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Review article

Islet transplantation

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Abstract: Recently, significant advances have been made in the number and purity of islets that can be retrieved from the human pancreas, thus enabling several centers to initiate or resume clinical trials of islet transplantation in type I diabetic patients. Although the success rate of islet transplantation is lower than that of pancreas transplantation in terms of achievement of insulin-independence, islet transplantation has significant potential advantages over vascularized pancreas transplantation: it is a simple and safe procedure; it has the potential to be performed on an outpatient basis; it offers access to cell banking after cryopreservation; it offers the potential advantages of pre-transplant reduction of immunogenecity; and it even offers the future feasibility of xenotransplantation. In this article, the current status of clinical trials and future perspectives of islet transplantation, including immunomodulation, immunotolerance, immunoisolation, and xenotransplantation, are reviewed.

Key words: islet, transplantation, xenotransplantation, bioartificial pancreas

Introduction

Clinical pancreas transplantation, not as an organ but as a tissue, was performed as early as 1894, when non vascularized minced fragments of fetal sheep pancreata were subcutaneously implanted to a diabetic patient.[1] This clinical attempt, however, failed without any endocrine function being observed. The first clinical transplantation of pancreas as an organ in patients with type I diabetes was reported by Kelly and coworkers in 1967.[2] A dramatic increase in the number of pancreas transplantations occurred beginning in the mid 1980s with the introduction of new immunosuppressive therapy. The success rate of pancreas transplantation was much improved, demonstrating long-term (more than 1 year) insulin-independence in more than 80% of recipients of pancreas grafts placed simultaneously with the kidney, in more than 70% of recipents of a pancreas after kidney, and in more than 60% of non-uremic recipents of a pancreas alone.[3]

On the other hand, clinical outcomes of islet grafts have been much less successful than those of whole pancreas grafts in type I diabetic patients, chiefly because of the difficulty in developing an effective islet isolation method.

In the 1980s, clinical trials with transplantation of pancreatic fragments (i.e., not isolated islets) were conducted.[4] Since 1985, applying the new techniques for islet isolation from the human pancreas, a few clinical trials of islet transplantation have been initiated.[5] Recently, significant advances have been made in the number and purity of islets that can be retrieved from the human pancreas,[6] thus enabling several centers to initiate or resume clinical trials of islet transplantation in type I diabetic patients.[7–10] Two hundred and thirty-six islet allotransplants have been performed in the past 8 years.[11] Although the success rate of islet transplantation is lower than that of pancreas transplantation in terms of achievement of insulin-independence, islet transplantation has significant potential advantages over vascularized pancreas transplantation: it is a simple and safe procedure; it has the potential to be performed on an outpatient basis; it offers access to cell banking after cryopreservation; it offers the potential advantages of pre-transplant reduction of immunogenecity; and it even offers the future feasibility of xenotransplantation. In this article, the current status of clinical trials and future perspectives of islet transplantation are reviewed.

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Clinical islet transplantation

Indications

Replacement of the patient's islets either by pancreas transplantation or by islet transplantation is the only treatment of type I diabetes to achieve an insulin-independent, constant normoglycemic state. According to the guidelines proposed by the American Diabetes Association,[12] pancreas transplantation should be considered appropriate therapy in the following circumstances:

(1) Panceas transplantation should be considered as an acceptable therapeutic alternative to continued insulin treatment in type I diabetic patients with end-stage renal disease who have had or plan to have a kidney transplant.
(2) In the absence of renal failure, pancreas transplantation should be considered a therapeutic alternative to insulin only in those unusual patients who exhibit a history of frequent acute and severe metabolic complications requiring medical therapy, clinical or emotional problems, or both, which make exogenous insulin therapy use unreasonable, or consistent failure of other insulin therapeutic approaches which result in frequent metabolic complications.

However, currently, indications for pancreas or islet transplantations exist almost exclusively in patients with end-stage renal disease who are waiting on dialysis for a kidney graft or in diabetic patients with established kidney graft obliged to be immunosuppressed. Future improvement in the results of clinical islet transplantation will enable us to extend the indications to non-uremic, non-kidney-transplanted type 1 diabetes patients.

Islet isolation

As first described by Lacy and Kostianovsky in 1967,[13] isolation of islet tissue was performed by collagenase digestion of pancreatic tissue. Many attempts have been made to transfer the techniques developed in rodents to large animal models or directly to clinical trials. A major problem has been that the pancreas of large mammals, including humans, is more fibrotic than in rodents. Prominent progress in this area has been the development of a technique for retrograde perfusion with collaganase solution into the ductal system of the pancreas before mechanical dispersion.[14] This technique was further developed in the dog, before being used on human pancreata, by Gray and Morris.[15] The procedure developed involved the injection of a high concentration of collagenase into the pancreatic duct under pressure, followed by incubation of the whole gland. The pancreas was then dispersed by teasing and shaking, and the islets were separated by filtration, followed by centrifugation on a Ficoll density gradient. A different approach to control collagenase digestion has been advocated by Scharp[16] and coworkers. They designed a digestion-filtration chamber that prevented islets isolated early in the procedure from becoming overdigested and subsequently disintegrating, by allowing the withdrawal of already isolated islets from the collagenase solution and the partially digested exocrine tissue. This procedure has subsequently been further improved, automated, and modified for use on the human pancreas.[6] A critical step in the islet isolation process has been the purification procedure. Simple sedimentation is the easiest, but also the most inefficient method. In rodent experiments, centrifugation on Ficoll gradients proved to be most effective compared with the use of sucrose or albumin. It was learned that the islet purification technique determines the mass and the viability of islets as well as the in purity, which may greatly affect the degree of islet implantation, islet immunogenicity, and safety for the islet recipient. Standard methods for purification are currently performed using Ficoll-sodium diatrizoate, nicodenz or bovine serum albumin, and a COBE 2991 cell-separator (COBE BCT, Inc. Lakewood. Co. USA) for large-scale purification of islet preparations from higher mammalian and human pancreata.[17,18]

Islet cryopreservation

Preservation of islets has been performed using culture techniques.[19] Long-term preservation of large amounts of isolated islets of Langerhans could be preferentially performed with cryopreservation techniques. Isolated adult islets of Langerhans derived from rodent pancreas can be frozen and stored at −196 °C for months or years without evident loss of viability.[19,20] Cryopreservation methods have recently been extended to long-term preservation of higher mammalian and human isolated islet tissue.[21] These data indicated that indefinite storage of islets is possible by cryopreservation and the methods have been used to successfully freeze islets from most species. Cryopreservation of islets is the only reliable mode for long-term storage, which will probably facilitate the clinical use of islet transplantation.

