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Pancreatic islet transplantation: toward definitive treatment for diabetes mellitus

Tadashi Takaki[1,2,3], Masayuki Shimoda[1,*]

[1] Department of Pancreatic Islet Cell Transplantation, National Center for Global Health and Medicine, Tokyo, Japan;
[2] Department of Cell Growth and Differentiation, Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan;
[3] Takeda-CiRA Joint Program for iPS Cell Applications (T-CiRA), Fujisawa, Kanagawa, Japan.

Abstract: Since the late 20th century, advances in pancreatic islet transplantation have targeted improved glycemic control and fewer hypoglycemic events in patients with type 1 diabetes, and some important milestones have been reached. Following the Edmonton group's success in achieving insulin independence in all transplanted patients with type 1 diabetes, clinical islet transplantation is now performed worldwide. β cell replacement therapy for type 1 diabetes was established based on the favorable outcomes of a phase 3, prospective, open-label, single-arm, clinical study conducted at 8 centers in North America, in which 42 of 48 patients who underwent islet transplantation from 2008 to 2011 achieved HbA1c < 7.0% (53 mmol/mol) at day 365, which was maintained at 2 years in 34 patients. In Japan, a phase 2 multicenter clinical trial of islet transplantation for type 1 diabetes patients is currently ongoing and will end soon, but the interim results have already led to positive changes, with allogeneic islet transplantation being covered by the national health insurance system since April 2020. Current efforts are being made to solve the problem of donor shortage by studying alternative donor sources, such as porcine islets and pancreatic progenitor cells derived from pluripotent stem cells. The results of clinical trials in this area are eagerly awaited. It is hoped that they will contribute to establishing alternative sources for insulin-producing β cells in the near future.

Keywords: type 1 diabetes, pancreas preservation, islet isolation, islet purification, instant blood-mediated inflammatory reaction, immunosuppression

Introduction

Whole pancreas transplantation has been performed since 1966 to facilitate endogenous insulin secretion and effectively manage blood glucose, but the mortality rate after 5 years still exceeds 10% (*1*). Pancreatic islet isolation techniques have been developed to provide a less invasive procedure (*2*) and a number of important milestones have been reached in its development. Islet transplantation was begun in the early 1970s using rat models (*3*), and the liver was considered to be the most appropriate site of engraftment *via* portal vein infusion (*4*). The first human islet transplantation was reported in 1977 in a preliminary report (*5*). Isolated islets from deceased donors were transplanted into the peritoneal cavity and portal vein of at least 7 diabetic patients, but none of them achieved insulin independence (*6*). In 1980, it was reported that 10 patients with chronic pancreatitis and unbearable pain underwent islet autotransplantation accompanied by total or distal pancreatectomy, with some patients successfully avoiding pancreatogenic diabetes after surgery (*7*). This confirmed that transplanted islets could indeed function as intended, but it was also clear that it can be difficult to adequately control rejection, inflammatory and autoimmune responses.

After methods were developed to isolate human pancreatic islets (*8*), in 1990 Scharp *et al.* reported insulin independence in a type 1 diabetes patient following allogeneic islet transplantation (*9*). A decade later, the Edmonton group, led by James Shapiro, reported that all 7 of their type 1 diabetes patients achieved insulin independence after allogeneic islet transplantation from an average of 2 donors using glucocorticoid-free immunosuppressants (*10*). Then, in 2005, the University of Minnesota group led by Bernard Hering reported that all 8 of their type 1 diabetes patients achieved insulin independence with transplantation from a single donor (*11*). This advancement led to a multicenter, phase 3, prospective, open-label, single-arm study being conducted at 8 centers in North America with publication of the results in 2016 (*12*). Between 2008 and 2011, 48 type 1 diabetes patients without stimulated C-peptide secretion had received islets from brain-dead donors; 42 patients achieved a HbA1c < 7.0% (53 mmol/mol) at day 365, and 34 maintained this level at 2 years with a marked reduction in severe hypoglycemia.

Based on the results of this trial, islet transplantation is now recognized as a standard treatment for unstable type 1 diabetes. A multicenter randomized controlled trial with type 1 diabetes patients in Europe later confirmed the safety and efficacy of islet transplantation compared with intensive insulin therapy (*13*). Other clinical trials have been completed or are underway (*14*), and bring promise of further developments in islet transplantation.

Here, we review some of the major achievements made thus far in pancreatic islet transplantation, including changes in islet preparation and improvements in immunosuppressive drugs. We discuss the current state of islet transplantation in Japan, and we consider future prospects using alternative islet sources and whether such developments can bring a definitive treatment for diabetes mellitus closer.

