior to isolation significantly reduced human islet yield and purity [200]. Consequently, preservation techniques that are sufficient for prolonged cold storage before vascularized pancreas transplantation are inadequate for even short periods of cold storage prior to islet isolation and transplantation.

Oxygenated pfc-based preservation

Islets are very vulnerable to irreversible damage after prolonged ischemia [187, 188, 189, 190, 181, 192]. Cold ischemia of the cadaveric pancreas is detrimental to islet yield [114, 201, 202, 203, 204, 205]. In vitro studies have shown a significant reduction in insulin release to glucose challenge even after short periods of cold storage in UW solution [114]. These observations have been seen in clinical practice as there have been no reports of successful single-donor islet transplants with prolonged cold storage beyond 10 h [205]. Ryan et al. have provided evidence of the detrimental impact of cold ischemia on post-transplant islet function [60]. The ischemic index, which takes into account the cold ischemia time for any given islet implant mass, had a positive correlation with insulin secretory response, as determined by the area under the curve (AUC).

Perfluorocarbons (PFCs) have a very high affinity for oxygen and release oxygen more effectively than hemoglobin into the surrounding tissue. In 1988 Kuroda et al. [206, 207, 208] developed a two-layer cold storage (TLM) method for vascularized pancreas preservation using PFC and EC solution (later changed to UW solution). Oxygen dissolved in the PFC diffuses through the undersurface of the partially submerged pancreas. Using substrates in the preservation media, the oxygenated graft continuously generates adenosine triphosphate (ATP), which is required to drive the sodium-potassium pump, thereby maintaining membrane integrity and minimizing ischemic cell swelling [186, 209].

Heat shock proteins are strongly expressed following canine pancreas transplantation and reperfusion, suggesting that they may prevent and/or repair reperfusion injury [210, 211]. PFCs also improve the viability of vascular endothelium and stabilize the microcirculation. Pancreas resuscitation can be further augmented by the addition of the thromboxane A_2 (TxA_2) synthetase inhibitor OKY046 to the preservation solution [212]. Although the immunosuppressive properties of PFCs precluded its use as a blood substitute, this feature may be beneficial for allogeneic organ preservation [213, 214].

Researchers at the University of Minnesota have demonstrated in experimental animal models and research human pancreata that the TLM can resuscitate and repair warm ischemically-damaged pancreata during preservation, improve islet yields, and improve islet engraftment [215, 216, 217, 218, 219, 220, 221]. TLM also maintains and repairs exocrine cell integrity and prevents trypsin activation, thereby enabling effective collagenase delivery and protecting the islets from enzymatic digestion [222]. Matsumoto et al. were the first to evaluate the efficacy of the TLM in the clinical setting of vascularized pancreas transplantation [214]. PFC had no adverse effect on the recipients. Morphological quality of the grafts after reperfusion was well-preserved compared to pancreata stored in UW solution alone. Preliminary data suggest that TLM-preserved pancreata are associated with a reduced incidence of acute rejection when compared to the UW control group [213]. The University of Minnesota group demonstrated in a canine autotransplant model that the TLM protects islets from ischemic damage [222]. The functional success rate was 89% without preservation, 33% after only 3 h of cold preservation in UW solution, and 83% after 3 h of preservation with the TLM. The functional success rates with the TLM and static UW preservation were the same (56%) when pancreata were stored for 24 h before islet isolation. Hiraoka et al. compared the efficacy of preservation techniques before islet isolation in a discordant xenogeneic (Lewis rat-to-diabetic nude mouse) islet transplant model [223, 224]. The functional success rate of islet transplants after 6 h of preservation was 100% with the TLM compared to 50% with static UW preservation. Intracellular ATP content was significantly higher with the TLM than with UW alone.

Hering et al. recently introduced PFC-based preservation before islet isolation and transplantation into clinical practice [225]. Their results clearly indicated that PFC had no adverse effect on in vivo graft function following intrahepatic transplantation.

We have demonstrated that pancreata preserved in UW solution for prolonged periods (>10 h) can be rescued by an additional 3 h of preservation with the TLM [226, 227, 228, 229]. The TLM had a positive effect on in vitro insulin secretory activity as compared to cold storage in UW solution alone [228, 229]. Furthermore, PFC-preserved allografts in the presence of effective immunosuppression improved glycemic control and decreased exogenous insulin requirements in all recipients.

Matsumoto et al. simplified the method by fully saturating the PFC with oxygen for 30 min at a flow rate of 100 ml/min [230]. Pre- and post-purification islet yields preserved by either method were significantly higher when compared with pancreata preserved in UW solution alone. The viability and function of islets preserved by both PFC methods were also significantly better as compared to simple cold storage.

Miyamoto et al. demonstrated that Kyoto solution combined with PFC improved porcine islet yields as compared to UW solution, which is known to inhibit collagenase activity [231]. If these results can be confirmed using human pancreas, the modified TLM could further increase islet yield from a single cadaveric pancreas.

Poor donor quality is the common reason for deferring a pancreas for whole organ transplantation. Pancreata from donors with multiple cardiac arrests, prolonged hypotensive episodes, a history of high dose vasopressor therapy or evidence of kidney or liver dysfunction are frequently rejected as potential pancreas or islet donors. By revising donor selection criteria and salvaging pancreata that would otherwise be discarded, the TLM has the potential to expand the donor pool by several-fold. Ricordi et al. studied the efficacy of PFC-based preservation on marginal (>50 years) human pancreata [232]. Islet yield was almost double that of the PFC control group (donor age: 20–50 years). All PFC-preserved grafts induced insulin independence. The in vivo response to glucose challenge was similar in each group.

Several centers (Minnesota, Miami, Edmonton) have incorporated PFC-based preservation into their existing protocols based on the findings described herein ([233] and personal communications). Refined procurement and preservation techniques will allow better allocation of pancreata among islet transplant laboratories and pancreas transplant centers. PFC-based preservation has the potential to expand the donor pool by using pancreata with cold ischemia times >10 h, marginalized pancreata from non-heart-beating (NHB) donors and pancreata from older donors (age >50 years).

Machine preservation

In 1967 Belzer demonstrated that canine kidneys could be safely stored for 72 h by continuous hypothermic perfusion with a perfusate containing ultrafiltrated cryoprecipitated plasma (CPP) [234]. Continuous hypothermic perfusion of the kidney remains the most reliable method to ensure normal renal function following transplantation [174]. This method simulates

metabolism by supplying oxygen and nutrients and removing metabolic waste products, while maintaining optimal tissue pH [174]. Machine perfusion has been shown to minimize ischemic and reperfusion injuries and to restore function in warm-ischemically damaged organs from non-heart-beating donors [235, 236, 237]. It also has the potential to extend the duration of cold ischemia to 24 h and reduce delayed graft function (DGF) [238]. There are no reliable donor factors that can accurately predict post-transplant function [159]. Machine preservation provides a means to objectively assess the suitability of a pancreas for clinical islet transplantation.

A number of commercial devices have been developed. Each configuration (hypothermia versus normothermia, continuous versus pulsatile, crystalloid and/or colloid perfusates versus blood, high- versus low-flow) has its own merits. Attempts to apply kidney perfusion technology to the pancreas have been unsuccessful [239, 240, 241, 242, 243, 244, 245, 246]. Most studies used an allograft model and therefore only short-term function could be determined reliably. The pancreas is a low-flow organ, requiring only a small proportion of the blood flow. Consequently, pancreatic edema secondary to excessive perfusion pressures is a major obstacle. Whole pancreas grafts tolerate higher flow rates compared to segmental grafts. Final outcomes were also affected by the composition of the perfusate and the profile of the pump waveform. The only reports of long-term function of machine-perfused canine autografts were by Florack et al. in 1982 [247, 248]. They concluded that pancreas preservation by cold storage in high osmolar silica gel filtered plasma (SGFP) was more reliable than pulsatile machine perfusion. At present, hypothermia is the most practical method because it is simple and less expensive.

Pilot studies are underway in our laboratory using a hypothermic, continuous low-flow preservation system to preserve whole pancreas grafts before islet isolation. We have demonstrated in a canine autotransplant model that it is possible to ameliorate hyperglycemia with islets harvested from a single machine-perfused graft preserved for 48 h (unpublished data).

Challenges for the future

Islet transplantation is a safe and effective strategy for β-cell replacement but many technical and scientific obstacles remain [58, 60]. Careful patient selection is essential to maximizing the risk-benefit ratio as islet transplantation becomes more widely available. With this in mind, we are developing a new scoring system, the lability index, to better select potential patients—particularly those with severe metabolic lability who may have been overlooked by the mean amplitude of glycemic excursion (MAGE) scoring system (unpublished data). Frequent blood glucose sampling (> 5 samples per day) has confirmed improvements in glycemic control after the first and second islet transplants (unpublished data).