Minimal requirements for islet preparations

Several quality control measures need to be evaluated to ascertain whether an islet preparation merits transplantation into a patient. Before islet transplantation, parameters such as islet yield (in terms of islet number and islet volume), islet purity, islet viability, and islet sterility must be assessed. The most crucial point will be the transplantability, in other words determination of

the in-vitro parameter that best predicts the in-vivo endocrine effect after islet transplantation.

Islet number

The minimal number of islets required to render an adult type 1 diabetic patient insulin-independent should be about 6000 IEQ/kg (IEQ; islets of 150 μm equivalent diameter).[22] Stained islets are counted according to diameter class, using a calibrated grid in the eyepiece of a phase contrast microscope. Particles smaller than 50 μm are not considered and islets larger than 350 μm are not further subdivided. The mean volume for each diameter class is assessed by using conversion factors to determine the equivalent number of islets of 150-μm-diameter.

Islet purity

The transplantation of highly purified islet preparations has potential advantages of increased safety,[23] reduced immunogenicity of the graft, and improved islet implantation. Islet preparations with high purity, of more than 80% (>80%; percentage of islet volume in total cell volume) should be used for transplantation purposes.[22]

Islet viability and endocrine function

Islet viability is a critical factor that determines the outcome of transplantation. For rapid assessment of islet viability before islet transplantation, a fluorometric assay, with inclusion and exclusion dyes that allow discrimination between intact and damaged cells, is now widely used and recommended.[24] It is crucial that the isolated islets be shown not only to be viable but also able to respond appropriately to a glucose challenge. The standard in assessing in-vitro islet endocrine function is the perfusion of islets with glucose, which provides a dynamic profile of the characteristics of glucose-mediated insulin release from pre-stored and newly synthesized insulin and of the ability of the islet endocrine cells to downregulate insulin secretion after the glycemic challenge is interrupted (return to baseline).[25] Standards for reporting results of perfusion studies are critical for the accurate comparison of data. The absolute levels of insulin secretion during the prechallenge baseline period, high glucose challenge, and the last period of perfusion after return to a low glucose concentration should be reported. The profile of insulin release is best reported as a plot that shows the release during the three consecutive periods. The stimulation index, estimated by determining the ratio between basal (last 15 min before high glucose and last 15 min after return to basal conditions) and stimulated insulin release (first 15 min and last 15 min of stimulation) identifies the secretory capacity.[26]

Islet sterility

Testing should start as usual with donor screening for viral antibodies (hepatitis A, B, and C; human immunodeficiency viruses 1 and 2; and cytomegalovirus). The demonstration that islets to be transplanted are free from bioburden risk is an important quality control test. It has been demonstrated that 42% of human donor pancreases had low-level contaminants, usually of gram-positive bacteria, and 15% had fungal contaminants. During islet isolation, most of these contaminants are eliminated.[27]

Transplantation sites

Many investigators have utilized animal models of diabetes and transplantation to investigate alternative sites of transplantation, such as kidney capsule, splenic parenchyma, and free dispersion of islets within the peritoneal cavity. Other possible routes are embolization of islets to the lung via systemic vein, direct injection into the liver or into the spleen, subcutaneous tissue, and intramuscular, intratesticular, intrapancreatic, intraprostatic, or an intrasalivary gland approach. Although each has potential benefits in terms of technical simplicity, intrahepatic infusion of islets (vascular access via the portal vein with final lodging of the islets in the liver) is almost exclusively employed in current islet transplantation in humans. For several reasons, the liver has been the major site for islet cell transplantation in patients. Percutaneous transhepatic catheterization provides relatively simple and inexpensive, non-surgical access to the liver. Transplantation of islets within the portal triad was thought, on theoretical grounds, to place the islets upstream from the hepatic veins, thus limiting systemic insulinization. Systemic drainage can result in hyperinsulinemia, which frequently occurs after subcutaneous insulin injections or whole pancraes transplantation. Using a dog model, it has previously been shown that hyperisulinemia following pancreas transplantation was accompanied by changes in aortic lipid metabolism; these alterations may be precursors of atherogenic lesions in the aorta of recipient dogs.[28]

Lipid metabolic indices in canine recipients of islet autografts, however, were unchanged.[29] In addition, the liver is considered to be an immunologically privileged site.[30] Detailed analysis of islet transplant recipients, as reported by the Islet Transplant Registry, has revealed that patients who were C-peptide-negative prior to transplant, and who subsequently became insulin-independent, received intrahepatic islet transplants via the portal vein.[11,31] It has been demonstrated that human islet allografts can survive for long periods, during which time functional competence is sustained and islet structural integrity is maintained. In fact, intact islets (which contained well granulated β-cells) were documented in the liver of a patient 5 years after combined liver/islet transplantation.[32] However, of the islets that are embolized to the liver, it is not known whether a fraction is lost prior to vascularization. Primary nonfunction has been postulated to contribute to the pos-

sible early islet loss.[33] In fact, it has been proposed that an inflammatory response after islet cell transplantation may result in the release of cytokines that are known to impair secretion and β-cell survival.[34]

Transplantation procedure

Human pancreata will be obtained at the time of cadaver donor procurement operations. At the donor operation, the liver, kidney, and pancreas will be obtained in a standard fashion. After dissection, the abdominal viscera will be perfused in situ with cold preservation solution. The liver, kidneys, and pancreas will be excised, stored in sterile solution, and kept on ice for transportation. Critical issues in human pancreas procurement are the warm ischemia time, which should be as short as possible; no overperfusion and no intrapancreatic venous hypertension; cold ischemia time of no more than 12 h; and a well preserved pancreatic capsule that allows subsequent intraductal collegenase distension.[35] At present, most centers with their own experience in human adult islet isolations prefer to start the pancreas dispersion procedure with intraductal administration of collagenase, followed by an automated digestion-filtration using recirculating collagenase solution.[6] Once the islets are isolated and purified, qualitative and quantitative tests on the islets will be performed. Insufficient yields of fresh islets will be augmented with cultured or cryopreserved islets. In patients with simultaneous islet-kidney transplants or simultaneous islet-liver transplants, the islets will be transplanted by infusion into a branch of the portal vein immediately after implantation of the donor kidney or liver into the recipient. In patients with islet-after-kidney transplants or islet transplants alone, intraportal implantation of islets will be performed through a non-surgical approach, of a percutaneous transhepatic catherization technique.