Transplant recipients

Islet allotransplantation has been performed in patients with brittle type 1 diabetes characterized by unstable glycemic control with frequent and unpredictable severe hypoglycemic episodes that cannot be controlled by optimal treatment from diabetologists. Unlike exogenous insulin, islets can physiologically produce and release insulin, resulting in strict blood glucose control. Current evidence-based guidelines propose a four-stage algorithm to treat type 1 diabetes, recommending that islet or pancreas transplantation be considered at the last stage in patients with persistent problematic hypoglycemia, defined as 2 or more episodes per year of severe hypoglycemia or as 1 episode associated with impaired awareness of hypoglycemia, extreme glycemic lability, or major fear and maladaptive behavior (*15*). If the recipient experiences severe chronic renal failure and requires a kidney transplant, a simultaneous kidney-islet transplantation or simultaneous pancreas-kidney transplantation is recommended. However, it is unclear if an islet transplantation should be performed in a patient with problematic hypoglycemia and an intermediate estimated glomerular filtration rate (eGFR) (30-60 mL/min) because the subsequent life-long administration of immunosuppressants might increase the risk of end-stage renal failure. Immunosuppression for the rest of the recipient's life also increases the risk of severe infections and malignancies. It is thus important to screen for endogenous infections and cancers (*16*).

Islet preparations

Pancreas preservation

In 2000, it was determined that single-donor islet transplants were viable after cold preservation of donor pancreases stored for less than 8 to 10 h, even in University of Wisconsin solution (UWS), a standard preservation solution for pancreas transplantation (*17*). A decade earlier, Kuroda *et al.* (*18*) reported that perfluorochemical (PFC), used as an oxygen-supplying agent for the cold storage of canine pancreas in Euro-Collins solution, enabled preservation up to 48 h in the two-layer cold storage apparatus, as tested by subsequent pancreas autotransplantation. Tanioka *et al.* (*19*) applied a UWS/PFC two-layer method (TLM) to preserve dog pancreas for 24 h before islet isolation, resulting in a huge increase in islet equivalents per gram pancreas compared with conventional simple cold storage in UWS. Hering *et al.* (*11,20*) used TLM for clinical islet transplants, but with less than 8 h of cold storage time. A few years later, two large-scale studies and a meta-analysis showed no beneficial effect of TLM on islet isolation and transplantation outcomes compared with UWS alone (*21-23*). However, several other meta-analyses reported that use of TLM produced a significantly higher islet yield (*24-26*). Moreover, a meta-analysis revealed that the beneficial effects of TLM on islet yield were correlated with a prolonged (> 20 h) cold ischemic time (*26*), which had been suggested by the previous meta-analysis (*23*).

As for the pancreas preservation solution, UWS had been used since the 1980s for clinical islet transplants (*27*) but had several disadvantages, including instability, high cost due to its short expiration date, and high viscosity. Noguchi *et al.* (*28*) reported increased islet yield from pancreases preserved using a TLM comprising extracellular-type trehalose-containing Kyoto (ET-Kyoto) solution with a trypsin inhibitor, namely, M-Kyoto solution. They subsequently found a higher viability of isolated islets with ulinastatin as the trypsin inhibitor than with gabexate mesilate or nafamostat mesilate (*29*).

To achieve a satisfactory distribution of collagenase in islet isolation, Sawada *et al.* (*30*) examined the importance of UWS injection into the rat pancreatic duct at the time of procurement, which protects the ducts from cold ischemic injury, leading to the clinical application of ductal preservation using M-Kyoto solution (*31*).

Islet isolation

The isolation of islets by incubation with collagenase originated from Moskalewiski's 1965 study in guinea pigs (*32*). For larger animals, a method for the preparation of viable islet cells from healthy dog pancreas was described in 1981, based on perfusion of the pancreatic duct with higher concentrations of collagenase, which resulted in improved islet yield and decreased acinar contamination (*33*). A method developed for the isolation of islets of Langerhans from the human pancreas was reported in 1984; this technique was based on injection of a high concentration of collagenase into the pancreatic duct under pressure, followed by a short incubation at 39°C (*34*). Finally, Ricordi *et al.* (*8*) published a semiautomated method

for the isolation of human pancreatic islets, leading to the first case of allogeneic islet transplantation for type 1 diabetes, which achieved a short period of insulin independence (*9*). The dissociation chamber at the core of the automated method was named the Ricordi chamber after Dr. Camillo Ricordi. The Ricordi Automated Method has since become the gold standard for human and large animal pancreatic processing, contributing to the success of clinical trials of islet transplantations and an increasing number of such trials worldwide (*35*).

Although the variable enzymatic composition of crude collagenases undermined the reproducibility of islet isolation procedures, Wolters *et al.* (*36*) found that only collagenase class I (CC-I), collagenase class II (CC-II), and a supplementary protease are essential for effective pancreas digestion, leading in 1995 to a commercially available purified enzyme blend, Liberase HI (Roche, Indianapolis, IN), which helped to significantly increase the number of human islet allotransplantations. In addition, Brandhorst *et al.* (*37*) revealed that a delicate ratio between CC-II and CC-I could reduce the demand on supplementary proteases, which has significant implications for the viability of isolated islets. Although Liberase HI was very effective, it was withdrawn from the market in 2007 because of the risk for potential transmission of bovine spongiform encephalopathy due to the use of bovine neural tissue during its production.