The first challenge is to obtain similar clinical success with single-donor grafts. The experience with islet autotransplantation after total pancreatectomy suggests that if ischemia and immune reactivity can be circumvented, fewer islets are required to induce and maintain normoglycemia [249]. Large animal studies with non-purified islet grafts suggest it may be possible to treat multiple recipients from a single pancreas [100]. A review of 111 live donor segmental pancreas transplants performed at the University of Minnesota [250] demonstrated that there was a modest increase in procedure-related complications to the donor but better screening has largely eliminated the risk. Live donation of a segmental pancreas graft for islet transplantation is an attractive alternative but the risk of inducing diabetes or other serious complications in an otherwise healthy donor is a major concern [251, 252, 253, 254]. Preliminary results suggest that more islets can be isolated from single human donor pancreata preserved by the two-layer method as compared to static UW preservation but this needs to be confirmed in a large prospective, randomized clinical trial.

The ultimate goal of organ transplantation is to eliminate the need for lifelong anti-rejection therapy. If long-term graft function can be maintained while avoiding serious side effects and the potential risks of malignancy and infection, the selection criteria could be revised to include all diabetics early in the course of the disease. The inability to detect early allograft rejection and the lack of specific serological markers in particular have been major obstacles [255]. Efforts to induce permanent function or stable tolerance in large animals, primates and humans have been technically challenging. Nonetheless, antigen-specific tolerance or near-tolerance strategies may soon be available [256, 257, 258]. The most promising therapies are the combination of anti-lymphocyte globulin with bone marrow or stem cell transplantation [39]. New calcineurin inhibitor-free protocols might provide similar protection from acute and chronic rejection and autoimmunity while optimizing graft function within the context of a subtherapeutic islet engraftment mass.

Even if single-donor islet transplantation becomes uniformly successful, only 0.5% of type 1 diabetics would benefit from an islet transplant due to limited supply of cadaveric pancreata. The final hurdle will be to explore other sources of insulin-producing, glucose-responsive cells to treat the more than 175 million diabetics worldwide. 'Islet farming' may be one solution. Embryonic stem cells have been transformed into islet-like clusters that can correct diabetes in mice [259]. Human embryonic stem cells have been induced to

secrete insulin, albeit in low concentrations and without glucose feedback [260]. Adult stem cells and ductal elements have been trans-differentiated into new islet-like cells or insulin-producing cells [261]. Other promising approaches include gene therapy [262, 263], transformation of hepatocytes to secrete a single-chain insulin analogue [264], expansion of cloned human insulin-producing cell lines [265], tissue engineering of non-β-cells to secrete insulin [266], and genetic engineering of intestinal mucosal K-cells to secrete insulin [267]. Xenotransplantation has great potential, but concerns regarding zoonotic viral transmission must be overcome [268, 269, 270, 271]. Transgenic pigs expressing human complement-regulatory proteins have been developed to overcome acute destructive pathways and chronic rejection, but large doses of cyclophosphamide are required [272, 273].

Some researchers transplant islets after culturing for a short period. Human islets cultured in modified serum-free media (M-SFM) have exhibited sustained viability and function after transplantation into non-obese diabetic (NOD) mice and humans [274, 275]. Others have cultured islets under conditions modified from the original insulin-transferrin-selenium-based cocktail described by Fraga (Ricordi and Shapiro, personal communications). The addition of nicotinamide to the culture media appears to be highly beneficial (unpublished data). If these findings can be confirmed in clinical models, extended (1–2 months) islet culture could significantly improve transplant outcomes by: (1) better matching the donor to the recipient, (2) pre-conditioning the recipient prior to transplantation, and (3) modifying the islets before transplant to promote engraftment and prolonged graft function.

With effective immunotherapy, long-term insulin independence can now be achieved in about 90% of recipients. Even though the risks associated with islet transplantation are significantly lower than those of pancreas transplantation, the trade-off of exchanging daily insulin injections for lifelong immunosuppression can not be justified in children or adolescents at this time. However, we will soon be undertaking a small collaborative study to determine the impact of de novo islet-alone transplantation in children who are at risk of premature death from severe metabolic lability, and other children who are already receiving immunosuppressive therapy because of a previous transplant (Hathout and Shapiro, personal communications). In the meantime, insulin therapy will continue to be the method of choice for the majority of type 1 diabetics. While preliminary clinical studies suggest that ten times more islets may be required to overcome the effects of peripheral insulin resistance, islet transplantation as a treatment option in type 2 diabetes must await the development of other tissue sources [276].

Extensive efforts are underway worldwide to characterize the endogenous components of the human pancreas. Future studies to determine the suitability of donor pancreata for islet transplantation will require sophisticated molecular and genetic assays of the integrity of the acinar, ductal and endocrine elements. With this information in hand, it will then be possible to selectively cleave islets from the ECM with bioengineered enzyme blends 'tailor-made' for each individual donor pancreas, thereby improving islet isolation efficiency, recovery, viability and ultimately post-transplant function.

Other innovative strategies currently under investigation include: pretreating islets to reduce their immunogenicity, protecting islets within immunoisolation devices, and transplanting islets into immunopriviledged sites [277, 278].

Prolonged insulin-independence has not been achieved in all recipients but islet transplantation has effectively eliminated glycemic lability and the sequelae of severe hypoglycemia. If excellent long-term blood glucose control can be maintained, we predict that positive protective effects on secondary neurovascular complications will emerge at 5–10 years post-transplant. None of our patients, whether or not they remain insulin-free, have requested to discontinue immunosuppression therapy, a true testament to the treatment's incredible impact on the management of diabetes. The continuing success of the Edmonton Protocol is most encouraging and is only one step forward in the quest to cure diabetes.

References

1. Gepts W. Role of cellular immunity in the pathogenesis of type 1diabetes. Curr Probl Clin Biochem 1983; 12:86.
2. Atkinson MA, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. Lancet 2001; 358 (9277): 221.
3. 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. N Engl J Med 1993; 329 (14): 977.
4. Hypoglycemia in the Diabetes Control and Complications Trial. The Diabetes Control and Complications Trial Research Group. Diabetes 1997; 46 (2): 271.