Human fetal pancreas transplantation

During the past decade, a large number of clinical trials with transplantation of human fetal pancreata have been performed, mostly in China and Russia. No patient so far has been reported to become insulin-independent after such a transplantation.[31] The use of aborted human fetuses as donors is undesirable, mainly for ethical, but also for legal reasons. Furthermore, since many fetal glands can be assumed to be needed to cure one patient, it may be less likely that the fetal pancreas will be of use in the future.

Categories of islet transplantation

Clinical islet transplantation can be subdivided into four categories:

(1) Islet allografts in type I diabetic patients
(2) Islet allografts in pancreatectomized patients
(3) Islet allografts in "insulin-requiring" diabetic patients (type II diabetic patients, patients with cystic fibrosis)
(4) Islet autografts in pancreatectomized patients.

Evaluation of success rates

Transplantation efficacy and the outcome of clinical isolated adult islet transplantation should be assessed in several ways: (1) patient life expectancy; (2) graft functional survival (i.e., insulin independence; significant C-peptide secretion); (3) normalcy of the patient's metabolic state; (4) impact on diabetic complications; and (5) the patient's quality of life. Currently, the limited number of adult islet allografts allows us to assess the efficacy of this appealing method for treating type I diabetes mellitus only in regard to its impact on patient survival, graft survival, and metabolic control.

Current status

Islet allotransplantation in type I diabetic patients

Islet allotransplantation has been pursued in the past two decades with the goal of improving the quality of life of diabetic patients and preventing secondary complications. Between 1974 and 1997, 373 adult islet allografts were performed worldwide.[11] Of the 373 allografts, 326 were adult islet allografts in type I diabetic patients. Adult islet allotransplants in type I diabetic patients showed a prominent increase from 1990, reaching a total of 236 cases for the past 8 years in 24 institutions (Table 1).[11] The International Islet Transplant Registry[11] showed a detailed analysis of 170 patients, transplanted between 1990 and 1997, with type I diabetes, who were C-peptide-negative prior to islet transplantation. Patient and graft survivals (basal C-peptide >0.5 ng/ml) at 1 year were 95% and 32%,

Table 1. Adult islet allografts in patients with type-1 diabetes 1990–1997[11]

• No. of patients		236
• Institutions	Giessen	51
	Minneapolis	31
	Pittsburgh	25
	Milan	20
	Miami	17
	St. Louis	14
	18 Additional institutions	78
• Insulin-independent		32/236 (14%)
• Insulin-independent at ≥1 year		17/209 (8%)
• Longest insulin-indepence follow-up		45 Months
• Insulin-independent after 1:1 tx		16/151 (11%)

1997 data on file incomplete

respectively (Fig. 1), and a 1-year isulin independence rate of 14% was observed (Table 1).

Four criteria have been found to be important for success: (1) if islets are isolated from organs preserved for less than 8 h; (2) if more than 6000 islet equivalents (number of islets if all had a diameter of 150 μm) per kg body weight are transplanted; (3) if islets are transplanted into the portal vein; and (4) if an anti-T-cell antibody preparation is used for immunosuppression.[11,31] The recipient is age, sex, or duration of diabetes, as well as the number of donor pancreata and islet purity, did not appear to affect 1-year graft survival.[31] The number of patients with C-peptide more than 0.5 ng/ml more than 1 year post-transplant was higher when all four criteria were met (46%) than when only one of them was (20%). Similarly, the percentage of insulin-independent patients was significantly higher when all four criteria were met (29%) versus only one (1%).[31] Insulin-independence has been achieved in type I diabetic patients at different institutions.[7,8,10,22,36–42] Of the reported achievements in clinical allotransplantation in type I diabetic patients, the recent outstanding outcome at the Giessen Center in Germany is noteworthy.[22,41,42] Bretzel and colleagues[41] have analyzed the recent outcome of islet allografts at the Giessen Center with simultaneous kidney transplantation (SIK; simultaneous islet and kidney transplantation) or after kidney transplantation (IAK; islet after kidney transplantation), demonstrating an excellent graft survival rate of 89% in patients with SIK and 54% in those with IAK. The 1-year insulin-independence rate was 33% in SIK patients and 23% in IAK patients. These encouraging results appear to be associated with well established islet quality control,[25] comprehensive immunosuppressive induction and maintenance therapy in the recipients, strategies aimed at improved islet engraftment, and strict posttransplant metabolic control. The longest duration of graft function and insulin-independence has been reported to be for more than 7 years and 2 months and more than 3 years and 5 months, respectively.[11]

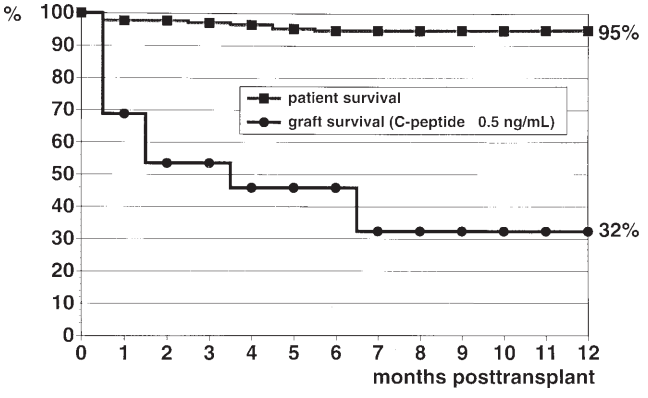

Fig. 1. One-year patient and graft survivals in 170 type I diabetic patients, transplanted between 1990 and 1997, who were C-peptide-negative prior to islet transplantation (from the International Islet Transplant Registry, 1998[11])