Shimoda *et al.* (*38*) examined various collagenases as alternatives to Liberase HI, such as collagenase NB1 (Serva, Heidelberg, Germany), Clzyme Collagenase HA (Vitacyte, Indianapolis, IN), and mammalian tissue-free Liberase MTF (Roche), and showed that each of the three enzymes achieved higher islet yields than Liberase HI. Although significant progress has been made in characterization of enzymes, further studies are necessary to determine the optimal enzyme blend.

Islet purification

In an earlier report, islets of rats purified from discontinuous sucrose gradients were not so sophisticated for *in vitro* metabolic studies of insulin biosynthesis (*2*). Thus, Lindall *et al.* (*39*) replaced the unpolymerized sucrose with layered isometric Ficoll, a polymer of sucrose, as the separating agent, and Scharp *et al.* (*40*) determined that rat islets obtained using dialyzed Ficoll exhibited improved insulin secretion. Meanwhile, Buitrago *et al.* (*41*) reported the rapid isolation of human and mouse pancreatic islets from collagenase-digested pancreas by sedimentation through a nontoxic low-osmotic density gradient medium, Percoll, which consists of colloidal silica particles coated with a layer of polyvinylpyrrolidone. A decade later, Scharp *et al.* (*42*) demonstrated that the Ficoll technique was much more effective for purifying human islets, providing about twice the yield of islets and insulin compared with the Percoll method. In 2000, the Edmonton protocol also adopted a Ficoll-based density gradient purification method with top loading using a semiautomated computerized COBE-2991 cell processor (*10*). Due to the suggestion that Ficoll is an islet toxin, Hering *et al.* investigated transplantation from a single donor using an iodixanol (OptiPrep^{TM})-based density gradient purification method and succeeded in achieving insulin independence (*11,43*). Later, Mita *et al.* (*44*) showed that the OptiPrep method significantly reduced the production of cytokines, including IL-1β, TNF-α, IFN-γ, IL-6, IL-8, MIP-1β, MCP-1, and RANTES, and improved β cell survival during pretransplant culture compared with the Ficoll purification method. In addition, Shimoda *et al.* developed a new purification method using top loading large bottles to reduce the shear stress seen with the COBE 2991 cell processor, thereby improving the quality and quantity of porcine and human islets after purification (*45,46*).

Instant blood-mediated inflammatory reaction

Despite considerable progress in the preparation of islets for transplantation, about 50% of transplanted islet cells are not detected in the liver in PET-CT images immediately after intraportal infusion of radio-labeled islets (*47*). In an *in vitro* model and pig-to-pig islet allotransplantation, Bennet *et al.* (*48*) observed macroscopic clotting within 5 min after the introduction of human islets into the blood, followed by continuous fibrin formation that generated a capsule surrounding the islets, complement activation, and infiltration with CD11+ leukocytes. They named this series of reactions 'instant blood-mediated inflammatory reaction' (IBMIR) and it could be prevented by a high heparin concentration in combination with the recombinant form of the complement inhibitor soluble complement receptor 1 (sCR1). Although it was recognized that IBMIR has a significant impact on the survival of transplanted islets, it is not easy to suppress IBMIR clinically. The addition of heparin is the current standard approach but it is insufficient.

Moberg *et al.* (*49*) determined that tissue factor (TF) from pancreatic islets serves as the main trigger of IBMIR and that the clotting reaction triggered by islets *in vitro* could be abrogated by blocking the active site of TF with specific antibodies or site-inactivated factor VIIa, a candidate drug for inhibition of TF activity. They also identified that the antioxidant nicotinamide inhibits TF and macrophage chemoattractant protein (MCP)-1 expression in isolated human pancreatic islets in an *in vitro* loop model (*50*). Their colleagues, Johansson *et al.* (*51*), revealed a negative correlation between thrombin-antithrombin complex generation 15 min after the first infusion of islets into the portal vein and fasting C-peptide production 7 days after transplantation.

Although Johansson *et al.* (*52*) reported in another study that low molecular weight dextran sulfate (LMW-DS; MW 5000) dose-dependently inhibited IBMIR by blocking coagulation and complement activation, a recent phase II, multicenter, open-label, active control, randomized study within the Clinical Islet Transplantation Consortium 01 study revealed that systemic LMW-DS treatment showed similar efficacy in preventing IBMIR and promoted islet engraftment to "state-of-the-art" treatment with heparin (*53*), leading to a reluctance to use LMW-DS because its target-activated partial thromboplastin time is 150 ± 10 s, which is 3 times longer than the 50 ± 5 s of heparin. This aspect is particularly important when performing total pancreatectomy followed by autologous islet transplantation. Notably, IBMIR also occurs in patients undergoing total pancreatectomy followed by autologous islet transplantation (*54*).

Islet surface engineering is another way to inhibit IBMIR. Cabric *et al.* (*55*) reported that heparin-coated islets protected against IBMIR in an allogeneic porcine model of islet transplantation. In addition to heparin-coating, other approaches reported to show protective effects against IBMIR include surface modification with polyethylene glycol (PEG)-lipid (*56*), surface modification with PEG-urokinase (*56*), co-immobilization of urokinase and the soluble domain of thrombomodulin (*57*), and immobilization of human soluble complement receptor 1 through PEG-conjugated phospholipid (PEG-lipid) (*58*). Further studies are thus required for the clinical application of islet transplantation.