5. Turner RC, Holman RR. The United Kingdom Prospective Diabetes Study. UK Prospective Diabetes Study Group. Ann Med 1996; 28 (5): 439.
6. Ryan EA. Pancreas transplants: for whom? Lancet 1998; 351 (9109): 1072.
7. American Diabetes Association. Position statement: pancreas transplantation in patients with diabetes mellitus. Diabetes Care 1992; (11): 1668.
8. Fioretto P. Reversal of lesions of diabetic nephropathy after pancreas transplantation. N Engl J Med 19xx; 339:69.
9. Chase HP, Lockspeiser T, Peery B, Shepherd M, MacKenzie T, Anderson J, Garg SK. The impact of the diabetes control and complications trial and humalog insulin on glycohemoglobin levels and severe hypoglycemia in type 1 diabetes. Diabetes Care 2001; 24 (3): 430.
10. von Mering J, Minkowski O. Diabetes mellitus after pancreas extirpation. Archiv fur Exper Path und Pharmakol 1889; 26:111.
11. Williams P. Notes on diabetes treated with extract and by grafts of sheep's pancreas. Br Med J 1894; 2:1303.
12. Minkowski O. Weitere Mitteilungen uber den diabetes mellitus nach extirpation des pancreas. Berl Klin Wochenschr 1892; 2:1303.
13. Ssobolew LW. Zur normalen and patholischen morphologie der inneren secretion der bauchspeicheldruse. Arch path anat klin med 1902; 168:91.
14. Pybus F. Notes on suprarenal and pancreatic grafting. Lancet 1924; 550.
15. Banting F. The beneficial Influences of certain pancreatic extracts on pancreatic diabetes. Delivered on December 30, 1921 at the American Physiological Society, Yale University, New Haven, Connecticut.
16. Paulesco NC. Action de l'extrait pancreatique. Comptes rendus des séances de la société de biologie 1921; 27:555.
17. Zuelzer GL. Uber versuche einer spezifischen ferment therapie des diabetes. Ztschr exper path ther 1908; 5:307.
18. Murlin JR, Kramer B. Effects of pancreatic extracts on glycosuria. J Biol Chem 1916; 15:365.
19. Kleiner IS. The action of intravenous injections of pancreas emulsions in experimental diabetes. J Biol Chem 1919; 40:153.
20. Scott EL. On the influence of intravenous injections of extract of pancreas on experimental pancreatic diabetes. Amer J Physiol 1922; 29:306.
21. Gley E. Action des extraits de pancréas sclerose sur des chiens diabetiques par extirpation du pancréas. C R Soc Biol 1922; 87:1322.
22. Banting FG, Best CH. The internal secretion of the pancreas. J Lab Clin Med 1922; 7:256.
23. Banting, FG, Best CH, Collip JB, Campbell WR, Fletcher AA. Pancreatic extracts in the treatment of diabetes mellitus: preliminary report. Can Med Assoc J 1922; 12:141.
24. Burrow GN, Hazlett BE, Phillips MJ A case of diabetes mellitus. N Engl J Med 1982. 306:340.
25. Bliss M. The discovery of insulin. Toronto. McClelland and Stewart Limited. 1982.
26. Calwell AR. Relation of small vessel complications to treatment of diabetes: a review. In: Small blood vessel involvement in diabetes mellitus. Siperstein MD, Calwell AR, Myer K (Eds). Washington, DC. American Institute of Biological Sciences. Science 1964; 253.
27. Hardin RC, Jackson RL, Johnson TL, Kelly HG. The development of diabetic retinopathy: effects of duration and control of diabetes. Diabetes 1956; 5:397.
28. Kimmelsteil P, Wilson C. Intercapillary lesions in the glomeruli of the kidney. Am J Pathol 1936; 12:83.
29. Le Compte PM. Vascular lesions in diabetes. J Chron Dis 1955; 2:178.
30. Marble A. Relation of control of diabetes to vascular sequelae. Med Clin North Am 1965; 49:1137.
31. Reckard CR, Ziegler MM, Barker CF. Physiological and immunological consequences of transplanting isolated pancreatic islets. Surgery 1973; 74 (1): 91.
32. Kemp CB, Knight MJ, Scharp DW, Ballinger WF, Lacy PE. Effect of transplantation site on the results of pancreatic islet isografts in diabetic rats. Diabetologia 1973; 9 (6): 486.
33. Najarian JS, Sutherland DE, Matas AJ, Steffes MW, Simmons RL, Goetz FC. Human islet transplantation: a preliminary report. Transplant Proc 1977; 9 (1): 233.
34. Larsen J, Lane J, Mack-Shipman L. Pancreas and kidney transplantation. Curr Diab Rep 2002; 2 (4): 359.
35. Largiader F, Kolb E, Binswanger U, Illig R. Successful allotransplantation of an island of Langerhans. Schweiz Med Wochenschr 1979; 109 (45): 1733.
36. White SA, London NJ, Johnson PR, Davies JE, Pollard C, Contractor HH, Hughes DP, Robertson GS, Musto PP, Dennison AR. The risks of total pancreatectomy and splenic islet autotransplantation. Cell Transplant 2000; 9 (1): 19.
37. Najarian JS, Sutherland DE, Matas AJ, Goetz FC. Human islet autotransplantation following pancreatectomy. Transplant Proc 1979; 11 (1): 336.
38. Pyzdrowski KL, Kendall DM, Halter JB, Nakhleh RE, Sutherland DE, Robertson RP. Preserved insulin secretion and insulin independence in recipients of islet autografts. N Engl J Med 1992; 327 (4): 220.
39. Brendel M. International Islet Transplant Registry Newsletter #9. Volume 8. No. 1. 2001.
40. Oberholzer J, Triponez F, Mage R, Andereggen E, Buhler L, Cretin N, Fournier B, Goumaz C, Lou J, Philippe J, Morel P. Human islet transplantation: lessons from 13 autologous and 13 allogeneic transplantations. Transplantation 2000; 69 (6): 1115.
41. Robertson GS, Dennison AR, Johnson PR, London NJ. A review of pancreatic islet autotransplantation. Hepatogastroenterology 1998; 45 (19): 226.
42. 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.
43. Robertson RP, Lanz KJ, Sutherland DE, Kendall DM. Prevention of diabetes for up to 13 years by autoislet transplantation after pancreatectomy for chronic pancreatitis. Diabetes 2001; 50 (1): 47.
44. Sutherland DE, Gruessner RW, Gores PF, Brayman K, Wahoff D, Gruessner A. Pancreas transplantation: an update. Diabetes Metab Rev 1995; 11 (4): 337.
45. Farney AC, Hering BJ, Nelson L, et al. No late failures of intraportal human islet autografts beyond 2 years. Transplant Proc 1998; 30 (2): 420.
46. Tzakis AG, Ricordi C, Alejandro R, et al. Pancreatic islet transplantation after upper abdominal exenteration and liver replacement. Lancet 1990; 336 (8712): 402.
47. Ricordi C, Tzakis AG, Carroll PB, et al. Human islet isolation and allotransplantation in 22 consecutive cases. Transplantation 1992; 53 (2): 407.
48. Ricordi C, Lacy PE, Finke EH, Olack BJ, Scharp DW. Automated method for isolation of human pancreatic islets. Diabetes 1988; 37 (4): 413.
49. Lakey JR, Warnock GL, Shapiro AM, et al. Intraductal collagenase delivery into the human pancreas using syringe loading or controlled perfusion. Cell Transplant 1999; 8 (3): 285.
50. Linetsky E, Bottino R, Lehmann R, Alejandro R, Inverardi L, Ricordi C. Improved human islet isolation using a new enzyme blend, liberase. Diabetes 1997; 46 (7): 1120.

