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REVIEW

The journey of islet cell transplantation and future development

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ABSTRACT

Intraportal islet transplantation has proven to be efficacious in preventing severe hypoglycemia and restoring insulin independence in selected patients with type 1 diabetes. Multiple islet infusions are often required to achieve and maintain insulin independence. Many challenges remain in clinical islet transplantation, including substantial islet cell loss early and late after islet infusion. Contributions to graft loss include the instant blood-mediated inflammatory reaction, potent host auto- and alloimmune responses, and beta cell toxicity from immunosuppressive agents. Protective strategies are being tested to circumvent several of these events including exploration of alternative transplantation sites, stem cell-derived insulin producing cell therapies, co-transplantation with mesenchymal stem cells or exploration of novel immune protective agents. Herein, we provide a brief introduction and history of islet cell transplantation, limitations associated with this procedure and methods to alleviate islet cell loss as a means to improve engraftment outcomes.

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Islet cell transplantation overview

Introduction and brief history

Globally, diabetes affects over 382 million people, with roughly 10% presenting with type 1 diabetes mellitus (T1DM) and is expected to rise to 592 million by 2035.[1] An annual 3% growth rate affords an escalating financial burden where the International Diabetes Federation estimates in Canada alone diabetes-related health care costs was $14 billion in 2015. These are expected to climb to a staggering $16 billion per annum by 2020.[2] Although the etiology of T1DM is incompletely elucidated, it is characterized as a multifactorial autoimmune disease resulting from specific immune-mediated destruction of pancreatic beta (β) cells within the islets of Langerhans. Classic symptoms include polyuria, polydipsia, and polyphagia with confirmation of diagnosis marked by hyperglycemia, low or indetectible serum C-peptide levels, elevated glycosylated hemoglobin (HbA_{1c}), and one or more positive autoantibody markers.[3] Those with T1DM must administer frequent exogenous insulin therapy to maintain normoglycemia. Continuous glucose monitoring systems (CGM) and insulin pumps may further help mitigate glycemic fluctuation. Recently, the FDA approved a closed-loop technology that infuses glucose regulatory hormones (insulin and glucagon) in response to glycemic fluctuations. While tighter glycemic control with medical intervention has been clearly shown to reduce secondary complications, it substantially increases risk of severe hypoglycemic reactions. T1DM is associated with a shortened life expectancy by 13 years.[4]

In consequence, the research community has focused on new avenues to arrest T1DM at the time of diagnosis. Intensive "new-onset" pilot trials conducted by TrialNet, a group of researchers aimed at identifying the prognosis and prevention of T1DM, have demonstrated means to sustained honeymoon periods and delayed diabetes onset.[5] In Brazil, Voltarelli and colleagues are currently conducting clinical trials aimed to reset the immune system in new-onset diabetes through administration of peripheral blood autologous bone marrow-derived hematopoietic stem cells coupled with immunodepleting conditioning (NCT00315133).[6-8] This approach led to impressive reversal in the diabetic state in 21 children and adolescents with new-onset T1DM, but was also associated with substantial side-effects. To date, no protocol has yet to eradicate exogenous insulin therapy entirely without substantial recipient risk. The growing

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prevalence of T1DM is concerning, and alternatives to insulin injections are needed desperately.