Islet allotransplantation in pancreatectomized patients

Between 1974 and 1997, a total of 47 islet allografts were performed in pancreatectomized patients.[11,18,43–47] Analysis of 15 allografts in pancreatectomized patients between 1990 and 1996 demonstrated an insulin-independence rate of 40% at 1 year posttransplant. Nine of the 15 patients in whom islets were simultaneously allografted with the liver became insulin-independent, 7 at the University of Pittsburgh,[43,44] 1 at the University at Milan,[45] and 1 at the Universities of Giessen and Wuerzburg.[46] Nearly all of these patients eventually died of recurrent metastatic disease while still being insulin-independent. The longest period of insulin independence was noted in the first Pittsburgh patient, who died of recurrent malignancy while being insulin-independent almost 5 years after a simultaneous islet-liver transplant.[44] At autopsy, insulin-containing islets were readily detected in liver specimens.

Islet autotransplantation

Islet cell autotransplantation is used for patients with intractable pain from small duct chronic pancreatitis or failed drainage procedures in whom total or near-total pancreatectomy may be the only treatment option. The results of 149 islet autografts performed at 21 different institutions between 1977 and 1994 have been published and/or have been reported to the Islet Transplant Registry.[11] In the great majority of patients, unpurified islet tissue was implanted into the liver via the portal vein. The University of Minnesota group has the largest experience.[48–50] The main predictor of insulin-independence was the number of islets transplanted. Of 14 patients who received more than 300000 islets, 74% were insulin-independent at more than 2 years posttransplant.[50] According to the recent International Islet Transplant Registry,[11] 69 islet autografts were performed between 1990 and 1996, demonstrating a rate of insulin-independence of 80% at 1 week or more, and a rate of 61% at 1 year or more posttransplant (Table 2). The longest period of insulin-independence in autografts has been reported to be more than 7 years (Table 2).

Perspectives of islet transplantation

Transplantation of isolated islets appears to be the most direct and appealing approach for treating type I diabetes mellitus. The field of islet cell transplantation has progressed rapidly over the past decade. Islet transplantation can reverse diabetes, and even in the absence of

Table 2. Islet autografts from 1990 to 1996[11]

• Institutions	Minneapolis	36
	Leicester	16
	Geneva	9
	Pittsburgh	5
	Three other Institutions	3
• No. of patients from 1990 through Dec. 31, 1996		69
• Insulin independence at ≥1 week[a]		33/41 (80%)
• Insulin independence at ≥1 year		20/33 (61%)
• Longest follow-up of insulin-independence		>7 years

[a] Only well-documented cases

insulin-independence, superior metabolic control can be achieved. Maintenance of normoglycemia without intensive insulin therapy, and eradication of severe hypoglycemia and diabetic ketoacidosis, while preserving normal Hemoglobin A_{1C} ($HgbA_{1C}$),[38,51] are also important goals of islet transplantation in terms of improvement of quality of life. However, at present, islet transplantation still requires major research breakthroughs, and several obstacles should be eliminated before this promising approach is universally recognized as a widespread and ultimate treatment of type I diabetes mellitus. With respect to obstacles to be eliminated, problems of human islet yield and purity are progressively being overcome.[6,16,17,18] The success rate of insulin-independence is greater in islet autografts than in islet allografts (Tables 1 and 2), indicating the problem of the immunological response to allogenic islets. The potential destruction of islets by the recipient immune system continues to play an important role in failure in considerable numbers of patients with intraportal islet allografts, because of alloreactivity and underlying autoimmune disease.[52] The invariable requirement of general immunosuppression also affects the survival of islet grafts. Another issue concerns the relative inadequacy of cadaveric donor organ availability. Aggressive research efforts are now in progress to develop immunoalteration or immunotolerance, to accomplish the creation of a bioartificial pancreas with immunoisolation barrier membranes, and to create xenogeneic islets or human/non-human engineered insulin-producing cells suitable for grafts with an excellent immunoisolation system.

Drug toxicity

The transplanted islet mass is subjected to the toxic side effects of the immunosuppressive drugs developed for solid-organ transplants. Many of the commonly used immunosuppressive drugs are diabetogenic. Although present-day immunosuppression is dangerous, extraordinary advances are being made in the field of immunology that should lead to the development of more selective and safer approaches. The well documented risk for the development of neoplasms is of special concern.[53] Glucocorticoids are particularly toxic and have adverse effects on islet function,[54] so there is hope that some steroid-sparing regimens, using such promising drugs as 15-deoxyspergualin, leflunomide, mycophenolate mofetile, and rapamycin, will turn out to be useful. Immune reactions against islets may differ from those found with solid-organ transplants and thus may require specially tailored drug regimens.

Immune-mediated destruction

In type I diabetes recipients of islet allografts, more than 50% of the grafts lose function either immediately after transplant or during the first 6 weeks.[52] If these early losses are excluded from the analysis of islet graft survival, approximately 80% the islet allografts would be functioning at 1 year. It has been suggested that the early response to islet allografts may include a nonspecific inflammatory reaction capable of inducing severe β-cell damage through the release of cytokines and free radicals.[55] β-Cells are extremely vulnerable to free-radical injury, possibly because of their low activities of mitochondrial superoxide dismutase and glutathione peroxidase, which act as scavengers.[56] Macrophages infiltrate freshly transplanted islet grafts and predominate in grafts exhibiting poor initial function after transplantation.[57] The immune-mediated destruction of free islets may also involve two other mechanisms in addition to non-specific inflammatory reaction; antigen-specific T-cell-mediated cytolysis[58] or autoimmune beta cell destruction.[59] The success of human islet autografts clearly indicates that the islet graft-directed immune response remains the most pressing issue. It seems plausible at this juncture that the function of islet transplantation in type I diabetes patients will depend on our ability to either potentiate and refine conventional techniques or, more likely, to develop alternative approaches for graft immunoprotection.