51. Robertson GS, Chadwick DR, Contractor H, James RF, London NJ The optimization of large-scale density gradient isolation of human islets. Acta Diabetol 1993; 30 (2): 93.
52. Bretzel RG, Brendel M, Eckhard M, et al. Islet transplantation: present clinical situation and future aspects. Exp Clin Endocrinol Diabetes 2001; 109 (suppl 2):S384.
53. Secchi A, Taglietti MV, Socci C, et al. Insulin secretory patterns and blood glucose homeostasis after islet allotransplantation in IDDM patients: comparison with segmental- or whole-pancreas transplanted patients through a long term longitudinal study. J Mol Med 1999; 77 (1): 133.
54. Shapiro AM, Hao E, Lakey JR, Finegood D, Rajotte RV, Kneteman NM. Diabetogenic synergism in canine islet autografts from cyclosporine and steroids in combination. Transplant Proc 1998; 30 (2): 527.
55. Hering B, Ricordi C. Islet Transplantation for patients with type 1 diabetes mellitus: results, research priorities and reasons for optimism. Graft 1999; 2:12.
56. Bennet W, Sundberg B, Groth CG, et al. Incompatibility between human blood and isolated islets of Langerhans: a finding with implications for clinical intraportal islet transplantation? Diabetes 1999; 48 (10): 1907.
57. Paraskevas S, Maysinger D, Wang R, Duguid TP, Rosenberg L. Cell loss in isolated human islets occurs by apoptosis. Pancreas 2000; 20 (3): 270.
58. Shapiro AMJ, Lakey JRT, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 2000; 343 (4): 230.
59. Shapiro AM, Suarez-Pinzon WL, Power R, Rabinovitch A. Combination therapy with low dose sirolimus and tacrolimus is synergistic in preventing spontaneous and recurrent autoimmune diabetes in non-obese diabetic mice. Diabetologia 2002; 45 (2): 224.
60. Ryan EA, Lakey JR, Rajotte RV, et al. Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol. Diabetes 2001; 50 (4): 710.
61. Ryan EA, Lakey JR, Paty BW, et al. Successful islet transplantation: continued insulin reserve provides long-term glycemic control. Diabetes 2002; 51 (7): 2148.
62. Bretzel RG, Hering BJ, Federlin KF. Islet cell transplantation in diabetes mellitus–from bench to bedside. Exp Clin Endocrinol Diabetes 1995; 103 (suppl 2): 143.
63. Bensley RR. Studies on the pancreas of the guinea pig. Am J Anat 1911–12; 12:297.
64. Hellerström C. A method for the microdissection of intact pancreatic islets of mammals. Acta Endocrinol 1964; 45:122.
65. Moskalewski S. Isolation and culture of the islets of Langerhans of the guinea pig. Gen Comp Endo 1965; 5:342.
66. Lacy P, Kostianovsky M. Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes 1967; 16:35.
67. Younoszai R, Sorensen R, Lindall A. Homotransplantation of isolated pancreatic islets. Diabetes 1970; 19 (suppl 1): 406.
68. Ballinger WF, Lacy PE. Transplantation of intact pancreatic islets in rats. Surgery 1972; 72 (2): 175.
69. Menger MD, Wolf B, Hobel R, Schorlemmer HU, Messmer K. Microvascular phenomena during pancreatic islet graft rejection. Langenbecks Arch Chir 1991; 376(4):214.
70. Vajkoczy P, Menger MD, Simpson E, Messmer K. Angiogenesis and vascularization of murine pancreatic islet isografts. Transplantation 1995; 60 (2): 123.
71. Menger MD, Vajkoczy P, Beger C, Messmer K. Orientation of microvascular blood flow in pancreatic islet isografts. J Clin Invest 1994; 93 (5): 2280.
72. Alajandro R, Cutfield RG, Sheinvold FL, et al. Natural history of intrahepatic canine islet cell autografts. J Clin. Invest 1986; 78:1339.
73. Ao, Z, Matayoshi K, Lakey JRT, Rajotte RV, Warnock GL. Survival and function of purified islets in the omental pouch of outbred dogs. Transplantation 1993; 56:524.
74. Hesse UJ, Sutherland DER, Gores PF, et al. Comparison of splenic and renal subcapsular islet autografting in dogs. Transplantation 1986; 8:590.
75. Brendel MD, Hering BJ, Schultz AO, and Bretzel RG. International Islet Transplant Registry Newsletter #8, 1999.
76. Mirkovitch V, Campiche M. Successful intrasplenic autotransplantation of pancreatic tissue in totally pancreatectomised dogs. Transplantation 1976; 21 (3): 265.
77. Warnock GL, Rajotte RV, Procyshyn AW. Normoglycemia after reflux of islet-containing pancreatic fragments into the splenic vascular bed in dogs. Diabetes 1983; 32 (5): 452.
78. Griffin SM, Alderson D, Farndon JR. Comparison of harvesting methods for islet transplantation. Br J Surg 1986; 73 (9): 712.
79. Alderson D, Kneteman NM, Scharp DW. The isolation of purified human islets of Langerhans. Transplant Proc 1987; 19 (1 Pt 2): 916–917.
80. Krestchner GJ, Sutherland DE, Matas AJ, Steffes MW, Najarian JS. The dispersed pancreas: transplantation without islet purification in totally pancreatectomized dogs. Diabetologia 1977; 13 (5): 495.
81. Lacy PE, Lacy ET, Finke EH, Yasunami Y. An improved method for the isolation of islets from the beef pancreas. Diabetes 1982; 31 (suppl 4): 109.
82. Gray DWR, McShane P, Grant A, Morris PJ A method for isolation of islets of Langerhans from the human pancreas. Diabetes 1984; 33 (11): 1055.
83. Horaguchi A, Merrell RC. Preparation of viable islet cells from dogs by a new method. Diabetes 1981; 30 (5): 455.
84. Noel J, Rabinovitch A, Olson L, Kyriakides G, Miller J, Mintz DH. A method for large-scale, high-yield isolation of canine pancreatic islets of Langerhans. Metabolism 1982; 31 (2): 184.
85. Rajotte RV, Warnock GL, Evans MG, Ellis D, Dawidson I. Isolation of viable islets of Langerhans from collagenase-perfused canine and human pancreata. Transplant Proc 1987; 19 (1 Pt 2): 918.
86. Warnock GL, Kneteman NM, Evans MG, Dabbs KD, Rajotte RV. Comparison of automated and manual methods for islet isolation. Can J Surg 1990; 33 (5): 368.
87. van Suylichem PT, Wolters GH, van Schilfgaarde R. Peri-insular presence of collagenase during islet isolation procedures. J Surg Res 1992; 53 (5): 502.
88. Warnock GL, Cattral MS, Rajotte RV. Normoglycemia after implantation of purified islet cells in dogs. Can J Surg. 1988; 31 (6): 421.
89. Ricordi C, Finke EH, Lacy PE. A method for the mass isolation of islets from the adult pig pancreas. Diabetes 1986; 35 (6): 649.
90. Gray DW, Warnock GL, Sutton R, Peters M, McShane P, Morris PJ. Successful autotransplantation of isolated islets of Langerhans in the cynomolgus monkey. Br J Surg 1986; 73 (10): 850.
91. Ricordi C, Lacy PE, Rajotte RV, Warnock GL. Automated islet isolation from human pancreas. Diabetes 1989; 38 (suppl 1): 140.
92. Ao Z, Lakey JR, Rajotte RV, Warnock GL. Collagenase digestion of canine pancreas by gentle automated dissociation in combination with ductal perfusion optimizes mass recovery of islets. Transplant Proc 1992; 24 (6): 2787.

93. Toomey P, Chadwick DR, Contractor H, Bell PR, James RF, London NJ Porcine islet isolation: prospective comparison of automated and manual methods of pancreatic collagenase digestion. Br J Surg 1993; 80 (2): 240.
94. Lakey JR, Warnock GL, Brierton M, et al. Development of an automated computer-controlled islet isolation system. Cell Transplant 1997; 6 (1): 47.
95. Klock G, Kowalski MB, Hering BJ, et al. Fractions from commercial collagenase preparations: use in enzymatic isolation of the islets of Langerhans from porcine pancreas. Cell Transplant 1996; 5:543.
96. Wolters GH, Vos-Scheperkeuter GH, Lin HC, van Schilfgaarde R. Different roles of class I and class II Clostridium histolyticum collagenase in rat pancreatic islet isolation. Diabetes 1995; 44: 227.
97. Gill JF, Chambers LL, Baurley JL, et al. Safety testing of Liberase, a purified enzyme blend for human islet isolation. Transplant Proc 1995; 27 (6): 3276.
98. Linetsky E, Selvaggi G, Bottino R, et al. Comparison of collagenase type p and liberase during human islet isolation using the automated method. Transplant Proc 1995; 27 (6): 3264.
99. Lakey JR, Cavanagh TJ, Zieger MA, Wright M. Evaluation of a purified enzyme blend for the recovery and function of canine pancreatic islets. Cell Transplant 1998; 7 (4): 365.
100. Payne WD, Sutherland DE, Matas AJ, Gorecki P, Najarian JS. DL-ethionine treatment of adult pancreatic donors. Amelioration of diabetes in multiple recipients with tissue from a single donor. Ann Surg 1979; 189 (2): 248.
101. Bai RX, Fujimori K, Koja S, et al. Effect of prophylactic administration of trypsin inhibitors in porcine pancreas islet isolation. Transplant Proc 1998; 30 (2) 349.
102. Heiser A. Isolation of porcine pancreatic islets: low trypsin activity during the isolation procedure guarantees reproducible high islet yields. J Clin Lab Anal 1994; 8 (6): 407.
103. Heiser A, Ulrichs K, Muller-Ruchholtz W. Prophylactic trypsin inhibition during the isolation procedure guarantees reproducible, high porcine islet yields. Xenotransplantation 1994; 1: 66.
104. Heiser A, Ulrichs K, Muller-Ruchholtz W. Isolation of porcine pancreatic islets: low trypsin activity during the isolation procedure guarantees reproducible high islet yields. J Clin Lab Anal 1994; 8 (6): 407.
105. Lakey JR, Warnock GL, Rajotte RV, et al. Improved outcome of pig islet isolation by Pefabloc inhibition of trypsin. Transplantation 1996; 61 (7): 1047.
106. Basir I, van der Burg MP, Scheringa M, Tons A, Bouwman E. Improved outcome of pig islet isolation by Pefabloc inhibition of trypsin. Transplant Proc 1997; 29 (4): 1939.
107. Lakey JR, Helms LM, Kin T, et al. Serine-protease inhibition during islet isolation increases islet yield from human pancreases with prolonged ischemia. Transplantation 2001; 72 (4): 565.
108. Rose NL, Palcic MM, Shapiro AMJ, Lakey JRT. Endogenous pancreatic enzyme levels show no significant effect on human islet isolation yield. Cell Transplantation 2003. In press.
109. Rose NL, Palcic MM, Lakey JR. Evaluating the effect of serine proteases on collagenase activity during human islet isolation. Cell Transplant 2002; 11 (8): 821.
110. Rose NL, Palcic MM, Helms LM, Lakey JR. Evaluation of Pefabloc as a serine protease inhibitor during human-islet isolation. Transplantation 2003; 75 (4): 462.
111. Johnson PR, White SA, London NJ. Collagenase and human islet isolation. Cell Transplant 1996; 5 (4): 437.
112. Gray DW, Sutton R, McShane P, Peters M, Morris PJ. Exocrine contamination impairs implantation of pancreatic islets transplanted beneath the kidney capsule. J Surg Res 1988; 45 (5): 432.
113. London NJM, Chadwick DR, Johnson PRV, et al. Approaches to islet purification. In: Lanza RP, Chick WI, eds. Pancreatic islet transplantation volume I: procurement of pancreatic islets. Austin, TX. Landes, 1994; 25.
114. Gotoh M, Maki T, Satomi S, Porter J, Monaco AP. Immunological characteristics of purified pancreatic islet grafts. Transplantation 1986; 42 (4): 387.
115. Downing R, Morrissey S, Kiske D, Scharp DW. Does the purity of intraportal islet isografts affect their endocrine function? J Surg Res 1986; 41 (1): 41.
116. Ulrichs K, Muller-Rucholz W. Mixed lymphocyte islet culture (MLIC) and its use in manipulation of human islet alloimmunogenicity. Horm Metab Res Suppl 1990; 25: 123.
117. Gray DW. The role of exocrine tissue in pancreatic islet transplantation. Transpl Int 1989; 2 (1): 41.
118. Gores PF, Sutherland DE. Pancreatic islet transplantation: is purification necessary? Am J Surg 1993; 166 (5): 538.
119. Gores PF, Najarian JS, Stephanian E, Lloveras JJ, Kelley SL, Sutherland DE. Transplantation of unpurified islets from single donors with 15-deoxyspergualin. Transplant Proc 1994; 26 (2): 574.
120. Farney AC, Najarian JS, Nakhleh RE, et al. Autotransplantation of dispersed pancreatic islet tissue combined with total or near-total pancreatectomy for treatment of chronic pancreatitis. Surgery 1991; 110 (2): 427.
121. Wahoff DC, Papalois BE, Najarian JS, et al. Autologous islet transplantation to prevent diabetes after pancreatic resection. Ann Surg 1995; 222 (4): 562.
122. Wahoff DC, Papalois BE, Najarian JS, Nelson LA, Dunn DL, Farney AC, Sutherland DE. Clinical islet autotransplantation after pancreatectomy: determinants of success and implications for allotransplantation? Transplant Proc 1995; 27 (6): 3161.
123. Wahoff DC, Papalois BE, Najarian JS, et al. Islet Autotransplantation after total pancreatectomy in a child. J Pediatr Surg 1996; 31 (1): 132.
124. Kretschmer GJ, Sutherland DE, Matas AJ, Cain TL, Najarian JS. Autotransplantation of pancreatic islets without separation of exocrine and endocrine tissue in totally pancreatectomized dogs. Surgery 1977; 82 (1): 74.
125. Mirkovitch V, Campiche M. Pancreatic transplantation: absence of diabetes in dogs after total pancreatectomy and intrasplenic autotransplantation of pancreatic tissue. Transplant Proc 1977; 9:321.
126. Mauer SM, Sutherland DE, Steffes MW, et al. Pancreatic islet transplantation. Effects on the glomerular lesions of experimental diabetes in the rat. Diabetes 1974; 23 (9): 748.
127. White SA, Robertson GS, London NJ, Dennison AR. Human islet autotransplantation to prevent diabetes after pancreas resection. Dig Surg 2000; 17 (5): 439.
128. Cameron JL, Mehigan DG, Broe PJ, Zuidema GD. Distal pancreatectomy and islet autotransplantation for chronic pancreatitis. Ann Surg 1981; 193 (3): 312-.
129. Toledo-Pereyra LH, Rowlett AL, Cain W, Rosenberg JC, Gordon DA, MacKenzie GH. Hepatic infarction following intraportal islet cell autotransplantation after near-total pancreatectomy. Transplantation 1984; 38 (1): 88.