Beta cell replacement therapy through islet transplantation (IT) provides a potential alternative to exogenous insulin. The history of IT extends 23 years before the discovery of insulin, when Watson-Williams and Harsant in 1893 in Bristol UK attempted to treat a 13 year old boy dying from acute ketoacidosis with transplantation of pieces of sheep's pancreas.[9,10] Although the patient had minor glycemic improvements, he ultimately died 3 days after this futile first attempt at xenotransplantion. The concept of isolating islets was not revisited till 1972, when Paul E. Lacy restored glycemic control with intraportal vein infusion of islets into chemically-induced diabetic rats.[11] In 1980, David Sutherland and John Najarian, two innovative surgeons working in Minnesota, demonstrated successful intraportal islet transplantation in 10 patients with surgical induced diabetes, where the patients' own islets (autografts) were infused back after islet isolation; ultimately 3 of these patients achieved insulin independence for 1, 9 and 38 months, respectively.[12] The development of the Ricordi® Chamber and the semi-automated method for islet isolation was developed by Camillo Ricordi while working in Paul Lacy's laboratory in St. Louis.[13] This semi-automated method remains state-of-the-art today, and is available commercially (BioRep, Miami, FL, USA). In 1990 David Scharp, also working with Camillo Ricordi and Paul Lacy in St. Louis, reported the first case of transient insulin independence after islet allotransplantation, in the context of recipient immunosuppression.[14] Despite substantial advances, fewer than 8% of the 267 islet transplant attempts between 1980 and 1999 resulted in insulin independence for longer than one year.[15] In 2000, the *Edmonton protocol* developed by Shapiro et al. made IT a feasible clinical procedure. The *Edmonton protocol* was ground-breaking as it utilized a corticosteroid-free immunosuppressive protocol by combining two potent immunosuppressants: sirolimus and tacrolimus, together with an anti-CD25 antibody to protect against rejection and recurrent autoimmunity. This protocol augmented the islet mass with two or more fresh islet preparations, infusing a total islet dose that was substantially higher than had been used previously in clinical islet trials (>13,000 islet equivalents (IE) kg^{-1} recipient body weight).[16] All seven-consecutive treated T1DM subjects remained insulin independent for >1 year with sustained C-peptide production after portal vein infusion.[16] A subsequent 5-year follow of the Edmonton protocol demonstrated that most subjects lost complete insulin independence by year 3–5, with only 10% remaining insulin free by 5 years. However 80% maintained strong C-peptide secretion, which was sufficient to correct the HbA1C <7%, and most importantly protected recipients from severe hypoglycemic events.[17] The success of the Edmonton protocol rejuvenated global interest in clinical IT and at least 30 new islet centres initiated activity. The Collaborative Islet Transplant Registry (CITR) in 2001 allowed progress to be tracked closely. The most recent CITR report registered 1,584 IT infusions in 819 patients between 1999 to 2013, and currently, 27 active registered centers are active.[18] IT has improved substantially over the past 17 years with multiple further refinements including more optimal islet preparation, culture, safer transplant techniques and more effective anti-inflammatory and immunomodulatory interventions. Likely cellular replacement therapies will become mainstay treatment, more practical and cost effective, for larger numbers of T1DM patients.

Islet cell transplantation procedure – isolation, purification and infusion

IT requires sequential steps including donor pancreas procurement, islet isolation, purification, culture and infusion. Attention to detail throughout all steps in this process are required to maximize islet integrity and survival. Organ donation from a multiorgan donor (neurological determination of death, or more recently also deceased cardiac death donors), after donor family consent. Donor characteristics, including age, body mass index and absence of diabetes in the donor (HbA1C <6.5%) may affect islet yield.[19] While obese donors previously provided the best islet mass, improvements in collagenase enzymes and purification protocols have improved the success of islet isolation from the younger, thinner donors too. After the pancreas is flushed and cooled with preservation solution University of Wisconsin (UW) or Histidine Ketoglutatate (HTK) solutions via intravascular flush, the pancreas is surgically removed and packaged for transport to the isolation centre. It is essential that the pancreatic capsule remains intact and uninjured if the pancreas is to be distended with collagenase

satisfactorily once the pancreas reaches the isolation laboratory. Once in the clean room facilities (clinical Good Manufacturing Practice (cGMP) approved), the duodenum, spleen and fatty tissues are dissected away from the pancreas, the pancreas transected at the neck or mid-body, and the pancreatic duct cannulated in both proximal and distal directions. The pancreatic duct is then perfused with cold then warmed collagenase solutions under pressure for 10 minutes to load the pancreatic acinar-islet interface with digestive enzyme. The pancreas is then chopped into multiple pieces (typically 9 or 10 large fragments), and transferred to the Ricordi Chamber where warm collagenase enzyme and serine protease solutions are recirculated while the chamber is shaken to facilitate separation of islets from their exocrine stromal matrix. The Ricordi Chamber serves to both mechanically and chemically digest islets. Once islets are liberated into the solution, the digestion is halted by cooling to 4°C and the enzyme is further quenched with the addition of collagenase binding proteins (human albumin solution). The islet digest is then purified on a COBE 2991 cell processor using a continuous density gradient of BioChrom Ficoll solution to separate islets from the exocrine tissue, the islets being less dense on centrifugation. Islets are then cultured for 24–72 hours at 20°C or 37°C (centre dependent) in media supplemented with insulin, transferrin and selenium. Before transplantation, the purified islet preparation must undergo detailed quality control testing to assess islet viability, purity, insulin content, cell number and insulin secretory response. Islets must have adequate purity (>50%), dose (>5000 IEQ kg^{-1}), a settled tissue volume <7cc, and be sterile on Gram stain.[20,21] The islet culture step may minimize the number of dying islets and acinar cells that are infused into the recipient, but these dying cells also create a toxic milieu for the remaining islets during culture.[19,22] Maximizing the infused islet mass, matched to the ABO-blood type of the recipient, is important as it decreases the need for multiple donor islet infusions. The fewer the donors required reduces the risk of HLA-recipient sensitization. Although considerable research efforts have been made in the field, the optimal protocol has still to be standardized.

