Clinical islet cell transplantation – recent advances

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Type-1 diabetes mellitus (T1DM) caused by autoimmune destruction of insulin-producing beta-cells essentially requires treatment with exogenous insulin therapy. Despite the education, technology and improvements in insulin formulations, patients face the ongoing life-threatening hypoglycaemia with poor quality of life as well as progressive disease leading to micro- and macro-vascular complications of diabetes. Islet transplantation offers an alternative therapeutic option for these patients. In this review, we discuss the recent advancements in this field tracking from the history of pancreatic islet transplantation to the present-day challenges of clinical islet cell transplantation. We summarize the cutting-edge clinical research with special reference to the results of current trials, including Clinical Islet Transplant Consortium, improvements in immunosuppressive protocols, the need for beta-cell replacement therapies, including the application of induced pluripotent stem cells and mesenchymal stem cells.

Keywords: Diabetes, insulin, islet cell transplantation, immunosuppression, stem cells.

Introduction

TRANSPLANTATION of islets isolated from pancreas is a promising treatment for patients suffering from severe form of type-1 diabetes mellitus (T1DM) to attain normoglycaemia. Although this treatment has been conceptualized for more than a century, its clinical practice has seen a remarkable increase only from the year 2000. This is the only procedure known to provide a 'cure' for T1DM in terms of its dependence on exogenous insulin use. Despite offering several advantages as a treatment for diabetes, it is also facing several hurdles in its broader application. This review covers the historical development of pancreatic islet transplantation and the recent progress made in this field.

History of pancreatic islet transplantation for type-1 diabetes

The introduction of insulin therapy in 1922 by Frederick Banting and Charles Best revolutionized the treatment of patients with diabetes and prolonged the lives of millions of people[1,2]. While insulin therapy treats patients with diabetes, it does not cure the disease, nor does it prevent the development of secondary complications associated with long-term diabetes. The need for a more permanent cure spurred researchers to explore other options. The pioneering experiments of Lacy and Kostianovsky in 1967 (ref. 3) showed that viable islets could be extracted from the pancreas of a rodent donor and reinfused in the portal vein of a diabetic rat recipient to achieve stable euglycaemia[3]. These experiments provided the basis for the emergence of islet isolation and transplantation. Subsequent discoveries related to enzymatic digestion and tissue purification paved the way for successful large-scale human islet isolation. The intraductal injection of collagenase proved an effective method for successful islet isolation from large animals and humans[4].

The first clinical islet transplant was performed at the University of Minnesota, USA by Najarian *et al.*[5]. Early islet transplants had very little success, as it was difficult to obtain sufficient amounts of human islets for allotransplantation, until a new method for the isolation of human islets from the pancreas was described by Ricordi *et al.*[6]. This approach allowed the extraction of consistently high numbers of purified and viable human islets for transplant. In subsequent years, attempts at transplanting islets into diabetic recipients were rather unsuccessful, except for the cases described in the early 1990s in Pittsburgh, USA, of patients with surgical rather than autoimmune diabetes who received multiorgan transplants, including islets. This first successful series of islet allografts provided the proof of concept. Despite these efforts, only 8% of all diabetic recipients of islet grafts worldwide could continue free of exogenous insulin[7].

Edmonton Protocol and later clinical trials

In 2000, researchers at the University of Alberta in Edmonton, Canada, reported successful reversal of diabetes in seven consecutive patients by pancreatic islet transplantation[8]. Their novel immunosuppressive regimen with meticulous preparation of islets, later named the 'Edmonton Protocol', revolutionized the field of islet transplantation. This protocol used improved techniques

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in pancreas procurement and isolation, with a focus on transplanting an adequate islet mass and using corticosteroid-sparing immunosuppression[9]. Specifically, the protocol involved harvesting the pancreas prior to multiorgan retrieval; avoiding its prolonged cold storage processing the pancreas for islet isolation immediately; avoiding animal serum products during isolation; infusing an islet mass of greater than 10,000 islet equivalents (IEQ)/kg of recipient body weight by infusing islets from 2–3 donors, and using an immunosuppressive protocol comprising induction therapy with a humanized interleukin-2 receptor antibody (daclizumab) and maintenance therapy comprising low-dose tacrolimus and sirolimus[8].

The International Trial of the Edmonton Protocol for Islet Transplantation was organized by the Immune Tolerance Network and was initiated by the National Institutes of Health (NIH), USA, in order to establish centres of excellence to conduct tolerance-based trials[9]. The trial consisted of a series of 36 T1DM patients across nine international islet centres who received islet transplants. Islets were isolated from the pancreas of deceased donors and transplanted after purification, without any culture period. The study results showed that 21 patients (58%) attained insulin independence with stable glycaemic control at any point throughout the trial. Among these patients, 16 (76%) required insulin again at 2 years; 5 of the 16 patients (31%) who reached the primary endpoint remained insulin-independent at 2 years[9]. Following the trial, high rates of insulin independence were observed 1 year post-transplant in leading islet transplant centres, and an international multicentre trial demonstrated the reproducible success of the approach[10,11]. In centres with the most experience with the procedures, approximately 80% of patients treated with islet transplantation could achieve insulin independence within the first year post-transplantation[10].