Immunomodulation and induction of immunotolerance

An attractive feature of free islets is the ability to immunomodulate the tissue prior to implantation; something which cannot be done effectively with the whole organ. Attempts to attenuate islet immunogenicity at a pretransplant level by different approaches, such as utilizing culture,[60] antibody,[61] or deoxyguanosine pretreatment,[62] or irradiation,[63] have so far provided convincing evidence of prolongation of function only in rodents. These protocols have been used in large animal models, but either no benefit,[64] or only slight prolongation of islet graft function has been observed.[65] Induction of immune tolerance would represent the endpoint for overcoming

the basic immunological problems associated with tissue/organ transplantation. Very impressive experimental results have been reported in which intrathymic preimmunization with islet cells or splenocytes and transient host immunosuppression[66,67] have indefinitely prolonged islet allograft survival in streptozotocin-treated rodents. The thymus, because of its peculiar anatomic configuration and central immunological role, could, in fact, offer a unique opportunity for the host to become unresponsive to subsequently grafted islets or other cell types of allogeneic donor origin implanted even in extrathymic sites. Very promising, yet fully experimental, are frontier studies on the possibility of inducing donor-specific unresponsiveness to islet allografts by creating microchimerism with peripheral infusion of donor bone marrow. The initial association of microchimerism with successful kidney and liver allotrasplantation[68] has been substantiated by clinical trials of renal allografts, using donor bone marrow transfusions, that have demonstrated the presence of chimerism in the absence of rejection episodes. The rationale for this clinical initiative is that peritransplant infusion of donor bone marrow cells offers a hope of reducing alloreactivity and the recurrence of autoimmunity.

Cryopreservation

A difficulty in envisioning large-scale islet cell transplantation has been the availability of islets themselves. Over the past decade investigators have improved the technique of cryopreservation, which will allow the collection and storage of viable human islets in tissue banks to allow pooled human grafts. Cryopreservation became available for rodent and canine islets in 1983 and subsequently for adult human islets[69] and human fetal islets.[70] The current process involves the addition of a cryoprotective additive (dimethylsulfoxide), freezing and thawing of tissue, and return to a physiological medium. The best results have been achieved with slow cooling to $-40\,^{\circ}C$, in combination with rapid thawing from $-196\,^{\circ}C$.[21] Using combinations of cryopreserved-pooled islets and fresh islets, researchers at the University of Alberta treated five patients.[71] One of their subjects, treated with 10 000 islet equivalents/kg became insulin-independent at 69 days and has remained insulin-independent for 2.5 years. Improvement in cryopreservation techniques will be indispensable for the future establishment of tissue banks for pooled xenogeneic islet cells or gene-manipulated insulin-producing cells, as well as human islets.

Transplantation site

Currently, the intrahepatic infusion of islets is exclusively employed in clinical trials in many institutions. However, the most appropriate site for implantation still remains to be determined. Although most of the data were derived from experimental studies, a number of pitfalls may be linked to the intrahepatic localization of islets. A significant failure rate of initially successful intraportal islet autografts has been reported compared with fairly successful intrasplenic islet autografts in dogs and sub-human primates.[72–74] Intrahepatic islets are localized downstream from the pancreas, which produces glucagon, somatostatin, and noradrenalin overflow, all having an inhibitory effect on beta-cell secretion.[75] Intrahepatic islets are reinnervated by the hepatic nerve fibers, which in the periportal areas, seem to be mainly noradrenergic and which therefore have an inhibitory action on insulin secretion.[57,76] Intrahepatic islets are exposed to Kupffer cells, the largest population of fixed tissue macrophages in the body.[77] Intraheptatic autologous islets do not restore a glucagon response to insulin-induced hypoglycemia,[49] in contrast with the increased glucagon response to hypoglycemia and increased rates of recovery from hypoglycemia observed after intrasplenic islet autotransplantation in totally pancreatectomized dogs.[78] Sites other than liver implantation sites, such as an intrasplenic site or omentum pouch should also be carefully investigated as possible optimal transplantation sites. Subcutaneous transplantation[79,80] will be the most interesting and promising procedure for future clinical islet transplantation.

Xenotransplantation

If islet transplantation is to become a widespread treatment for type I diabetes, solutions must be found for increasing the availability of insulin-producing tissue, since islet tissues, as well as other transplant organs, are limited on the basis of human organ donation. In an attempt to overcome the serious problem of donor supply, insulin-producing tissues from abundant and accessible sources will be considered for clinical transplantation. Alternative sources of insulin-producing tissues could be: (1) porcine,[81–89] and bovine[90] islets, (2) fish-brockman bodies,[91] (3) genetically engineered insulin-secreting cell lines,[92–95] and (4) in vitro production of human fetal,[96] or adult[97] β-cells. Among these candidates for alternative sources, porcine islets or genetically engineered insulin-secreting β-cells will be the most promising source for clinical application in the near future.

Isolation of adult pig islets

Pigs are an attractive source of donors for islet tissues, chiefly because of their abundance and because of the structural similarity between human and porcine insulin.[98] Moreover, pigs are omnivores, and their glucose levels are similar to those of humans. Another attractive features is that pigs can be subjected to genetic manipu-

lation, which means that transgenic pigs can be developed with genes expressed in their β-cells that could help resist immune attack and even enhance insulin secretion. However, the establishment of an isolation method for adult pig islets has been tremendously difficult, because of the marked fragility of the islets and the rapid dissociation of the pancreas into single cells during the isolation procedure.[81,82] In 1990, Ricordi et al.[83] improved the isolation procedure, introducing an automated method. Finke et al.[84] have described a superior method for the large-scale isolation of islets from the adult porcine pancreas, and they minimized the warm ischemia time to 8–15 min. We have a achieved excellent isolation and recovery of pure islets, performing cannulation of the pancreatic duct in the slaughterhouse at the beginning of the storage period before transportation to the laboratory.[85] We have also developed a two-stage digestion procedure,[89] employing acidic oxidative potential water.[88] Further studies must be done regarding various immunosuppressive regimens, pretransplant immunomodulation protocols,[99,100] and immunoisolation techniques for the prevention of islet xenograft rejection.