Currently intraportal islet infusion remains the gold standard site for implantation. To date, this is the only site that has reliably led to high rates of insulin independence in patients with T1DM. The portal vein may be safely accessed by a minimally invasive percutaneous transhepatic access route. The advantage of this approach is that patients do not require surgery or general anesthesia. The early disadvantage was that some patients developed intraperitoneal bleeding from the liver surface after the portal catheter was withdrawn. Refinement in this technique with occlusion and obliteration of the catheter track using soluble hemostatic paste agents (Avitene paste or D-STAT) have virtually eliminated this complication in the larger centre experience. Administration of therapeutic heparin at 70 units per kg recipient weight delivered intraportally with the islets, and a heparin infusion initiated at 3–5 units/kg/hour then adjusted to main a PTT between 60–80 seconds has further eliminated the risk of branch vein portal venous thrombosis, another recognized complication of this procedure. Maintaining a purer islet preparation of typically 2–3ccs of islet tissue coupled with systemic heparinization has been an important component in mitigating this thrombotic risk.[20] Although intrahepatic infusion is associated now with minimal complications, the intravascular site fails to provide an optimal environment for islet survival, and it has been estimated that >60% of the infused islet mass is destroyed within minutes to hours by innate immune responses.[23] Means to optimize islet survival throughout all steps in the islet preparation process is seen as key to the success of this approach (Fig. 1).

Current limitations and possible alleviations in clinical islet transplantation

Instant blood-mediated inflammatory reaction (IBMIR)

The innate destruction of transplanted islet tissue occurs through an intense reaction called the instant blood-mediated inflammatory reaction (IBMIR). The process is triggered by exposed tissue factor on the islet surface, which attracts platelets that bind and undergo their release reaction, and a cascade of clot adherence and intense inflammatory cellular recruitment follows.[24,25] Potential means to reduce inflammatory islet stress and protect islets can be achieved through addition of anti-inflammatory agents during islet culture and systemically to the recipient, administration of anticoagulants, or islet coating with a variety of protective macromolecules.[26] The infusion of anticoagulation agents such as dextran sulfate or heparin has been shown to improve islet

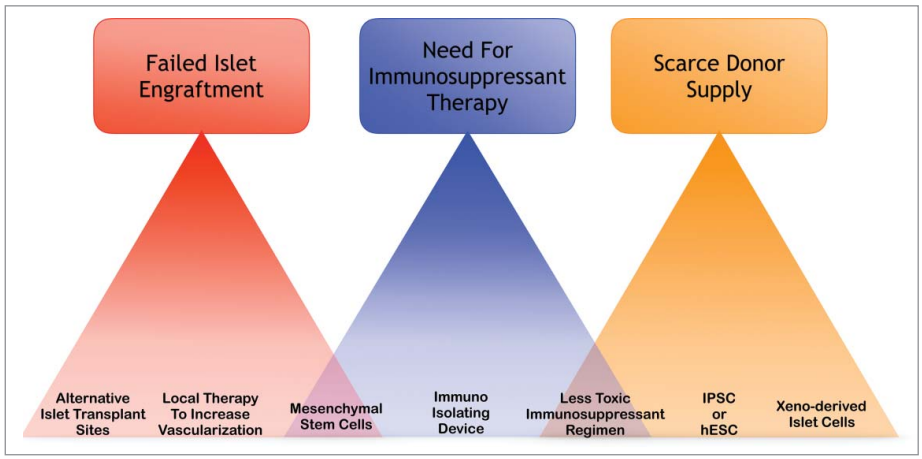

Figure 1. Drawbacks of islet transplantation. Islet transplantation is a temperate alleviation from exogenous insulin injections. Drawbacks include, but not limited to failed engraftment, the need for a lifelong immunosuppressant therapy, and scarce donor supply. Displayed in colors are possible avenues to help alleviate these drawbacks.

survival by downregulating the IBMIR response in the experimental setting, but remains to be validated in clinical studies.[27-29]

Alternative transplantation sites

As islets are infused intraportally, they embolize and become trapped within the portal sinusoidal capillaries. This may render islets ischemic, and apoptotic or necrotic islet death may ensue.[119,20-30] The inability to locate, visualize or biopsy human islets within the intrahepatic site creates a challenge, and has hampered progress as the scale and relative nature of the various insults affecting islet survival cannot be quantified easily. Several investigators have searched for more favorable extrahepatic sites that might obviate IBMIR and provide more ready access for biopsy, imaging and retrieval. Such sites have included the renal subcapsular space,[31] striated muscle,[32] pancreas,[33] omentum,[34] eye chamber,[35] and testis.[36]Although these alternative sites can reverse hyperglycemia in small animal models, thus far only the omentum has recently allowed a small number of subjects to become insulin independent for short periods of time. Thus far, all attempts to develop a clinically applicable site that is proven to be superior to the intraportal site have remained elusive. The renal subcapsular site in favored and most efficient for islet implantation in rodents over the intraportal site, but this does not translate in species larger than mice and rats.[37]The subcutaneous space remains an attractive consideration as alternative embryonic stem cell products are being developed for the clinic, mainly because this site is easily retrievable. However, the limited vascularization and low oxygen tension of this site poses challenges. Efforts to improve islet survival within the subcutaneous space may be achieved by the use of a prevascularized technique, which harnesses the natural foreign body reaction, achieved by pre-implanting a catheter.[38] Our laboratory developed a "deviceless" method that implants islets into a prevascularized subcutaneous site created by the temporary placement of a 5 or 6F hollow nylon medical-grade catheter used in angiography.[39] The deviceless method was found to be highly effective in reversing diabetes in full dose and marginal mass islet transplants with human or mouse islets, and with human derived-pancreatic endoderm cells.[39,40] We have yet to test this approach in larger animals or in patients, so the utility of this approach remains unknown in clinical translation.