Immunosuppression

The development of immunosuppressive agents has contributed greatly to transplant efficacy. Various immunosuppressive agents are listed in Table 1, and their applications in major clinical islet transplantation are shown in Table 2.

Early days of immunosuppression

Steroidal hormones, first extracted by Edward C. Kendall in 1934, have been used as anti-inflammatory agents for various inflammatory and autoimmune diseases since the successful use of cortisone to treat rheumatoid arthritis in 1948 by rheumatologist Phillip S. Hench (*59*). Meanwhile, Elion *et al.* (*60*) described the growth-inhibitory effect of 6-mercaptopurine (6-MP) on *Lactobacillus casei* in 1953 and Schwartz *et al.* (*61*) reported its suppressive effect on the formation of humoral antibody when given simultaneously with the antigen in 1958. Azathioprine (also called BW 57-322), a prodrug of 6-MP, was subsequently synthesized by George H. Hitchings and Gertrude Elion in 1957 (*62*). In contrast, cyclosporine (cyclosporin A), one of the most successful antirejection drugs to date, was obtained from the fermentation broth of the fungi *Trichoderma polysporum* and *Cylindrocarpon lucidum* in 1970 by Borel and Stahelin in an attempt to develop a new antifungal treatment. Its immunosuppressive properties were identified in 1971 and were accompanied by only weak myelotoxicity, a possible adverse effect of azathioprine (*63*).

Classical immunosuppressants in organ transplantation

The era of transplant therapy began with kidney transplantation. Because the first kidney transplantation between genetically unrelated individuals was performed in 1962 using prednisone and azathioprine for immunosuppression (*64*), this combination of glucocorticoid and azathioprine became the standard antirejection regimen. In 1966, Starzl *et al.* (*65*) used a horse antilymphocyte globulin (ALG) preparation in patients receiving a kidney transplantation as an adjunct to the standard immunosuppressive drugs, azathioprine and prednisone, whose doses were reduced in half the patients. The results of this study meant that this T cell-depleting polyclonal antibody, ALG, became one of the standard agents for the induction therapy, before being supplanted in the early 1980s by antithymocyte globulin (ATG) to avoid variations between lots of ALG (*66*). Moreover, since cyclosporine was introduced to clinical organ transplantation by Calne *et al.* in 1978 (*67*), the use of steroids was successfully reduced or avoided in cadaveric renal transplantation (*68*). Meanwhile, the use of high-dose intravenous methylprednisolone in renal transplantation was established; this strategy was associated with a very rapid lympholytic effect, a low incidence of complications, and the avoidance of tapering steroid doses (*69*).

Expansion of immunosuppression in islet transplantation

In the context of the immunosuppressive drugs used in organ transplantation, the first case of insulin independence after islet transplantation into the portal vein from cadaveric pancreases was achieved in 1990 in a type 1 diabetic patient premedicated with methylprednisolone 1 mg/kg and azathioprine 1 mg/kg and given Minnesota antilymphoblast globulin 20 mg/kg for 7 days starting 1 day after transplantation in addition to the stable cyclosporine dosage of 500 mg once daily for the existing kidney transplant (*9*).

A decade later, in 2000, three new immunosuppressive drugs (daclizumab, tacrolimus, and sirolimus) enabled 7 consecutive type 1 diabetes patients to achieve insulin independence over a median follow-up of 11.9 months after transplantation from 2 to 4 donor islets, without the use of the diabetogenic immunosuppressive drug, glucocorticoids, in the Edmonton protocol (*10*). Daclizumab, like basiliximab, is a monoclonal antibody