130. Mittal VK, Toledo-Pereyra LH, Sharma M, Ramaswamy K, Puri VK, Cortez JA, Gordon D. Acute portal hypertension and disseminated intravascular coagulation following pancreatic islet autotransplantation after subtotal pancreatectomy. Transplantation 1981; 31 (4): 302.
131. Traverso LW, Abou-Zamzam AM, Longmire WP Jr. Human pancreatic cell autotransplantation following total pancreatectomy. Ann Surg 1981; 193 (2): 191.
132. Shapiro AM, Lakey JR, Rajotte RV. Portal vein thrombosis after transplantation of partially purified pancreatic islets in a combined human liver/islet allograft. Transplantation 1995; 15; 59 (7): 1060.
133. Kneteman NN, Shapiro AM. Portal venous pressure changes after sequential clinical islet transplantation. Transplantation 2002; 74 (7): 913.
134. Walsh TJ, Eggleston JC, Cameron JL. Portal hypertension, hepatic infarction, and liver failure complicating pancreatic islet autotransplantation. Surgery 1982; 91 (4): 485.
135. Froberg MK, Leone JP, Jessurun J, Sutherland DE. Fatal disseminated intravascular coagulation after autologous islet transplantation. Hum Pathol 1997; 28 (11): 1295.
136. Offord RE, Halban PA. Isolation of pancreatic islets of Langerhans by filtration on nylon mesh. Biochem Biophys Res Commun 1978; 82 (4): 1091.
137. Cavanagh TJ, Dwulet FE, Fetterhoff TJ, et al. Collagenase selection. In: Lanza RP, Chick, WL, eds. Pancreatic islet transplantation volume I: procurement of pancreatic islets. Austin, TX: Landes, 1994; 39.
138. Pipeleers DG, Pipeleers-Marichal MA. A method for the purification of single A, B and D cells and for the isolation of coupled cells from isolated rat islets. Diabetologia 1981; 20 (6): 654.
139. Scharp D, Lacy P, Ricordi C, et al. Human islet transplantation in patients with type 1diabetes. Transplant Proc 1989; 21 (1 Pt 3): 2744.
140. Hering BJ, Gramberg D, Ernst E, et al. Isokinetic gradients: a new approach to reduce islet graft immunogenicity. Transplant Proc 1993; 25 (1 Pt 2): 959.
141. Ricordi C, Rastellini C. Automated method for pancreatic islet separation. In: Ricordi C ed. Methods in islet implantation. RG Landes, Austin, TX. 1995. 433.
142. Lindall A, Steffes M, Sorenson R. Immunoassayable insulin content of subcellular fractions of rat islets. Endocrinology 1969; 85 (2): 218.
143. 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.
144. Nash JR, Horlor M, Bell PR. The use of ficoll in the separation of viable islets of Langerhans from the rat pancreas. Transplantation 1976; 22 (4): 411.
145. Lakey JR, Cavanagh TJ, Zieger MA. A prospective comparison of discontinuous EuroFicoll and EuroDextran gradients for islet purification. Cell Transplant 1998; 7 (5): 479.
146. Lake SP, Anderson J, Chamberlain J, Gardner SJ, Bell PR, James RF. Bovine serum albumin density gradient isolation of rat pancreatic islets. Transplantation 1987; 43 (6): 805.
147. Alejandro R, Strasser S, Zucker PF, Mintz DH. Isolation of pancreatic islets from dogs. Semiautomated purification on albumin gradients. Transplantation 1990; 50 (2): 207.
148. van der Vliet JA, Meloche RM, Field MJ, Chen DJ, Kaufman DB, Sutherland DE. Pancreatic islet isolation in rats with ductal collagenase distention, stationary digestion, and dextran separation. Transplantation 1988; 45 (2): 493.
149. Tze WI, Wong FC, Tingle AJ The use of hypaque-ficoll in the isolation of pancreatic islets in rats. Transplantation 1976; 22 (2): 201.
150. Rahdt G. Isolation of functionally intact pancreatic islets by centrifugation in metrizamide gradients. Hoppe Seylers Z Physiol Chem 1977; 358 (10): 1369.
151. Buitrago A, Gylfe E, Henriksson C, Pertoft H. Rapid isolation of pancreatic islets from collagenase digested pancreas by sedimentation through percol at unit gravity. Biochem Biophys Res Commun 1977; 79 (3): 823.
152. Hering BJ, Muench KP, Schelz J, et al. The evaluation of neutral density separation utilizing ficoll-sodium diatrizoate and nycodenz and centrifugal elutriation in the purification of bovine and canine islet preparations. Horm Metab Res Suppl 1990; 25:57.
153. Lake SP, Bassett PD, Larkins A, et al. Large-scale purification of human islets utilizing discontinuous albumin gradient on IBM 2991 cell separator. Diabetes 1989; 38 (suppl 1): 143.
154. Brandhorst H, Brandhorst D, Brendel MD, Hering BJ, Bretzel RG. Assessment of intracellular insulin content during all steps of human islet isolation procedure. Cell Transplant 1998; 7 (5): 489.
155. Ricordi C, Lakey JR, Hering BJ Challenges toward standardization of islet isolation technology. Transplant Proc 2001; 33 (1–2): 1709.
156. Benhamou PY, Watt PC, Mullen Y, et al. Human islet isolation in 104 consecutive cases. Factors affecting isolation success. Transplantation 1994; 57 (12): 1804.
157. Nagase H, Woessner Jr JF, Matrix metalloproteinases. J Biol Chem 1999; 274:21491.
158. Gomez DE, Alonse DF, Yoshiji H, Thorgeirson UP. Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. Euro J Cell Biology 1997; 74:111.
159. Lakey JR, Warnock GL, Rajotte RV, et al. Variables in organ donors that affect the recovery of human islets of Langerhans. Transplantation 1996; 61 (7): 1047.
160. Kneteman NM, Lakey JRT, Warnock GL, Rajotte RV. Human islet isolation after prolonged storage. Diabetes, Nutrition and Metabolism. 1992; 5:3.
161. Fujioka T, Terasaki PI, Heintz R, et al. Rapid purification of islets using magnetic microspheres coated with anti-acinar cell monoclonal antibodies. Transplantation 1990; 49 (2): 404.
162. Davies JE, James RF, London NJ, Robertson GS. Optimization of the magnetic field used for immunomagnetic islet purification. Transplantation 1995; 59 (5): 767.
163. Brunicardi FC, Suh E, Kleinman R, et al. Selective photodynamic laser treatment of dispersed pancreatic tissue for islet isolation. Transplant Proc 1992; 24 (6): 2796.
164. Brunicardi FC, Oh Y, Shevlin L, Suh E, Kleinman R, Stein E, Lipaz G, Plant DV, Imagawa D, Fetterman HR, et al. Laser destruction of human nonislet pancreatic tissue. Transplant Proc 1994; 26 (6): 3354.
165. Nason RW, Rajotte RV, Procyshyn AW, et al. Purification of pancreatic islet cell grafts with radiation. Transplant Proc 1986; 18:174.
166. Liu C, Benson CT, Gao D, Haag BW, McGann LE, Critser JK. Water permeability and its activation energy for individual hamster pancreatic islet cells. Cryobiology 1995; 32 (5): 493.
167. Liu C, McGann LE, Gao D, Haag BW, Critser JK. Osmotic separation of pancreatic exocrine cells from crude islet cell preparations. Cell Transplant 1996; 5 (1): 31.
168. Evans MG, Rajotte RV, Warnock GL, Procyshyn AW. Cryopreservation purifies canine pancreatic microfragments. Transplant Proc 1987; 19 (4): 3471.