A Canadian based biotechnology company, Sernova Corp., developed a permanent plastic mesh-based device with removable plugs called the Cell Pouch™. After Health Canada approval, the device was loaded with human islets and implanted into 3 patients treated at the University of Alberta. While the device was able to reverse diabetes successfully in mice, none of the patients demonstrated any islet function, and had only *de minimus* islet survival upon device explantation.[41,42] Considerable further research is required to refine these and other approaches if they are to be useful in the clinic. Another promising alternative IT site is the omentum based on the expansive surface area, rich blood supply, portal drainage and potential for minimal access surgery. By folding the omentum upon itself, additional surface for oxygenation and metabolic exchange may be accomplished.[43] The

University of Miami is currently completing a Phase I/II clinical trial with this approach, transplanting human allogenic islets coated in autologous plasma and placed using laparoscopic instruments onto the wrapped omentum (NCT02213003). Recently, a 43-year-old diabetic woman was rendered normoglycemia with this approach, and its clinical investigation is ongoing.[44] The intramuscular site is a vascular enriched site that has comparable blood flow to the native pancreas.[45] In Sweden, a 7-year-old girl receiving intramuscular autologous islets after a total pancreatectomy was reported; this subject had detectible C-peptide but failed to gain insulin independence.[46]

Encapsulation technologies

The potential to shield transplant islets or stem cells from immune attack through micro or macroencapsulation approaches is a concept that has been explored extensively over the past seven decades. Encapsulation utilizes selectively permeable membranes that permit passive diffusion of glucose, insulin, oxygen, carbon dioxide and other nutrient exchange while preventing direct cell-cell contact with immune cells. Factors to consider in evaluating such devices include the site of transplantation, the device configuration, the materials used, and their ability to promote neovascularization and biocompatibility.[47]

Macroencapsulation involves encapsulating multiple islets within a device >1mm diameter and is usually placed in an extravascular space.[48] The use of macroencapsulation dates back to the 1950's when Algire, Prehn, and Weaver transplanted thyroid tissue within a device made of lucite rings, membrane filters, and lucite-acetone seal.[49,50] Several studies demonstrate islet cell survival within macroencapsulation devices in mice, but translation to larger animals or humans is often limited by fibroblastic overgrowth around the device.[51] In the 1990's a double-membrane sealed device called the Theracyte™ device was developed by Baxter Healthcare and showed initial promise, but failed to maintain euglycemia.[50,52,53] In 2013, Ludwig et al. used an oxygenated macro-chamber (Beta-O_2) to implant human islets without immunosuppression beneath the abdominal wall skin of patients. Human islets were stabilized in an alginate matrix.[54] Preliminary published data confirmed that patients had detectible human C-peptide in the complete absence of immunosuppression, but none were insulin independent.[55] ViaCyte Inc. created a macroencapsulation device termed Encaptra™, which also has an outer plastic weave support matrix and an inner thin immune barrier layer to protect implanted cells. In 2014, ViaCyte Inc. launched a Phase I/II combination trial of human embryonic stem cells derived product (PEC-01, derived from Cyt49 cells, implanted within Encaptra™) (VC-01™, NCT02239354). In 2017 ViaCyte further initiated a second trial using a perforated macroencapsulation device containing PEC-01 cells, in which it is anticipated that cell survival will be improved by more optimal neovascularization, but recipients in that trial will require full systemic immunosuppression (PEC-direct™ (VC-02™, NCT03163511). Islet Sheet Medical developed a flat sheet device made of ultra-thin biocompatible polymer which showed early promise in small animal models, but failed to be replicated in larger animal studies.[48] The concept of macroencapsulation has been around for several decades, but is still plagued by cell survival challenges as cells are cut off from physiologic gaseous and nutrient exchange. Ongoing studies will help to define the utility of such approaches with the hope that transplants could be conducted without need for chronic immunosuppression. Importantly, these devices have not been tested thoroughly in human patients with autoimmune diabetes, and it remains unknown how effective they may be in preventing recurrent autoimmunity.