Table 1. List of immunosuppressants

Generic name	Effects	*Ref.*
Glucocorticoids	Genomic actions mediated through the classic glucocorticoid receptor (GR), a member of the nuclear receptor family of ligand-activated transcription factors, and rapid non-genomic actions *via* cytosolic GR or membrane-bound GR.	(*98*)
High-dose methylprednisolone	Rapid non-genomic actions mediated *via* cytosolic GR or membrane-bound GR.	(*69,98*)
Antilymphocyte globulin (ALG)	Polyclonal antibodies generated in animals by inoculation with human lymphoid cells.	(*66*)
Antithymocyte globulin (ATG)	Polyclonal antibodies generated in animals by inoculation with human thymocytes.	(*66*)
Azathioprine	A long-lived prodrug of 6-mercaptopurine (6-MP).	(*62*)
6-mercaptopurine (6-MP)	A purine antagonist.	(*60*)
Mycophenolate mofetil (MMF)	A relatively selective inhibitor of T and B cell proliferation; it is a prodrug of mycophenolic acid (MPA) synthesized to improve oral bioavailability.	(*74,78*)
Mycophenolic acid (MPA)	A 5-fold more potent inhibitor of the type II isoform of inosine-5′-monophosphate dehydrogenase (IMPDH), which is expressed in activated T and B lymphocytes, compared with the housekeeping type I isoform of IMPDH, which is expressed in most cell types, resulting in inhibition of T and B cell proliferation.	(*74,78*)
Cyclosporine (cyclosporin A, CsA)	Binds preferentially to cytosolic cyclophilin A, the predominant cyclophilin found within T cells, inhibiting calcineurin, a calcium/calmodulin-dependent serine threonine phosphatase. Inhibition of the dephosphorylation of the nuclear factor of activated T cells (NFAT) inhibits NFAT translocation from the cytoplasm to the nucleus, resulting in decreased transcription of interleukins (IL-2, IL-3, IL-4), TNF-alpha, and interferon-gamma (IFN-γ).	(*72,74*)
Tacrolimus (FK506)	Binds to FK506-binding proteins, particularly FKBP12, provoking inhibition of calcineurin, a phosphatase also inhibited by CsA.	(*72,73*)
Sirolimus (rapamycin)	Binds to FKBP12 to form a complex that binds to and inhibits the serine/threonine kinase mammalian target of rapamycin (mTOR), resulting in cell cycle arrest, repression of tumor cell growth, and inhibition of T and B cell proliferation.	(*74,76*)
Everolimus	A derivative of sirolimus.	(*76*)
Muromonab-CD3 (OKT3)	A mouse monoclonal antibody to CD3 primarily expressed on T cells that binds to CD3, thereby depleting T cells from the circulation and induces transient cytokine release syndrome.	(*70*)
hOKT3γ1(Ala-Ala)	A human IgG1-based OKT3 with amino acids 234 and 235 of the Fc region changed to alanine; this preserves the immunosuppressive effects of OKT3 but reduces the inductive effect on cytokine release syndrome.	(*77*)
Daclizumab	A monoclonal antibody targeting CD25, the alpha subunit of the IL-2 receptor that is expressed on activated T lymphocytes, leading to inhibition of T cell proliferation.	(*70*)
Basiliximab	Another monoclonal antibody targeting CD25, which inhibits T cell proliferation.	(*70*)
Etanercept	A monoclonal antibody to TNF-alpha that is particularly toxic to β cells.	(*99*)
Anakinra	An IL-1 receptor antagonist that promotes the survival of transplanted human islets; it has an additive effect with etanercept.	(*99*)
Alemtuzumab	A humanized, recombinant antibody to CD52, a cell surface glycosylphosphatidylinositol (GPI)-anchored glycoprotein expressed on T and B lymphocytes, monocytes, macrophages, and natural killer cells.	(*74,100*)
Efalizumab	A monoclonal antibody to CD11a, the alpha subunit of LFA-1, that inhibits the binding of intercellular adhesion molecule 1 (ICAM-1) to LFA-1, thereby suppressing the activities of LFA-1 expressed on all leukocytes. Efalizumab was withdrawn in 2009 due to concerns about the development of progressive multifocal myeloencephalopathy.	(*83*)
Belatacept	A fusion protein composed of the Fc fragment of a human IgG1 immunoglobulin linked to the extracellular domain of CTLA-4 (cytotoxic T lymphocyte-associated protein 4), also known as CD152, which binds the ligands CD80 and CD86 on antigen-presenting cells, thereby inhibiting T cell activation.	(*83*)

targeting CD25 – the alpha subunit of the IL-2 receptor, which is expressed on activated T lymphocytes – and has fewer adverse effects compared with other monoclonal antibodies, such as the cytokine release syndrome caused by muromonab-CD3, a mouse monoclonal antibody against CD3 (trade name: Orthoclone OKT3) (*70*). Tacrolimus, also known as FK-506, is a macrolide compound derived from the fermentation broth of the fungus *Streptomyces tsukubaensis*, which was isolated from a soil sample collected near Mount Tsukuba in Japan by Fujisawa Pharmaceutical Company in 1984 (*71*). Although tacrolimus, as well as cyclosporine, is a calcineurin inhibitor that blocks dephosphorylation of the transcription factor nuclear factor of activated T cells, reducing the activity of genes encoding IL-2 and other related cytokines, it has been shown to be superior to cyclosporine in terms of graft survival and acute rejection (*72*). However, a recent report described detrimental effects of tacrolimus on β cells (*73*). Sirolimus, also called rapamycin, is another macrolide compound isolated from a fungus (*S. hygroscopicus*) from soil collected in the Vai Atoreregion on Easter Island (Rapa Nui) between December 1964 and February 1965; its immunosuppressive effect attracted attention in the 1980s after its structural homology with tacrolimus was noted, leading to its clinical use in organ transplantation in 1999 (*74*). The use of sirolimus enabled the glucocorticoid-free Edmonton immunosuppression protocol (*10*). However,