169. Soon-Shiong P, Heintz P, Terasaki P. An immunological method of islet cell purification using anti-acinar cell monoclonal antibodies. Transplant Proc 1988; 20 (1 suppl 1): 61.
170. Matas AJ, Sutherland DE, Steffes MW, Najarian JS. Short-term culture of adult pancreatic fragments for purification and transplantation of islets of Langerhans. Surgery 1976; 80 (2): 183.
171. Jiao L, Gray DW, Gohde W, Flynn GJ, Morris PJ. In vitro staining of islets of Langerhans for fluorescence-activated cell sorting. Transplantation 1991; 52 (3): 450.
172. Salvalaggio PR, Deng S, Ariyan CE, et al. Islet filtration: a simple and rapid new purification procedure that avoids ficoll and improves islet mass and function. Transplantation 2002; 74 (6): 877.
173. Rabkin JM, Olyaei AJ, Orloff SL, et al. Distant processing of pancreas islets for autotransplantation following total pancreatectomy. Am J Surg 1999; 177 (5): 423.
174. D'Alessndro MD, James H, Southard JH, Love RB, Belzer FO. Organ preservation. Surg Clin North Am 1994; 74 (5): 1083.
175. Kneteman NM, Lakey JR, Kizilisik TA, Ao Z, Warnock GL, Rajotte RV. Cadaver pancreas recovery technique. Impact on islet recovery and in vitro function. Transplantation 1994; 58 (10): 1114.
176. Lakey JR, Kneteman NM, Rajotte RV, Wu DC, Bigam D, Shapiro AM. Effect of core pancreas temperature during cadaveric procurement on human islet isolation and functional viability. Transplantation 2002; 73 (7): 1106.
177. Kalayoglu M, D'Alessandro AM, Knechtle SJ, et al. Preliminary experience with split liver transplantation. J Am Coll Surg 1996; 182 (5): 381.
178. Sollinger HW, Vernon WB, D'Alessandro AM, Kalayoglu M, Stratta RJ, Belzer FO. Combined liver and pancreas procurement with Belzer-UW solution. Surgery 1989; 106 (4): 685.
179. Nghiem DD. A technique for concomitant whole duodenopancreatectomy and hepatectomy for transplantation in the multiple organ donor. Surg Gynecol Obstet 1989; 169 (3): 257.
180. Marsh CL, Perkins JD, Sutherland DE, Corry RJ, Sterioff S. Combined hepatic and pancreaticoduodenal procurement for transplantation. Surg Gynecol Obstet 1989; 168 (3): 254.
181. Delmonico FL, Jenkins RL, Auchincloss Jr H, et al. Procurement of a whole pancreas and liver from the same cadaveric donor. Surgery 1989; 105 (6): 718.
182. Nakazato PZ, Concepcion W, Bry W, et al. Total abdominal evisceration: an en bloc technique for abdominal organ harvesting. Surgery 1992; 111 (1): 37.
183. Manax WG, Largiader F, Lillehei, RC. Whole canine organ preservation prolonged in vitro by hypothermia and hyperbaria. JAMA 1966; 196:1121.
184. Idezuki Y, Dietzman RH, Feemster JA, Ersek RA, Lillehei RC. Successful twenty-four hour preservation of pancreaticoduodenal allograft with hypothermia and hyperbaric oxygen. Surg Forum 1968; 19:221.
185. Idezuki Y, Goetz FC, Lillehei RC. Experimental allotransplantation of the preserved pancreas and duodenum. Surgery 1969; 485.
186. Belzer FO, Southard JH. Principles of solid-organ preservation by cold storage. Transplantation 1988; 45 (4): 673.
187. Belzer FO, Ploeg RJ, Knechtle SJ, et al. Clinical pancreas preservation and transplantation. Transplant Proc 1994; 26 (2): 550.
188. Carlsson PO, Palm F, Andersson A, Liss P. Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site. Diabetes 2001; 50 (3): 489.
189. Carlsson PO, Palm F, Andersson A, Liss P. Chronically decreased oxygen tension in rat pancreatic islets transplanted under the kidney capsule. Transplantation 2000; 69 (5): 761.
190. Dionne KE, Colton CK, Yarmush ML. Effect of hypoxia on insulin secretion by isolated rat and canine islets of Langerhans. Diabetes 1993; 42 (1): 12.
191. Munn SR, Kaufman DB, Field MJ, Viste AB, Sutherland DE. Cold-storage preservation of the canine and rat pancreas prior to islet isolation. Transplantation 1989; 47 (1): 28.
192. Tanioka Y, Sutherland DE, Kuroda Y, Suzuki Y, Matsumoto I, Deai T. Preservation of dog pancreas before islet isolation with the two-layer method. Transplant Proc 1998; 30 (7): 3419.
193. Gruessner AC, Sutherland DER. Analysis of United States (US) and non-US pancreas transplants reported to the United Network for Organ Sharing (UNOS) and the International Pancreas Transplant Registry (IPTR) as of October 2001. In: Carina MJ, Terasaki P, eds. Clinical transplants 2001 Los Angeles, UCLA Immunogenetics Center 2001, 41.
194. Hesse UJ, Sutherland DE, Gores PF, Najarian JS. Experience with 3, 6, and 24 hours' hypothermic storage of the canine pancreas before islet cell preparation and transplantation. Surgery 1987; 102 (3): 460.
195. Ohzato H, Gotoh M, Monden M, Kanai T, Yamamoto H, Kawai M, Dono K, Ukei T, Umeshita K, Mori T. Intraductal injection of collagenase solution at the time of harvesting: a possible solution for preservation and collagenase digestion. Transplant Proc 1990; 22 (2): 782.
196. Kneteman NM, Wagner T. Critical components of storage fluid for pancreas preservation before islet isolation. Transplant Proc 1992; 24 (6): 2824.
197. Contractor HH, Johnson PR, Chadwick DR, Robertson GS, London NJ. The effect of UW solution and its components on the collagenase digestion of human and porcine pancreas. Cell Transplant 1995; 4 (6): 615.
198. Kneteman NM, Lakey JR, Kizilisik TA, Ao Z, Warnock GL, Rajotte RV. Cadaver pancreas recovery technique. Impact on islet recovery and in vitro function. Transplantation 1994; 58 (10): 1114.
199. Lakey JR, Rajotte RV, Warnock GL, Kneteman NM. Lakey JR. Human pancreas preservation prior to islet isolation. Cold ischemic tolerance. Transplantation 1995; 59 (5): 689.
200. 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.
201. Robertson GSM, Chadwick D, Thirdborough S, at al. Human islet isolation: A prospective randomised comparison of pancreatic vascular perfusion with hyperosmolar citrate or University of Wisconsin solution. Transplantation 1993; 56:650.
202. Benhamou PY, Watt PC, Mullen Y, et al. Human islet isolation in 104 consecutive cases. Factors affecting isolation success. Transplantation 1994; 57 (12): 1804.
203. Ketchum RJ, Nicolae M, Jahr H, et al. Analysis of donor age and cold ischemia as; factors in cadaveric human islet isolation. Transplant Proc 1994; 26:596.
204. Lakey JR, Rajotte RV, Warnock GL, Kneteman NM. Human pancreas preservation prior to islet isolation: Cold ischemic tolerance. Transplantation 1995; 59:689.