Neonatal porcine islets

There are complex arguments about the optimal source of porcine islet tissue, with various reasons being put forth to support the use of mid-fetal, late-fetal, neonatal, market-weight, and older pigs. Although most of the data show that better islet yields are obtained from older pigs, improvements are being made in harvesting islets from younger market-weight pigs, which would be a more practical and less expensive source of tissue. The potential use of porcine fetal pancreas tissue is attractive because of the capacity for growth and ease of maintaining sterility. In addition, the procedure for obtaining this tissue is less traumatic than that used for adult pancreas, so the cells are hardier when placed into cultured or transplanted. These fetal pancreatic cell preparations are very complex; fortunately, the exocrine cells spontaneously die off when cultured or transplanted, but the surviving population consists of a mixture of mesenchymal, precursor, protodifferentiated, and mature islet cells. There is a great need to learn more about how these cells develop so as to maximize growth capacity and optimize function when they are transplanted. Much work has been done on pancreases removed at the mid-fetal stage of 60–90 days' gestation, which can normalize glucose levels in recipient mice with diabetes.[101–103] Neonatal porcine islets have an inherent ability for growth both in vitro and in vivo, and one approach to more rapidly correct diabetes is to enhance the growth and proliferation of new β-cells in vitro prior to transplantation so that the islets contain a majority of endocrine cells.[104] The neonatal porcine pancreas could be used for the isolation of a large number of functionally viable islet cells, and, because of their ready availability and inherent capacity to proliferate and differentiate, they constitute an attractive source of insulin-producing tissue for studies of islet cell neogenesis or as a source of xenogeneic islet cells for clinical transplantation. Before neonatal porcine islets can be considered for application in humans, key immunological problems need to be solved. Especially, xenotransplantation between discordant species (e.g., pig-to-human) has been hindered by the occurrence of hyperacute rejection. The mechanisms responsible for xenograft rejection are complicated, but are being rapidly elucidated because of the drive to use xenografts for heart, liver, and islet transplantation. With organ transplants, hyperacute rejection occurs, mediated by preformed antibodies that bind the Gal alpha (1, 3) Gal epitope (also known as the Gal epitope) of transplanted cells and act with complement to cause rapid cell death, with the most serious target being endothelial cell.[105,106] This hyperacute rejection phenomenon may be less of a problem with islet transplantation because islet cells seem to have very little of the Gal epitope,[107] and because the vascularization of transplanted islets seems to come entirely from recipient endothelial cells.[108] Hyperacute rejection may be more of a problem for fetal or neonatal pancreas cells, because duct cells, which are precursor cells for islet formation, appear to express the Gal epitope.[109] Unfortunately, the xenograft rejection process is far more complex than just Gal epitope-dependent hyperacute rejection; there seem to be other antibody-and complement-mediated reactions, as well as a variety of cell-mediated assaults that provide a serious challenge to the success of these transplants.[105]

Clinical islet xenotransplantation

Fetal porcine islet cells have been used for the treatment of type I diabetes mellitus by Groth and colleagues in Sweden.[110–112] Although there was no obvious clinical evidence to indicate the effectiveness of this treatment for glucose metabolism, all patients tolerated the procedure well and no adverse side effects were observed. In several patients, porcine C peptide, measured with a radioimmunoassay specific for porcine C peptide, was detected for several months after transplantation, thereby suggesting a release of insulin from the graft. This clinical trial demonstrates that xenogeneic transplantation of fetal porcine islet-like cell clusters to humans could be performed, provided that allergic, hemodynamic, or coagulation disturbances were not induced by the xenogeneic cells, and that transfer of viruses or other microorganisms could be excluded. A recent report from the same institution shows that there is no evidence of infection with porcine endogenous retrovirus in these recipients.[111] The immunologic problems should be extensively examined and overcome before porcine islet-cell xenotransplantation

is universally accepted as an effective treatment for type I diabetes mellitus.

Genetically engineered β-cell lines

Much attention is being focused on the general problem of β-cell growth, development, and function in the hope of finding new sources of insulin-producing cells for transplantation. Because the β-cell mass cannot be expanded in a meaningful way either in vivo or with tissue culture, an increasing number of investigators are working on such basic problems as embryology of the endocrine pancreas,[113] differentiation of duct cells,[114] mechanisms of β-cell replication,[115] and apoptosis of β-cells.[116] Even in adulthood, new β-cells are constantly produced either by differentiation of pancreatic duct cells or through replication of preexisting β-cells.[114] The hope is that with the right combination of growth and differentiation factors, or with some genetic manipulation, β-cell expansion could provide cells for transplantation. The most adequate approach to expansion is to create β-cell lines. Insulin-secreting pancreatic β-cell lines represent a promising approach for the treatment of insulin-dependent diabetes mellitus. Such cell lines can provide an abundant and reproducible source of β-cell material for transplantation. A number of highly differentiated β-cell lines have been developed using transgenic mice.[92,95,113,116] These cells produce insulin in amounts comparable to that in normal pancreatic islets, and they release it in response to physiological insulin secretagogues. The development of approaches to tightly regulate cell replication has made it possible to use these cells in restoring and maintaining euglycemia in diabetic animals. Cell engineering with adenovirus genes that reduce cell immunogenicity allowed successful transplantation across allogeneic barriers without immunosuppression or immunoisolation.[117] Although the development of human β-cell lines by genetic engineering still awaits further and extensive investigation, research on the creation of genetically engineered β-cells will, in the future, hold the promise of replacing insulin injections as an accurate, convenient and safe method for the long-term maintenance of euglycemia in type I diabetic patients.

Immunoisolation

Among the alternative strategies being developed for immunoprotection of islet grafts without immunosuppression, immunoisolation within selective permeable and highly biocompatible artificial membranes actually seems to incorporate safer and simpler features.[118,119] The principle of the bioartificial pancreas is that the permeability of the membrane would be open enough to allow nutrients and oxygen to reach the islets and for insulin to be released into the bloodstream, but restrictive enough to exclude immune cells and even antibodies (Fig. 2). Remarkably, islets completely separated from their normally rich blood supply and innervation can survive and function well inside such devices. Two major types of immunoisolatory membranes are available at present; microencapsulation and macroencapsulation.