Table 2. List of major clinical islet transplantation trials

Year of publication (*Ref.*)	Induction	Maintenance	Insulin independence
1990 (*9*)	Cyclosporin, ALG, methylprednisolone, and azathioprine.	Cyclosporin and azathioprine.	1 patient achieved insulin independence at day 10-22.
2000 (*10*)	Daclizumab, sirolimus, and low-dose tacrolimus.	Sirolimus and low-dose tacrolimus.	All recipients (7 of 7) achieved insulin independence over a median follow-up of 11.9 months with 2 to 4 donor islets.
2004 (*20*)	hOKT3γ1(Ala-Ala) and sirolimus.	Sirolimus and low-dose tacrolimus.	Mean time to insulin independence was 35 ± 7 days in 4 of 6 patients with islets from a single-donor pancreas.
2005 (*11*)	ATG, methylprednisolone, daclizumab, and etanercept.	Sirolimus and reduced-dose tacrolimus. Tacrolimus was gradually replaced by MMF at 1 month posttransplantation.	5 of 8 recipients remained insulin-independent for longer than 1 year with only 1 islet infusion.
2008 (*79*)	ATG, methylprednisolone, and etanercept. Basiliximab instead of ATG and methylprednisolone before the second islet infusion.	Cyclosporine and everolimus. If deemed clinically appropriate, everolimus was replaced with MMF.	Of 6 recipients transplanted from 1 or 2 donor islets, 5 (83%) achieved insulin independence at 1 year and 4 continued to be insulin-independent at a mean of 3.4 ± 0.4 years.
2010 (*83*)	ATG and methylprednisolone before the first islet transplant and basiliximab before the second islet transplant.	Efalizumab or belatacept, followed by additional sirolimus and MMF. Tacrolimus and MMF.	All recipients (5 of 5) achieved insulin dependence over 1 year with 1 or 2 islet transplants.
2013 (*80*)	ATG, tacrolimus, and MMF. Basiliximab instead of ATG for the third islet transplant.	Tacrolimus converted to sirolimus 6 months after the final transplant, if tolerated.	Insulin independence was achieved 1 year after the first islet infusion with 1 to 3 islet infusions in 52.9% (7 of 19 recipients).
2016 (*12*)	ATG and etanercept. Basiliximab replacing ATG at subsequent transplant.	Sirolimus and low-dose tacrolimus.	Insulin independence was achieved at day 365 in 52.1% (25 of 48 recipients). About half of the recipients received 1 islet infusion.
2020 (*82*)	Anakinra, etanercept, and ATG (first 7 patients) or alemtuzumab (next 2 patients). Steroids for premedication before ATG.	Tacrolimus and MMF.	Insulin independence was achieved at 1 year with 1 or 2 islet infusions in 44% (4 of 9 recipients).
2020 (*87*)	Reparixin or placebo along with ATG or basiliximab (plus bolus of methylprednisolone prior to ATG) before the first islet infusion and basiliximab before the second islet infusion.	A cell proliferation inhibitor (either sirolimus or MMF) and a calcineurin inhibitor (preferentially tacrolimus, but cyclosporine could also be used).	Insulin independence was achieved 1 year after the last islet infusion with 1 or 2 islet infusions in 32.0% (8 of 25 recipients) in the reparixin group vs. 31.3% (5 of 16 recipients) in the placebo group.

the rate of insulin independence at 2 years was only 14% (*75*), partly because sirolimus has detrimental effects on pancreatic β cells (*76*).

After establishment of the Edmonton protocol

To improve long-term outcomes in clinical islet transplantation, various new immunosuppressants have been developed and used. Although muromonab-CD3, a mouse monoclonal antibody to CD3 (Orthoclone OKT3), is effective for reversing renal transplant rejection episodes, it can induce cytokine release syndrome, which is characterized by flu-like symptoms and, occasionally, severe hypotension, bronchospasm, tachycardia, and even death (*70*). To reduce this human anti-mouse antibody response, Xu *et al.* (*77*) constructed hOKT3γ1(Ala-Ala), a human IgG1-based OKT3 with amino acids 234 and 235 of the Fc region changed to alanine, which prevents binding to the Fc receptor. Hering *et al.* (*20*) applied hOKT3γ1(Ala-Ala) along with sirolimus for induction immunosuppression to allogeneic islet transplant, followed by sirolimus and low-dose tacrolimus for maintenance immunosuppression. This protocol enabled 4 of 6 patients with type 1 diabetes to achieve insulin independence at a mean time of 35 ± 7 days after transplantation.

However, Hering *et al.* had previously reported in 2005 (*11*) that the first single-donor, marginal-dose islet transplantation restored insulin independence in all 8 treated patients with type 1 diabetes. This protocol comprised rabbit antithymocyte globulin (rATG), methylprednisolone, daclizumab, and etanercept for

induction immunosuppression and sirolimus and reduced-dose tacrolimus for maintenance immunosuppression, with tacrolimus gradually replaced by mycophenolate mofetil (MMF) at 1-month posttransplantation. The results suggest the importance of inhibiting tumor necrosis factor with etanercept in the peritransplant period and replacing tacrolimus. Furthermore, 5 of the 8 recipients remained insulin-independent for longer than 1 year.