A series of major clinical trials have been performed by the Clinical Islet Transplant (CIT) Consortium (CIT website: http://www.citisletstudy.org/). This Consortium is a network of clinical centres and a data coordinating centre established in 2004 to conduct studies of islet transplantation in patients with T1DM. Studies conducted by the CIT Consortium are focused on improving the safety and long-term success of methods for transplanting islets[12]. Seven CIT clinical trials have been completed or are nearing completion, including phase 3 clinical trials under way to support the biological license application mandate from the US Food and Drug Administration (FDA)[13]:

- CIT-01: Open randomized multicentre study to evaluate the safety and efficacy of low-molecular-weight sulphated dextran in islet transplantation (Nordic countries).
- CIT-02: Strategies to improve long-term islet graft survival (University of Miami and University of Illinois, Chicago, USA).
- CIT-03: Peritransplant deoxyspergualin in islet transplantation in T1DM (University of California-San Francisco, University of Minnesota, and Northwestern University, USA).
- CIT-04: Islet transplantation in T1DM with LEA29Y (belatacept) maintenance therapy (University of Alberta, Canada and Emory University, USA).
- CIT-05: B-lymphocyte immunotherapy in islet transplantation: toward calcineurin inhibitor-free immunosuppression (University of Pennsylvania, USA).
- CIT-06: Islet transplantation in T1DM kidney allograft recipients: efficacy of islet after kidney transplantation (all North American sites).
- CIT-07: Islet transplantation in T1DM (all North American sites; phase-III clinical trial for obtaining biological licensure).

The results of these clinical trials will be available to public in near future (http://www.citisletstudy.org/).

As of 2014, over 750 islet allotransplants have been performed across more than 30 international islet processing facilities. A report from the Collaborative Islet Transplant Registry[14] stated that 44% of islet allotransplant recipients were insulin-independent three years after receiving an islet graft from 2007 to 2010 (refs 15, 16). However, few islet processing facilities perform islet allotransplants in the United States because of classification of the treatment as an experimental therapy and not a clinical therapy. As a result, acquiring sufficient funding to continuously perform islet allotransplants has proven difficult. Islet facilities in other countries, most notably in Europe, are not subject to the FDA and have established their own clinical islet transplant programmes. The University of Alberta, Canada, is currently the most active centre, having performed 66 islet transplants in 2013 alone[16]. The Edmonton group has reported that after 400 islet preparations in over 200 patients, 79% of recipients showed full or partial graft function[15].

Results from recent phase 3 trials of human islet transplantation

In T1DM patients, the impaired awareness of hypoglycaemia (IAH) and severe hypoglycaemic events (SHEs) cause substantial morbidity and mortality. The current therapies are ineffective in preventing these hypoglycaemic episodes, leaving an enormous number of T1DM patients at high risk. According to the regulations by the FDA, for clinical islet transplantation to be considered as a standard therapy, it is mandatory to demonstrate its efficacy using clinical trials. The CIT consortium trials were designed for this purpose and results of these trials have offered promising data on the significant decrease in SHEs in T1DM patients[17]. This multicentre single-arm, phase-3 study of the investigational product purified

human pancreatic islets (PHPI) was conducted at eight centres in North America and 48 adults experiencing T1DM for over 5 years were included in the study. These adults had absent or zero stimulated C-peptide and documented with IAH and SHEs in spite of expert care. Each individual in this study received one or more transplants of PHPI, under proper immunosuppression.

In the CIT consortium trial, the primary end-point was proposed as the accomplishment of haemoglobin A1c (HbA1c) <7.0% (53 mmol/mol) at 1 year and freedom from SHEs from day 28 to day 365 after first transplant. The primary end-point was effectively met by 87.5% of patients at 1 year and by 71% at 2 years. The median HbA1c was 5.6% (38 mmol/mol) at both 1 and 2 years. The hypoglycaemia awareness was significantly ($P >$ 0.0001) restored with good improvement in Clarke and HYPO scores in the 48 adults with T1DM. Also, no study-related deaths or disabilities were reported.

This study also reported that immunosuppression administration significantly decreased the renal function and also the development of donor-specific antibodies developed in two cases amongst 48 T1DM patients. Hence, the study proposed strongly that islet transplantation ought to be considered for patients with T1DM and IAH, where other less invasive current treatments have been incapable of counteracting SHEs.

Hurdles faced during pancreatic islet transplantation

Despite the promising results shown by this procedure, broader implementation of pancreatic islet transplantation is facing several hurdles. These include non-availability of suitable donor pancreas; difficulties in the technical aspects of the isolation procedure; improvement in the quality and nature of isolated islets; incendiary response during the peritransplant period due to incompatibility between islets and blood, known as instant blood-mediated inflammatory reaction (IBMIR), and the moderate, long-term survival of transplanted islets.

In the vast majority of the cases of islet transplantation, pancreas from the heart-beating, brain-dead cadaveric donor is preferred due to the better outcome over the organs from non-heart-beating donors. The damage to the islet begins from the donor organ procurement mainly due to the infiltration of immune cells and pro-inflammatory cytokines immediately after brain death[18].