205. Hering BJ, Ricordi C. Islet transplantation for patients with type 1 diabetes: results, research priorities and reasons for optimism. Graft 1999; 2:12.
206. Kuroda Y, Kawamura T, Suzuki Y, Fujiwara H, Yamamoto K, Saitoh Y. A new, simple method for cold storage of the pancreas using perfluorochemical. Transplantation 1988; 46 (3): 457.
207. Fujino Y, Kuroda Y, Suzuki Y, et al. Preservation of canine pancreas for 96 hours by a modified two-layer (UW solution/perfluorochemical) cold storage method. Transplantation 1991; 51 (5): 1133.
208. Kuroda Y, Fujino Y, Morita A, Tanioka Y, Ku Y, Saitoh Y. Oxygenation of the human pancreas during preservation by a two-layer (University of Wisconsin solution/perfluorochemical) cold-storage method. Transplantation 1992; 54 (3): 561.
209. Fujino Y, Kuroda Y, Suzuki Y, et al. Preservation of canine pancreas for 96 hours by a modified two-layer (UW solution/perfluorochemical) cold storage method. Transplantation 1991; 51 (5): 1133.
210. Fujino Y, Suzuki Y, Tsujimura T, et al. Possible role of heat shock protein 60 in reducing ischemic-reperfusion injury in canine pancreas grafts after preservation by the two-layer method. Pancreas 2001; 23 (4): 393.
211. Kuroda Y, Fujita H, Matsumoto S, et al. Protection of canine pancreatic microvascular endothelium against cold ischemic injury during preservation by the two-layer method. Transplantation 1997; 64 (7): 948.
212. Matsumoto S, Kuroda Y, Suzuki Y, Ku Y, Fujita H, Saitoh Y. Thromboxane A2 synthesis inhibitor OKY046 ameliorates vascular endothelial injury of pancreas graft during preservation by the two-layer UW solution/perfluorochemical method at 20 degrees C. Transplant Proc 1997; 29 (1–2): 1359.
213. Lane TA, Lamkin GB. Paralysis of phagocyte migration due to an artificial blood substitute. Blood 1984; 64 (2): 400.
214. Kuroda Y, Morita A, Fujino Y, Tanioka Y, Ku Y, Saitoh Y. Successful extended preservation of ischemically damaged pancreas by the two-layer (University of Wisconsin solution/perfluorochemical) cold storage method. Transplantation 1993; 56 (5): 1087.
215. Kuroda Y, Matsumoto S, Fujita H, et al. Resuscitation of ischemically damaged pancreas during short-term preservation at 20 degrees C by the two-layer (University of Wisconsin solution/perfluorochemical) method. Transplantation 1996; 61 (1): 28.
216. Matsumoto S, Kuroda Y, Fujita H, et al. Extending the margin of safety of preservation period for resuscitation of ischemically damaged pancreas during preservation using the two-layer (University of Wisconsin solution/perfluorochemical) method at 20 degrees C with thromboxane A2 synthesis inhibitor OKY046. Transplantation 1996; 62 (7): 879.
217. Deai T, Tanioka Y, Suzuki Y, Kuroda Y. The effect of the two-layer cold storage method on islet isolation from ischemically damaged pancreas. Kobe J Med Sci 1999; 45 (3–4): 191.
218. Matsumoto S, Qualley S, Rigley T et al. Prolonged preservation of the human pancreas prior to islet isolation using the two-layer (University of Wisconsin solution [UW]/perfluorochemical) method. Transplantation 2000; 69:8213.
219. Tanioka Y, Sutherland DE, Kuroda Y, et al. Excellence of the two-layer method (University of Wisconsin solution/perfluorochemical) in pancreas preservation before islet isolation. Surgery 1997; 122 (2): 435.
220. Matsumoto S, Rigley TH, Qualley SA, Kuroda Y, Reems JA, Stevens RB. Efficacy of the oxygen-charged static two-layer method for short-term pancreas preservation and islet isolation from nonhuman primate and human pancreata. Cell Transplant 2002; 11 (8): 769.
221. Iwanaga Y, Suzuki Y, Okada Y, et al. Ultrastructural analyses of pancreatic grafts preserved by the two-layer cold-storage method and by simple cold storage in University of Wisconsin solution. Transpl Int 2002; 15 (8): 425.
222. Matsumoto S, Kandaswamy R, Sutherland DE, et al. Clinical application of the two-layer (University of Wisconsin solution/perfluorochemical plus O_2) method of pancreas preservation before transplantation. Transplantation 2000; 70 (5): 771.
223. Hiraoka K, Trexler A, Fujioka B, et al. Optimal temperature in pancreas preservation by the two-layer cold storage method before islet isolation. Transplant Proc 2001; 33:891.
224. Hiraoka K, Kuroda Y, Suzuki Y, et al. Outcomes in clinical pancreas transplantation with the two-layer cold storage method versus simple storage in University of Wisconsin solution. Transplant Proc 2002; 34 (7): 2688.
225. Hering BJ, Kandaswamy R, Harmon JV et al. Insulin independence after single-donor islet transplantation in type 1 diabetes with hORT3gamma-1 (ala-ala), sirolimus, and tacrolimus therapy. Am J Transplant 2001; 1 (suppl): 180.
226. Stevens RB, Matsumoto S, Lawrence O, et al. Ischemically damaged human pancreas can be resuscitated by the two-layer method before islet isolation: Implications for clinical islet transplantation. Am J Transplant 2001; 1 (suppl 1): 321.
227. Ricordi C, Fraker C, Szust J et al. Towards making every pancreas count: significant improvement in human islet isolation from marginal (older) donors following addition of oxygenated perfluorocarbon to the cold storage solution. Am J Transplant 2002; 2 (suppl 3): 229.
228. Tsujimura T, Kuroda Y, Kin T, et al. Human islet transplantation from pancreases with prolonged cold ischemia using additional preservation by the two-layer (UW solution/perfluorochemical) cold-storage method. Transplantation 2002; 74 (12): 1687.
229. Lakey JR, Tsujimura T, Shapiro AM, Kuroda Y. Preservation of the human pancreas before islet isolation using a two-layer (UW solution-perfluorochemical) cold storage method. Transplantation 2002; 74 (12): 1809.
230. Matsumoto S, Kuroda Y. Perfluorocarbon for organ preservation before transplantation. Transplantation 2002; 74 (12): 1804.
231. Miyamoto M, Morimoto Y, Balamurugan AN, et al. Improvement of modified two-layer preservation method (PFC/Kyoto solution) in islet isolation from breeder pigs. Transplant Proc 2000; 32 (7): 1660.
232. Fraker CA, Alejandro R, Ricordi C. Use of oxygenated perfluorocarbon toward making every pancreas count. Transplantation 2002; 74 (12): 1811.
233. Hering BJ, Matsumoto I, Sawada T, et al. Impact of two-layer pancreas preservation on islet isolation and transplantation. Transplantation 2002; 74 (12): 1813.
234. Belzer FO, Ashby BS, Dunphy JE. 24-hour and 72-hour preservation of canine kidneys. Lancet 1967; 2 (7515): 536.
235. Burdick JF, Rosendale JD, McBride MA, Kauffman HM, Bennett LE. National impact of pulsatile perfusion on cadaveric kidney transplantation. Transplantation 1997; 64 (12): 1730.
236. Rolles K, Foreman J, Pegg DE. A pilot clinical study of retrograde oxygen persufflation in renal preservation. Transplantation 1989; 48 (2): 339.
237. Hassanein W, Zellos L, Tyrrell TA, et al. Continuous perfusion of donor hearts in the beating state extends preservation time and improves recovery of function. J Thorac Cardiovasc Surg 1998; 116:821.