The alternative approach of microencapsulation involves coating of individual islets or islet clusters in an immunoprotective envelope. In 1964, Chang et al. described microencapsulation.[56] and in 1980 Lim and Sun demonstrated islet survival with alginate-polylysine-polyethyleneimine microcapsules.[57] Alternative microencapsulation materials have been explored, including polyethylene glycol, poly methyl methacrylates, alginate, agarose, or chitosan.[48,58] In one study, Vegas et al. maintained normoglycemic for 174 days and suppressed perivascular overgrowth in mice.[59] Human islets have heterogeneous diameters ranging from <50-500μm, and manufacturing a microencapsulation system that accommodates this range has proven to be a challenge.[60] Conformal coating has generated much interest, but even this approach frequently leads to islets that breech the microencapsulation barrier, and when transplanted leave exposed donor antigens accessible to the recipient immune system. Shielding of direct immune cell-to-cell contact may be an over-simplistic approach as it overlooks the cytokine and damage associated membrane

products (DAMPs) small molecule cross-talk between contained damaged and dying donor cells that can be sensed by the recipient immune system. Clinical trials of encapsulated pig islets by Diabecell® generated much interest, but the final results of those trials are disappointing as insulin requirements remain unchanged from baseline and pig C-peptide was undetectable.[61,62] Although microencapsulation holds potential promise, materials and nutrient exchange remain suboptimal to sustain islet survival. Ongoing studies will determine if some of these barriers may be overcome with more refined biomaterials and technologies.

Islet graft revascularization

The islets of Langerhans constitute ~2% of the total pancreatic mass but receive up to 20% of the pancreatic blood flow.[63] Revascularization is imperative for islet survival after transplantation. Islets have a dense network of sinusoidal capillaries that drain into peripheral venules.[64] The process of islet isolation strips off these capillary networks, and islets must therefore neovacularize if they are to survive. Angiogenesis begins between the first and day post-transplant, and expands for the first 14 days, as new arteriolar vessels grow in from recipient origin.[65] Vascular remodeling may then continue for up to 3 months.[65]

Islets and vascular endothelial cells express high levels of vascular endothelial growth factor (VEGF) that serve to recruit neovascularization.[64,66] Supplementation of VEGF in the islet graft may have both positive and negative impact, as VEGF also recruits and amplifies inflammation that can also be destructive to islet survival. Cheng et al. utilize an adenovirus containing cDNA from human VEGF isoforms and transplants transfected islets into diabetic nude mice and found improved islet revascularization with normoglycemia.[67] VEGF also stimulates release of interleukin, increasing blood flow to ischemic tissue.[68] Hepatocyte growth factor (HGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), and matrix metalloproteinase (MMP) may also expedite the islet vascularization process.[69,70] Basterrechea et al. applied a plasma-based scaffold containing fibroblasts to augment subcutaneous IT function in mice.[71]

Brief overview of oxidative stress

Oxidative stress is associated with release of free radicals especially reactive oxygen species (ROS).[72] Islets are especially vulnerable to pro-inflammatory cytokines (e.g. TNFα), free radicals (i.e. H_2O_2 or peroxynitrite) and superoxide dismutases (SODs).[73] Treatment of islet preparations with potent antioxidants may mitigate oxidative stress. Supplementation with glutathione (GSH) was able to decrease apoptosis and reduce intracellular ROS during islet isolation.[74] This supplementation may have the converse detrimental effect of disrupting VEGF synthesis and thereby impede neovascularization.[75] An antioxidant metalloporphyrin analog BMX-010 improved islet function and survival and was non-toxic to islets.[72] A pilot study using BMX-010 is currently underway at the University of Alberta to evaluate the impact of this agent in improving single donor islet engraftment.[76] Controlling oxidative stress could provide promise for improved islet survival.

Mesenchymal stem cells to improve islet engraftment

Mesenchymal stem cells (MSCs) were first identified by Friedenstein et al. in rat bone marrow in 1966.[77] MSCs are non-hematopoietic precursor cells that can differentiate into mesoderm lineages: osteocytes, chondrocytes, myocytes, and adipocytes.[78] MSCs may be isolated from amniotic fluid,[79] skeletal muscle,[80] adipose tissue,[81] umbilical cord,[82] and human umbilical cord perivascular cells.[83] MSCs may be transdifferentiated into insulin-producing cells, but have yet to be rendered as fully functional β-like cells.[84] MSCs also secrete trophic factors that may stimulate and support tissue regeneration,[85] and also hold immune regulatory properties that could also suppress allograft rejection[86] (Fig. 2).