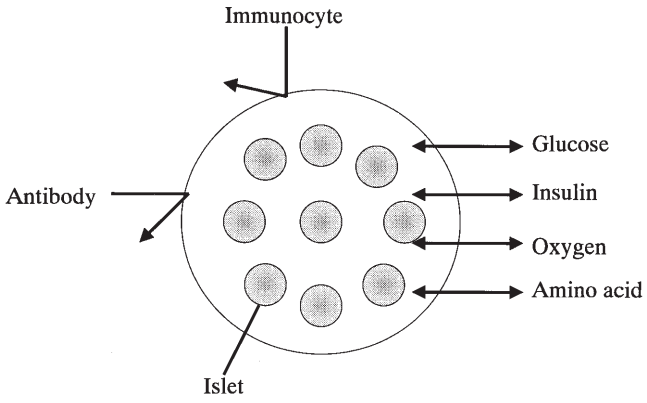

Fig. 2. Scheme that illustrates the principle of the bioartificial pancreas with immunoisolatory membrane

Microencapsulation

The most widely used approach is to contain islets within a bead of alginate gel and then coat the bead with poly-L-lysine or some other material to provide permselectivity and strength.[120–125] These capsules were originally made with a diameter of around 800 μm, but can now be produced in the range of 300 μm or even as a conformal coating adherent to the surface of the islet. Another promising approach is to use polyethylene glycol as a conformal coating photopolymerized to the surface of the islet with eosin Y.[125] The possibility of using other materials is also being explored.[124] Interest in the potential of microencapsulation has been enhanced by a report in which monkeys with spontaneous diabetes given adult porcine islets contained in alginate/polylysine capsules were cured for periods as long as 803 days without immunosuppression.[121] Although a preliminary clinical transplantation of microencapsulated allografts in diabetic patients has been performed, this clinical trial provided encouraging but only partial results.[123] There are still unresolved problems concerning the microcapsules, such as biocompatibility, diffusion limitation, fragility of microcapsules, size of microcapsules, and xenogeneic immunorejection. Our previous report showed that agarose was superior to both alginate and other materials in regard to both biocompatibility and diffusion efficiency.[126]

Although the conventional agarose-only microcapsule is effective in allotransplantation,[127] it is not as effective in xenotransplantation. Thus, additional immunosuppression or immunomodulation methods are needed in conventional microcapsules to prolong the

survival of xenografts.[128] In order to solve these problems, we have recently developed a new type of agarose microcapsule, in which the previous agarose-only microcapsule is modified to possess a three-layer coating for greater effectiveness in xenogenic islets transplantation.[80,129,130] Five percent polystyrene sulfonic acid (PSSa) was added to the agarose solution to increase the polymer concentration, resulting in better immunoisolation and greater physical strength. The PSSa is used because it mixes easily with agarose and does not interact with it. Moreover, PSSa is thought to be able to suppress complement activity, and, accordingly, to protect the encapsulated islet from immunorejection even if the antibody should enter the microcapsule. A polybrene coating was added to give the microcapsule greater stability and to prevent PSSa leakage by the formation of an agarose/polybrene polyion complex on the surface of the agarose/PSSa membrane. However, the use of the polybrene coating raised the problem of poor biocompatibility as a result of exposure of the cationic surface, and so carboxymethyl cellulose (CMC), an anion that is biocompatible with surrounding tissues, was selected as the outermost coating.[129] An in-vivo xenotransplantation (rat-to-mouse) study has demonstrated that the improved three-layer agarose microcapsules can effectively prolong xenograft survival times without the use of immunosuppressive drugs (Fig. 3).[129] The results of the Intravenous Glucose Tolerance Test (IVGTT) study have also clearly demonstrated that transplantation of islets in microcapsules induced excellent improvement in the glucose curves of the recipients.[129] Furthermore, it has been demonstrated that the improved three-layer agarose microcapsules can function effectively for a long period after xenotransplantation with a smaller number of islets than was used in other studies.[130]

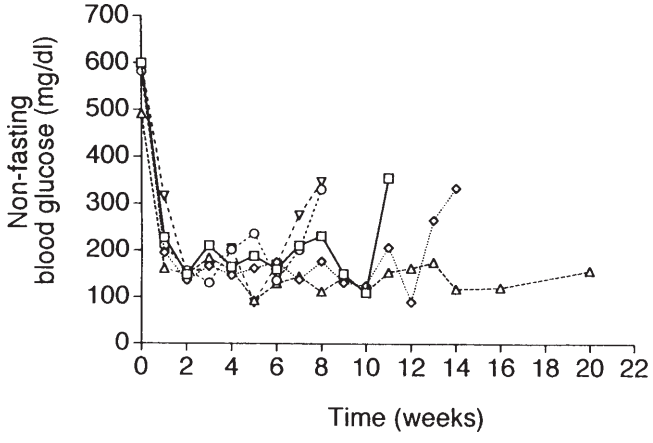

Fig. 3. Changes in non-fasting blood glucose levels in diabetic recipient mice after xenotransplantation with three-layer agarose microcapsules enclosing isolated rat islets. *Squares*, *circles* and *triangles* indicate changes of non-fasting blood glucose levels in each diabetic recipient. (From the ref. 129, with permission)

Macroencapsulation

There are two forms of macroencapsulation of islets, the intravascular device and the diffusion chamber.

Intravascular device. This device is derived from the principle of dialysis cartridges, in which islets are seeded in the space between hollow fibers that are perfused with blood. The original perfusion devices consisted of bundles of capillary fibers seeded on their outside surfaces with isolated islet cells. A device consisting of a single coiled membrane with a diameter of 5–6 mm has been developed.[131,132] One dog that received these devices containing bovine islets demonstrated excellent control of fasting glucose levels for almost 2 months without exogenous insulin.[131] Results with porcine islets showed a substantially decreased exogenous insulin requirement for up to 9 months.[132] However, a number of issues remained that appeared to limit the therapeutic potential of this approach. Perhaps most importantly, data suggested that the size and geometry of perfusion devices imposed a critical limitation on the amount of islet tissue that could be transplanted into a patient with a single device. Two perfusion devices would be required. Nevertheless, these studies represent an important step toward developing simpler, more viable strategies for transplanting islets using encapsulation technologies.