MMF is a prodrug of mycophenolic acid (MPA), which was first discovered as a fermentation product of *Penicillium brevicompactum* and related fungi in 1893 (*74,78*). Because MPA was found to be a 5-fold more potent inhibitor of the type II isoform of inosine-5′-monophosphate dehydrogenase (IMPDH), which is expressed in activated T and B lymphocytes, than of the housekeeping type I isoform of IMPDH, which is expressed in most cell types, MMF was developed as an effective immunosuppressive prodrug of MPA that preferentially inhibits the proliferation of human T and B lymphocytes.

A similar immunosuppression protocol using rATG and etanercept for induction and sirolimus and low-dose tacrolimus for maintenance was adopted in a phase 3 trial of human islet transplantation for type 1 diabetes complicated by severe hypoglycemia (*12*). Insulin independence was achieved in 52.1% of the patients at day 365, about half of whom received only 1 islet infusion. Moreover, the same group from the University of Minnesota reported in 2008 that, of 6 patients who received transplants from 1 or 2 donor islets, 5 achieved insulin independence at 1 year and 4 continued to be insulin-independent at a mean of 3.4 ± 0.4 years posttransplant (*79*). Their protocol used rATG, methylprednisolone, basiliximab, and etanercept for induction immunosuppression and cyclosporine and everolimus, a derivative of sirolimus, for maintenance immunosuppression. If deemed clinically appropriate, everolimus was replaced with MMF, suggesting that it is important to avoid tacrolimus and sirolimus, both of which have diabetogenic effects (*72,76*).

In contrast, a multicenter Australian trial (*80*) of islet transplantation that used rATG, tacrolimus, and MMF for induction and tacrolimus and MMF for maintenance with conversion of tacrolimus to sirolimus 6 months after the final transplant if tolerated achieved insulin independence in 52.9% of patients at 1 year after the first islet infusion, despite the use of 1 to 3 islet infusions, implying the beneficial effect of the addition of an anti-TNF agent, as used in the previous report from the University of Minnesota group. On the other hand, Matsumoto *et al.* (*81*) reported in 2011 the favorable effects of the IL-1 receptor antagonist anakinra in combination with etanercept for induction. This was followed by a recent phase 1/2 clinical trial (ClinicalTrials.gov, NCT00530686) (*82*). Although double blockade of TNF-α and IL-1 by etanercept and anakinra in the peritransplantation period was shown to be safe in this recent trial, only 4 of the 9 patients achieved insulin independence with 1 or 2 islet infusions at 24 months after the transplantation, suggesting that other strategies are required to maintain long-term islet function.

Distinct from the above-mentioned clinical studies, Posselt *et al.* (*83*) of the University of California reported calcineurin inhibitor-free immunosuppressive regimens using efalizumab, an anti-leukocyte functional antigen-1 (anti-LFA-1) antibody, or belatacept, a fusion protein composed of the Fc fragment of a human IgG1 immunoglobulin linked to the extracellular domain of CTLA-4 (cytotoxic T lymphocyte-associated protein 4; also known as CD152), which achieved insulin independence for over 1 year with 1 or 2 islet transplants. The immunosuppressants were rATG and methylprednisolone for induction and efalizumab or belatacept for maintenance, followed by additional sirolimus, and MMF. The results demonstrated the considerable benefits of efalizumab and belatacept for both the engraftment and survival of transplanted islets.

Efalizumab is a humanized monoclonal antibody against CD11a, the alpha subunit of LFA-1, that inhibits the ability of intercellular adhesion molecule 1 (ICAM-1) to bind to LFA-1, thereby suppressing the activities of LFA-1 expressed on all leukocytes. However, efalizumab was withdrawn from clinical use in 2009 due to concerns about the development of progressive multifocal myeloencephalopathy caused by reactivation of latent JC virus infection (*83*).

Belatacept, a fusion protein composed of the Fc fragment of human IgG1 linked to the extracellular domain of CTLA-4, binds to the ligands CD80 and CD86 on antigen-presenting cells, thereby inhibiting T cell activation. Because this drug has been successfully used in a phase 3 kidney transplantation study (*84*), it would also be an attractive immunosuppressive agent for pancreatic islet transplantation. Moreover, in the above-mentioned multicenter Australian trial, 1 recipient remained insulin-independent after switching to belatacept and MMF 6 months after the final islet transplantation because the patient could not change from MMF and tacrolimus to MMF and sirolimus due to severe anemia and edema (*80*). As discussed by Barlow *et al.* (*76*), the reason MMF is increasingly used instead of sirolimus in clinical islet programs, despite the detrimental effect of MMF on glucose-stimulated insulin secretion (GSIS) in human islets, may be the adverse effects of sirolimus rather than its toxicity to β cells.