Trophic factors

MSCs may promote angiogenesis through gene expression of cytokines, including VEGF, fibroblast growth factor (FGF), Angiopoietin-1 (Ang-1), matrix metalloproteinase (MMPs) and transforming growth factor-β (TGF-β).[87,88] Such growth factors may facilitate islet survival. MSCs can migrate to sites of injury and release paracrine factors that regulate local inflammation, and may promote revascularization and repair at the transplant site.[89-91] Ongoing studies will help define the potential role of MSC co-transplantation aimed at improving islet survival.[92]

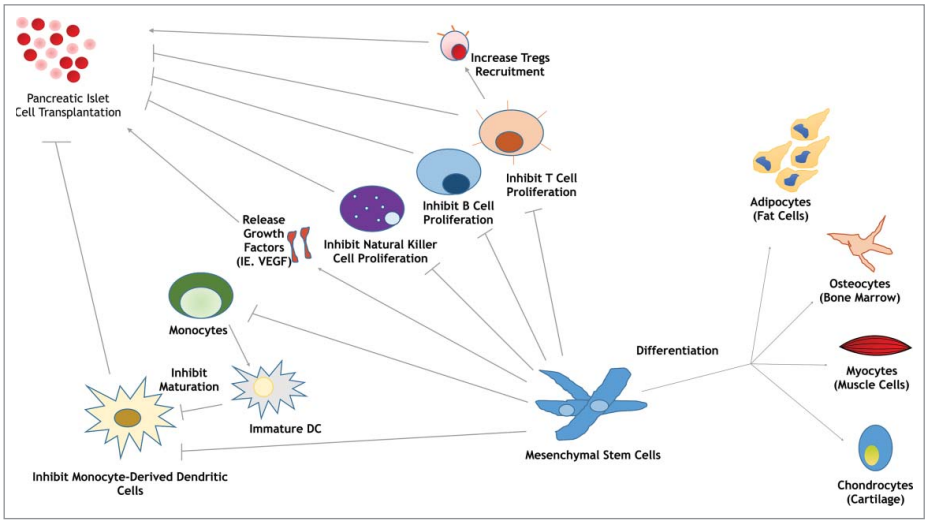

Figure 2. Mesenchymal stem cell differentiation and co-transplantation. The benefits of MSCs effectiveness to ameliorate islet cell transplantation and MSCs capability to differentiate is displayed. The multipotent capability of MSC can differentiate into mesodermal lineages such as adipocytes, osteocytes, myocytes, and chondrocytes. MSCs co-transplanted with pancreatic islets can decrease the proliferation of natural killer cells, dendritic cells, monocytes, B cells and T cells. The inhibition of T cells promotes regulatory T cells (Tregs). Alongside, MSCs release trophic factors that can improve islet engraftment.

Immunomodulatory effects of MSCs

MSCs may immunomodulate both innate and adaptive immune responses in experimental islet transplantation, both through direct and indirect antigen presentation.[88,93,94] MSCs suppress T lymphocyte proliferation and have low human leukocyte antigen (HLA) Class I expression. Low but inducible Class II MHC expression by MSCs could further modulate allogeneic rejection.[95] MSCs may decrease B cell proliferation,[96] natural killer cells,[97] and monocyte-derived dendritic cells.[93] MSCs suppress T cell reactivity and proliferation, and increase recruitment of T-regulatory cells (Tregs).[98] MSC secretion of matrix metalloproteinases 2 and 9 may block T cell expansion and activation.[99] Tregs are master regulators of immune reactivity and involution, and could potentially facilitate immune tolerance induction or minimize the need for chronic long term immunosuppression in transplant recipients.[100] Berman et al. noted Tregs recruitment occurred with co-transplantation of allogeneic islets and third-party MSCs, and led to prolonged islet survival.[101] MSCs decrease T cells through reduced differentiation, maturation, and dendritic cell (DC) function.[102] CD11c (DCs phenotype derived from monocytes) and CD83 (mature DCs phenotype) can be down-regulated in mice using co-transplantation of pancreatic islets with MSCs.[103] Co-transplantation of bone marrow MSCs co-cultured with different agonist antibodies including anti-CD40, or anti-IL-4 markedly inhibited B cell and immunoglobulin production.[96] MSCs alter natural killer (NK) cell function by suppressing proliferation and cytotoxicity. Spaggiari et al. found that MSCs inhibited NK cell function.[97] The role of MSCs in cross-regulation of cytokine production and immune cell function merits ongoing intense research.[104]

Reversing autoimmunity in type 1 diabetes

All future strategies that aim to reverse diabetes with cellular replacement of insulin secreting cells will require some adjunctive strategy to prevent recurrent autoimmune destruction of the newly transplanted cells. TrialNet is a group of international scientists that have focused efforts in reversing autoimmunity in T1DM. Over 500 strategies have proven effective in NOD autoimmune mouse models, but very few have translated to clinical benefit. This suggests that the mouse model is an inadequate representation of the human disease, and that strategies that interrupt autoimmunity in mice are inadequate when applied to the far more complex human immune system. Haller et al. gave newly diagnosed T1DM subjects autologous umbilical cord blood and reported lower HbA_{1c} and reduced insulin requirement.[105] This study is small and underpowered, and lacked appropriate control groups to demonstrate clear efficacy in the intervention arm. Voltorelli et al., and more recently Couri et al. infused with hematopoietic stem cells after myeloablative conditioning in children

with new onset T1DM, and reported remarkably high rates of insulin independence with restoration of endogenous C-peptide production.[7] This approach was associated with infectious and other complications related to the conditioning regimen, including sterility. Bluestone et al. infused autologous polyclonal reactive *ex vivo* expanded Tregs into patients with new onset T1DM and markedly prolonged the honeymoon period with that approach.[106,107]