Diffusion chamber. The principle of the diffusion chamber is the use of hollow fibers filled with hundreds or thousands of islets either in packed form or dispersed in a "spacer" matrix which separates single islets from each other and improves the diffusional supply with oxygen and nutrients. These devices (diffusion chambers) were successfully placed into the free peritoneal cavity or into the subcutaneous tissue,[133,134] and this was followed by a correction of the diabetic state for several months. These devices are typically tubular or planar designs, although the most significant progress has been achieved with cylindrical polyacrylonitrile-polyvinyl chloride (PAN-PVC) membranes having a smooth outer skin.[134–137] Porcine, bovine, and canine islets placed within these chambers restored normoglycemia in streptozotocin-induced diabetic rats for more than a year without immunosuppression.[137] These membranes solved many of the problems associated with diffusion chambers (e.g., fibrosis, abscess formation, adhesions).[138] However, a number of unsolved issues critical to the wide-scale clinical success of these devices must be carefully addressed. These include long-term biocompatibility, membrane breakage, and suitability for retrieval. We have developed two types of diffusion chamber: the tube type mesh-reinforced polyvinyl alcohol tube; MRPT and the bag type (mesh-reinforced polyvinyl alcohol bag; MRPB). A polyvinyl alcohol

hydrogel membrane was employed in both types of diffusion chambers as the membrane material for a bioartificial pancreas. An in-vitro study of the permeability of this membrane clearly demonstrated that glucose, insulin, and nutrients passed through the membrane easily, whereas the passage of immunoglobulin G was completely blocked, indicating that this membrane could effectively prevent immunorejection of a transplanted hybrid artificial pancreas.[139,140] The function of this membrane has been further improved.[126,141] An in-vivo transplantation study revealed that the MRPT with entrapment of islets exhibited excellent function and survival in allotransplantation,[139] as well as in isotransplantation.[142] Figure 4 shows the normalization of nonfasting serum glucose levels in a long-surviving diabetic recipient rat after the intraperitoneal transplantation of MRPT without immunosuppressants.[139] Serum glucose levels were elevated right after the removal of the MRPT 3 months posttransplant (Fig. 4), indicating the successful functioning of the bioartificial pancreas. We have developed an innovative type of diffusion chamber (MRPB) in which the tube type of MRPT was modified to a bag type device, which is, presumably, superior in terms of permeability, biocompatibility and flexibility.[143–145] This MRPB proved to retain superior ability as a diffusion chamber, demonstrating long survival and excellent function even after discordant xenotransplantation (dog or pig to rat) without any immunosuppressants.[143,144] We have recently employed a novel β — cell line (MIN6)[80,92] as a bioreactor enclosed in a MRPB diffusion chamber.[145] MIN6 was established from insulinomas obtained by targeted expression of the simian virus 40 T-antigen gene in transgenic mice. MIN6 retains the ability to secrete insulin in response to physiological glucose concentrations.[90] The MRPB can perfectly immunoisolate enclosed MIN6 cells from immunoglobulin G and lymphocytes, both of which are potent factors in causing the xenogeneic rejection response. Xenotransplantation with MRPB containing MIN6 cells resulted in a rapid and significant decrease of serum glucose levels in the diabetic recipient rats, with a sustained normalization of serum glucose levels for long periods without any immunosuppressants.[145] In the near future, the employment of pig islets or gene-manipulated β — cell lines for diffusion chambers such as the MRPB will solve the current serious problem of the insufficient supply of donor organs.

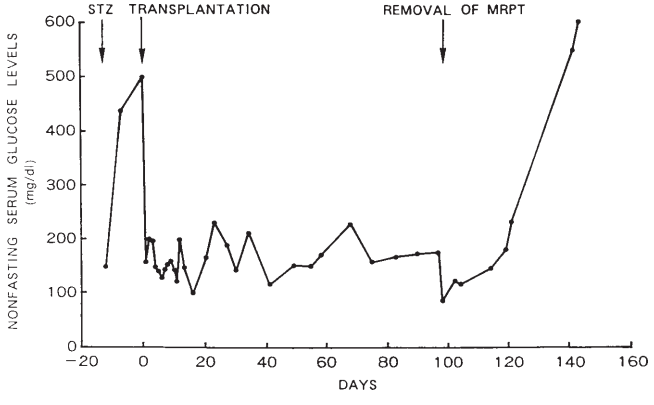

Fig. 4. Changes in non-fasting serum glucose levels in a long-surviving diabetic recipient rat with mesh-reinforced polyvinyl alcohol tube (*MRPT*) transplantation. *STZ*, Streptozotocin (From the ref. 139, with permission)

Conclusions

Today, islet transplantation has become clinically applicable, resulting in long-term insulin-independence in some type I diabetic patients, and detectable C-peptide in an increasing number of patients. Patients with detectable C-peptide can show improved diabetes management with normalization of glycohemoglobin and eradication of severe hypoglycemia. Although the obstacles that prevent consistent success with islet transplantation need to be eliminated, this may not be a critical challenge and appears to be solvable. It seems likely that pancreas transplantation may be replaced with islet transplantation in the near future. We should, however, notice that the avoidance of general immunosuppression is definitely the ideal scenario for islet transplantation. Toward this goal, while the developments in immune tolerance induction hold widespread interest and promise, islet graft immunoisolation with highly biocompatible and immunoselective bioartificial pancreas could offer a more suitable opportunity to target the basic immunological problem. In order to overcome the serious problem of the limitation of human pancreata supplies, insulin-producing tissues from other abundant and accessible sources must be considered. Porcine islets or genetically engineered insulin-secreting β-cell lines will be the most promising sources which will allow the widespread application of islet transplantation. It is anticipated that research breakthroughs and further progress in the development of immunoisolation systems with xenogeneic islets from defined pigs or genetically engineered cell lines will provide a new era of diabetes managemant, accomplishing the longstanding final goal of the cure of diabetes mellitus.

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