Because the CXCL1–CXCR1/2 axis is another therapeutic target of inflammatory cytokines (*85*), reparixin, an inhibitor of CXC chemokine receptor types 1 (CXCR1) and 2 (CXCR2), was used to enhance pancreatic islet survival after transplantation in a phase 2, randomized, open-label, pilot study involving a single infusion of allogeneic islets (*86*). However, a recent

phase 3, multicenter, randomized, double-blind, parallel-assignment study (ClinicalTrials.gov, NCT01817959) showed no significant effects of reparixin on stimulated C-peptide production during the mixed-meal tolerance test at 75 ± 5 days after the first transplant and 365 ± 14 days after the last transplant, on the rate of early insulin independence or at 1 year after islet transplantation, or on any other secondary measures of glycemic control (*87*). Although the results were disappointing, there was a higher tendency for recipients in the reparixin group to achieve insulin independence after the first islet infusion, suggesting that the combination of ATG and reparixin was favorable. Further studies using new immunosuppressive agents and their combination with conventional immunosuppressants hold promise for achieving more efficient islet transplantation.

Islet transplantation in Japan

The clinical islet transplantation program in Japan has been run by the Japan Society for Pancreas and Islet Transplantation since 1997 (*88*). Following the first islet transplantation in Japan conducted by a Kyoto University group in 2004 (*31*), 18 patients with type 1 diabetes had received isolated islets from 33 non-heart-beating donors by 2007. All patients showed functioning islets, and 3 patients who received multiple transplants achieved temporary insulin independence (*89*). However, most recipients failed to maintain detectable C-peptide levels 5 years after transplantation. In April 2007, islet transplantation was suspended because the collagenase used in the islet isolation was purified from culture supernatants of *Clostridium histiolyticum* grown in medium containing brain heart infusion broth, which is a potential source of transmissible bovine spongiform encephalopathy (*88*). After the development of a mammalian-free collagenase, a phase 2, multicenter, clinical trial of islet transplantation for type 1 diabetes patients (UMIN Clinical Trials Registry: UMIN000003977) was started in 2012 based on the protocol used in phase 3 trials in North America (*12*) is currently ongoing and will end soon. Because sirolimus has not been approved in Japan as an immunosuppressant for transplantation, MMF has been used from the beginning of the maintenance period. From 2013, brain-dead donors as well as non-heart-beating donors were approved as sources of transplantable islets. The success rate of the islet isolation was very high according to an interim report (*90*) and the outcomes were favorable, which led to allogeneic islet transplantation being covered by the national health insurance in Japan beginning in April 2020 (*https://www.mhlw.go.jp/content/12400000/000602944.pdf*).

Alternative islet sources

Because of the overwhelming shortage of allogeneic donor islets, alternative islet sources are desirable. Pigs could provide an alternative source of organs and cells, even after taking into consideration the potential risks of xenotransplantation and porcine endogenous retrovirus infection (*91,92*). The first transplantation of porcine fetal islet-like cell clusters into diabetes patients using immunosuppressants was reported in 1994 (*93*). Four of the 10 insulin-dependent diabetic kidney transplant patients excreted small amounts of porcine C-peptide in urine for 200-400 days but were unable to reduce their insulin injection dose. Meanwhile, transplantation of alginate-poly-L-ornithine-alginate (APA)-encapsulated neonatal porcine islets to 14 patients with unstable type 1 diabetes was performed in New Zealand from 2009 to 2011 without immunosuppressants. The results showed no detection of porcine endogenous retrovirus DNA and RNA, decreased frequency of unaware hypoglycemic events, and a slightly decreased dose of daily injected insulin in the patients transplanted with a lower number of islets, implying that too many transplanted islets compromised oxygen and nutrition supply (*94*). A modified protocol performed in Argentina successfully reduced HbA1c in all 8 patients, with a reduction in daily insulin dose in 5 of the patients (*95*).

Stem cell-derived pancreatic progenitors and insulin-producing cells are also promising sources of cells for β cell replacement therapies for type 1 diabetes. These cells are often combined with macroencapsulation, which provides immune isolation and eliminates the need for immunosuppressive drugs. Moreover, there is the option for them to be removed from the recipient in case of emergency, mainly because of tumorigenicity derived from undifferentiated embryonic or pluripotent stem cells that might be included in the transplanted cell clusters. A phase 1/2 clinical trial using human embryonic stem cell-derived pancreatic progenitors in a macroencapsulation device (VC-02 by ViaCyte, Inc.) is ongoing in the United States, having started in 2017 (ClinicalTrials.gov, NCT03163511). Although stem cell-derived pancreatic progenitors and insulin-producing cells are more resistant than mature islets to hypoxia and nutrient deprivation, long-term viability has not yet been achieved. Further studies are being conducted. Amino acid supplementation during transplantation and devices that can supply oxygen are promising avenues of research (*96,97*).

Conclusion

Transplantation of pancreatic islets from allogeneic donors has been widely performed. Although Japan was slow to establish islet transplantation as a standard treatment for type 1 diabetes, the national health insurance system in Japan has recently begun to cover allogeneic islet transplantation from deceased donors. The shortage of donor islets has yet to be solved, but several clinical trials using xenogeneic and stem cell-

derived sources are ongoing and their results are eagerly awaited. It is hoped that they will contribute to establishing alternative sources for insulin-producing β cells in the near future.

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**Address correspondence to*:
Masayuki Shimoda, Department of Pancreatic Islet Cell Transplantation, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan.
E-mail: mshimoda@hosp.ncgm.go.jp