Alternative islet cell sources

The available organ donor supply will never be sufficient to match the potential demand if cellular replacement therapies are to play a greater role in the treatment of all patients with T1DM and T2DM. Thus, alternative strategies including gene therapy, xenotransplantation and stem cell transplantation are being explored to bridge this gap. Transfecting non-islet cells to contain and express glucose-regulated insulin is an attractive approach that has been tested in keratinocytes,[108] adipose-derived stem cells,[109] and hepatocytes.[110] Hepatocytes share a common endodermal origin with pancreatic islets, and hepatocyte adenoviral transfection with genes encoding for human proinsulin have demonstrated ability to secrete human insulin and C-peptide and maintained normoglycemia in small and large animal models.[111] If a glucose-sensitive promoter could be included in these constructs, and if the vectors were less antigenic, their potential could be substantial in the future management of all forms of clinical diabetes.

Xenotransplantation

Xenotransplant sources of islet replacement have been considered for many years. Neonatal or adult pig islets provide an attractive source.[112] First-in-human trials by Carl Groth and colleagues in 1994 involved transplantation of fetal pig islets placed beneath the kidney capsule of human kidneys in patients with diabetes and renal failure.[113] These studies were remarkable as porcine C-peptide was detectable for >300 days in many subjects, and no serious side effects were observed. However, no reduction in insulin requirement and no insulin independence was ever observed. The opportunity to genetically manipulate the pig genome initially with knock-out constructions for decay accelerating factor, and Gal epitopes, and more recently the potential to humanize the pig genome using CRISP-Cas9 technologies, offers great potential.[114-117] These technologies have been used recently to eliminate porcine endogenous retroviruses from the pig genome. CRISPR-Cas9 was used to inactivate 62 copies of the PERV pol gene in a porcine cell line and resulted in >1000-fold reduction in PERV transmission to human cells.[118] Nui et al. formulated PERV-inactivated pigs with this approach. In New Zealand, encapsulated neonatal porcine islets have been transplanted into non-immunosuppressed T1DM patients but with minimal if any detectable function to date.[119] Detectable porcine C-peptide has been strikingly absent in these subjects, and insulin requirement reduction has been modest, suggesting that these cells are non-functional to date. A similar study in Argentina with encapsulated porcine islets showed similar findings, but claimed modest reduction in HbA_{1c} and some correction of hypoglycemic unawareness, but these studies lack sufficiently rigorous controls to validate that the function is all derived from the transplanted xenogenic cells.[120] Ongoing studies are required to validate such approaches, and the further application of CRISP-Cas9 to humanize the porcine genome will generate more promise, but additional ethical challenges too.

Pluripotent stem cell transplantation

Human embryonic stem cells (hESCs)

Human embryonic stem cell (hESC) and induced pluripotent stem cells (iPSC) are being intensively investigated for their ability to differentiate into insulin producing cells. Essential expression of a series of transcription factors including pancreatic homeodomain transcription (PDX1), the homeobox transcription factor NKX61, and MafA have been used to generate pancreatic progenitor cells.[121-123] In 2004, Kubo et al.[124] successfully differentiated hESCs into pancreatic endoderm cells (PEC). In 2006, a California-based company called ViaCyte Inc. generated PEC-01 cells that experimentally displayed positive C-peptide, proinsulin, and key transcription factors that led to regulated insulin production after transplantation and *in vivo* differentiation.[125] ViaCyte Inc. utilizes a single pluripotent embryonic stem cell line, termed CyT49, that differentiates into PEC-01 cells. The PEC-01 cell population is intended to mature into glucose-responsive and insulin-producing cells and continues to differential *in vivo* after implantation. Two clinical trials of Viacyte's PEC-Encap™ (VC-01™) and PEC-Direct™ (VC-02™) utilize these hESC-derived pancreatic endoderm cells contained in a macroencapsulation device. In trial VC-01, the device has an intact membrane

that prevents direct immune cell-to-cell contact, whereas in VC-02 the device has laser microperforations designed to improve neovascularization and stem cell survival, but recipient subjects in this second trial require full dose immunosuppression. Ongoing data is eagerly awaited to validate the safety and preliminary efficacy of these promising approaches.