238. Cecka JM, Terasaki PI. The UNOS scientific renal transplant registry. United Network for Organ Sharing. Clin Transpl 1995; 1.
239. Serrou B, Solassol C, Michel H, Gelis C, Pujol H, Romieu C. Eight- and twenty-four-hour canine pancreas preservations using a simple gel cooling technique. Transplantation 1973; 16 (5): 398.
240. Westbroek DL, De Gruyl J, Dijkhuis CM, et al. Twenty-four-hour hypothermic preservation perfusion and storage of the duct-ligated canine pancreas with transplantation. Transplant Proc 1974; 6 (3): 319.
241. Tersigni R, Toledo-Pereyra LH, Pinkham J, Najarian JS. Pancreaticoduodenal preservation by hypothermic pulsatile perfusion for twenty-four hours. Ann Surg 1975; 182 (6): 74378.
242. Brynger H. Twenty-four-hour preservation of the duct-ligated canine pancreatic allograft. Eur Surg Res 1975; 7 (6): 341.
243. de Gruyl J, Westbroek DL, Macdicken I, Ridderhof E, Verschoor L, van Strik R. Cryoprecipitated plasma perfusion preservation and cold storage preservation of duct-ligated pancreatic allografts. Br J Surg 1977; 64 (7): 490.
244. Toledo-Pereyra LH, Valjee KD, Chee M, Lillehei RC. Preservation of the pancreas for transplantation. Surg Gynecol Obstet 1979; 148 (1): 57.
245. Toledo-Pereyra LH, Chee M, Condie RM, Najarian JS, Lillehei RC. Forty-eight hours hypothermic storage of whole canine pancreas allografts. Improved preservation with a colloid hyperosmolar solution. Cryobiology 1979; 16 (3): 221.
246. Baumgartner D, Sutherland DE, Heil JE, Zweber B, Awad EA, Najarian JS. Cold storage of segmental canine pancreatic grafts for 24 hours. J Surg Res 1980; 29 (3): 248.
247. Florack G, Sutherland DE, Heil J, Zweber B, Najarian JS. Long-term preservation of segmental pancreas autografts. Surgery 1982; 92 (2): 260.
248. Florack G, Sutherland DE, Heil J, Squifflet JP, Najarian JS. Preservation of canine segmental pancreatic autografts: cold storage versus pulsatile machine perfusion. J Surg Res 1983; 34 (5): 493.
249. Robertson RP. Pancreatic islet transplantation for diabetes: successes, limitations, and challenges for the future. Mol Genet Metab 2001; 74 (1–2): 200.
250. Sutherland DE, Gruessner RW, Dunn DL, Matas AJ, Humar A, Kandaswamy R, Mauer SM, Kennedy WR, Goetz FC, Robertson RP, Gruessner AC, Najarian JS. Lessons learned from more than 1,000 pancreas transplants at a single institution. Ann Surg 2001; 233 (4): 463.
251. Griffin SM, Alderson D, Farndon JR. Comparison of harvesting methods for islet transplantation. Br J Surg 1986; 73 (9): 712.
252. Gruessner RW, Kendall DM, Drangstveit MB, Gruessner AC, Sutherland DE. Simultaneous pancreas-kidney transplantation from live donors. Ann Surg 1997; 226 (4); 471.
253. Humar A, Gruessner RW, Sutherland DE. Living related donor pancreas and pancreas-kidney transplantation. Br Med Bull 1997; 53 (4); 879.
254. Sutherland DE, Goetz FC, Najarian JS. Living-related donor segmental pancreatectomy for transplantation. Transplant Proc 1980; 12 (4 suppl 2): 19.
255. Roep BO, Stobbe I, Duinkerken G, van Rood JJ, Lernmark A, Keymeulen B, Pipeleers D, Claas FH, de Vries RR. Auto- and alloimmune reactivity to human islet allografts transplanted into type 1 diabetic patients. Diabetes 1999; 48 (3): 484.
256. Knechtle SJ, Hamawy MM, Hu H, Fechner Jr JH, Cho CS. Tolerance and near-tolerance strategies in monkeys and their application to human renal transplantation. Immunol Rev 2001; 183: 205.
257. Tzakis AG, Kato T, Nishida S, Levi D, Madariaga J, De Faria W, Nery J, Neff G, Kirk AD, Ruiz P. Campath-1H in intestinal and multivisceral transplantation; preliminary data. Transplant Proc 2002; 34 (3): 937.
258. Shapiro AM, Geng Hao E, Lakey JR, Finegood DT, Rajotte RV, Kneteman NM. Defining optimal immunosuppression for islet transplantation based on reduced diabetogenicity in canine islet autografts. Transplantation 2002; 74 (11): 1522.
259. Soria B, Roche E, Berna G, Leon-Quinto T, Reig JA, Martin F. Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. Diabetes 2000; 49 (2): 157.
260. Rafaeloff R, Pittenger GL, Barlow SW, et al. Cloning and sequencing of the pancreatic islet neogenesis associated protein (INGAP) gene and its expression in islet neogenesis in hamsters. J Clin Invest 1997; 99 (9): 2100.
261. Bonner-Weir S, Taneja M, Weir GC, et al. In vitro cultivation of human islets from expanded ductal tissue. Proc Natl Acad Sci USA 2000; 97 (14): 7999.
262. Soldevila G, Buscema M, Marini V, et al. Transfection with SV40 gene of human pancreatic endocrine cells. J Autoimmun 1991; 4 (3): 381.
263. Wang S, Beattie GM, Mally MI, Lopez AD, Hayek A, Levine F. Analysis of a human fetal pancreatic islet cell line. Transplant Proc 1997; 29 (4): 2219.
264. Lee HC, Kim SJ, Kim KS, Shin HC, Yoon JW. Remission in models of type 1 diabetes by gene therapy using a single-chain insulin analogue. Nature 2000; 408 (6811): 483.
265. Halvorsen TL, Beattie GM, Lopez AD, Hayek A, Levine F. Accelerated telomere shortening and senescence in human pancreatic islet cells stimulated to divide in vitro. J Endocrinol 2000; 166 (1): 103.
266. Levine F, Leibowitz G. Towards gene therapy of diabetes mellitus. Mol Med Today 1999; 5 (4): 165.
267. Cheung AT, Dayanandan B, Lewis JT, et al. Glucose-dependent insulin release from genetically engineered K cells. Science 2000; 290 (5498): 1959.
268. Patience C, Le Tessier P, Takeuchi Y, Weiss R. Endogenous retroviruses; a potential problem for xenotransplantation? Ann N Y Acad Sci 1998; 862; 67.
269. Wilson CA, Wong S, Muller J, Davidson CE, Rose TM, Burd P. Type C retrovirus released from porcine primary peripheral blood mononuclear cells infects human cells. J Virol 1998; 72 (4): 3082.
270. Blusch JH, Patience C, Takeuchi Y, et al. Infection of nonhuman primate cells by pig endogenous retrovirus. J Virol 2000; 74 (16): 7687.
271. van der Laan LJ, Lockey C, Griffeth BC, et al. Infection by porcine endogenous retrovirus after islet xenotransplantation in SCID mice. Nature 2000; 407 (6800): 90.
272. Vial CM, Ostlie DJ, Bhatti FN, et al. Life supporting function for over one month of a transgenic porcine heart in a baboon. J Heart Lung Transplant 2000; 19 (2): 224.
273. Cozzi E, White DJ The generation of transgenic pigs as potential organ donors for humans. Nat Med 1995; 1 (9): 964.

274. Fraga DW, Sabek O, Hathaway DK, Gaber AO. A comparison of media supplement methods for the extended culture of human islet tissue. Transplantation 1998; 65 (8): 1060.
275. Gaber AO, Fraga DW, Callicutt CS, Gerling IC, Sabek OM, Kotb MY. Improved in vivo pancreatic islet function after prolonged in vitro islet culture. Transplantation 2001; 72 (11): 1730.
276. Ricordi C, Angelico MC, Alejandro R, et al. Liver-islet transplantation in type 2 diabetes. Transplant Proc 1997; 29 (4): 2240.
277. Biancone L, Ricordi C. Pancreatic islet transplantation: an update. Cell Transplant 2002; 11 (4): 309.
278. Lanza RP, Chick WL. Immunoisolation: at a turning point. Immunol Today 1997; 18 (3):135.