ViaCyte cells are considered 'Stage 4' and are immature at the time of transplantation. The advantage of this approach is that the metabolic demands of cells at this stage may be less than their fully mature metabolically active counterparts. Furthermore, expression of Class II HLA antigens may be less, and so the cells may be less immunogenic. This remains to be proven; however, such cells take 2–3 months to fully mature in mice, and are not expected to work instantly when transplanted into patients. Those with longstanding T1DM may be more than happy to wait the 2-3-month maturation period which may be inconsequential. However, other groups including Rezania et al. have further differentiated these types of cells to a more mature 'Stage 7' phenotype, which are more mature and engraft faster after implantation in mice.[121] Paglucia et al. used similar 'Stage 7' cells to avert diabetes onset in mice, and demonstrated more robust function *in vitro*.[126] Russ et al. confirmed earlier diabetes reversal with similar cellls.[127] Doug Melton and colleagues within the Harvard Stem Cell Institute and Semma Therapeutics have used a 6-step protocol to create more mature human β cells from hESC-derived cells.[126,128] The potential risk of teratogenicity warrants caution in all approaches that use hESC-derived product. Whether a benign teratoma would have serious consequences in patients, or whether unregulated growth would pose high risk of hypoglycemia remains to be tested, but these remain of concern in first-in-human trials. There are potential ethical and religious considerations when hESCs are used, as the starting cell population is derived from discarded human embryos taken at the blastocyst stage. ViaCyte's Cyt49 and subsequent PEC-01 derivation was obtained from just one human discarded embryo. Many, but not all might consider that a small ethical price to pay for the potential to provide a limitless cell source for future diabetes treatments.

Induced pluripotent stem cells (iPSCs)

In 2006, Shinya Yamanaka's group from Kyoto Japan developed a protocol for dedifferentiating and transdifferentiating adult human pluripotential stem cells.[129] The Yamanaka genetic factors Oct3/4, Sox2, Klf4 and c-Myc allowed human skin fibroblasts to dedifferentiate and to mature into human cardiomyocytes. Ongoing intense research will determine the utility of generating patients' own β cells with the iPS approach. Controlling recurrence of islet autoimmunity will be key to the success of this approach. Patients will not require immunosuppression, but the costs associated with good manufacturing practice (GMP) manufacture of individualized stem cells could be astronomical, and there are still many hurdles to cross.

Post-Transplant limitations

Protecting against immunosuppressant-related toxicity

Islet and future stem cell therapies will not be considered truly 'curative' until such treatments can be delivered and maintained without need for chronic immunosuppression. Antirejection drugs paralyze immune responses to alloantigens effectively, but also increase the risk of life-threatening infection or malignancy. Furthermore, the most potent antirejection drugs (tacrolimus and cyclosporine) have direct toxicity to β cells.[130-132] Approaches that limit need for immunosuppression will lower the barrier for future patients with diabetes being considered for cellular therapy. Gala-Lopez et al. found that an anti-aging glycopeptide (AAGP) protected human islets from tacrolimus toxicity and promoted graft survival and function.[133] Ongoing work in the area of tolerance induction using myeloablative chemotherapy or non-ablative cellular therapeutics including facilitating cells or Tregs could transform future opportunities across all aread of transplantation.

Conclusion

T1DM remains a chronic autoimmune disease resulting from permanent destruction of β-cells. Improvements in new insulin formulations, continuous insulin and now coupled glucagon infusion pumps, and continuous glucose monitoring systems represent advances in care, but are still cumbersome, imprecise and costly. Cellular replacement with IT has also advanced considerably, and this therapy is of proven benefit in protecting against hypoglycemia, correcting HbA1C,

and in many cases providing sustained periods of insulin independence. The need for lifelong immunosuppressive therapy and attendant risks of infection, cancer and nephrotoxicity pose their own unique additional challenges, making this treatment unattractive for all but those with severe risk of brittle hypoglycemia. IT success is also hampered by limited islet survival after implantation, resulting from a combination of innate immune attack through IBMIR, recurrent autoimmune islet destruction or alloimmune rejection. Optimizing neovascularization with better control of angiogenesis, suppressing inflammation and reducing oxidative stress all offer to further improve outcomes with IT. Access to human islets from available scarce organ donors makes cellular replacement therapy impractical if indications are to be broadened to include patients with both T1DM and T2DM. Alternative cell sources are therefore required. Intense efforts to improve islets derived from xenogenic sources are underway, and in parallel remarkable progress has occurred in the science and application of pluripotential stem cells, which are now entering early pilot clinical trials. The possibility that cellular transplantation could be accomplished with less need for immunosuppressant's remains a tangible possibility, and advances in immune regulation control with Treg infusions, MSC co-transplantation and other innovative approaches are underway. Further, advances in vascularized macroencapsulated oxygenated devices offer potential to shield transplanted cells from immune attach. It remains to be seen whether any of these approaches will prove to be as promising in patients as they have offered to date in mice.

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