One of the main reasons for destruction of significant mass of transplanted islets is IBMIR, triggered by the tissue factor (TF) expressed on islets. In IBMIR, the discharged cytokines and chemokines, in relationship with antigen-presenting cells (APCs) assume a key role in the dynamics and pathogenies of this inflammation process. Furthermore, the primary damage to the islets is due to the invasion of leukocytes and macrophages before engraftment[19,20]. IBMIR is likewise characterized by coagulation, discharge of chemokine and complement activation. It attracts innate immune cells, resulting in the release of proinflammatory cytokines prompting the islet damage further by apoptosis[21].

The occurrence of IBMIR under allogeneic and xenogeneic combinations is well studied[19,20], but its importance and existence in autologous islet infusion has not been clinically or experimentally illustrated[22]. Recently, our group observed IBMIR in patients undergoing total pancreatectomy followed by autologous islet transplantation. There was immense augmentation of proinflammatory markers and islet damage markers during the initial period of 0–3 h after infusion of islets. Markers for coagulation, for instance, thrombin–antithrombin and proinflammatory cytokines such as interleukin IL- 6, IL-8 and IP-10 in conjunction with C-peptide, showed rapid increase indicating the inflammatory provocative response to the transplanted islets.

Further examination on the presentation of islets to autologous blood utilizing a miniaturized *in vitro* tube model resulted in the expression of TF, verified by our previous *in vivo* findings[22]. Hence, it was evident that IBMIR is an issue in allogenic and xenogenic islet transplantation as well as in autologous islet transplantation.

Another significant segment of IBMIR was the activation of complement cascade which ultimately resulted in the elevation of concentration of complement proteins in the islet transplant recipient serum[21,23]. The increased concentration of C3a and C5a complement proteins further enriched the cascade of events via leukocyte accumulation, upregulation of adhesion molecule on the endothelium and platelets, and production of proinflammatory cytokines and reactive oxygen species (ROS)[23]. Previous demonstration showed that the classical complement pathway was also activated on the surface of human islets that reacted with ABO-compatible blood involving IgG, IgM and other complement proteins, namely C3, C4 and C9, thus emphasizing the significance of immunoglobulins in the activation of complement cascade[23]. In contrast to other complement proteins, C5a has been proposed as a critical molecule responsible for the initiation of coagulation and inflammation by intervening TF expression in neutrophils[24,25]. Compstatin – a complement inhibitor treatment significantly improved the graft survival under *in vivo* conditions[26]. In contrast, there was no significant impact of complement proteins during IBMIR in clinical autologous islet transplantation[22].

Alternative sites for transplantation

The experts in the clinical islet transplantation, favour the portal vein as the ideal or perfect site for such transplantation, despite the fact that there is significant early loss of viable islets because of low oxygen tension, induction

of IBMIR and inflammatory cytokines during peritransplant. The success in islet transplantation mostly depends on the mass of viable and functional islets that survive after peritransplant period and hence in many centres, researchers are working to find out alternate sites that can be ideal for islet transplantation. So far, islets have been experimentally transplanted into the portal vein, kidney subcapsule, spleen, pancreas, peritoneum, omentum, gastrointestinal wall, testis, thymus, bone marrow, anterior chamber of the eye and intramuscular spaces[27]. Despite moderate success in such investigations, portal vein transplantation is still at the top clinically.

Next to portal vein, the peritoneum and omental pouch offer favourable circumstances over other sites of transplantation. The omentum has numerous blood vessel pedicles, various lymphatic vessels, exclusive portal drainage, high vascular density and neoangiogenesis capability[27]. In rodents, enough evidence is available for better function of islets transplanted in the omentum[28–30]. Recently, the omental pouch has been used as islet transplantation site in diabetic cynomolgus monkey, a non-human primate model. The islets transplanted in the omentum showed granulated, vascularized, insulin-positive islets encompassed by T-cell subsets and macrophages. Compared to portal vein, the islets transplanted in the omentum showed delayed engraftment but similar levels of C-peptide production were eventually achieved[30,31].

Evolution of and advancements in immunosuppressive protocols

Early immunosuppressive protocols consisted of treatment with azathioprine, cyclosporine and corticosteroids. Although azathioprine did not appear to have any adverse effects on islet function or insulin sensitivity, it is a relatively weak immunosuppressant. The introduction of cyclosporine, a potent immunosuppressive drug that blocks the clonal expansion of resting T-cells, was revolutionary in whole-organ transplants[32]. However, cyclosporine has been shown to exhibit diabetogenic potential in patients[33]. Further studies revealed harmful effects on mouse islets[34], rat islets[35] and human islets[36] when exposed to cyclosporine *in vitro*. Corticosteroids are also potent immunosuppressive drugs and act on the entire immune system. Like cyclosporine, corticosteroids also carry diabetogenic side effects for patients and suppress the entire immune system. Avoiding corticosteroid treatment is critical in the success of islet transplantation. In fact, introduction of novel immunosuppressive protocols has dramatically increased the success of clinical islet transplantation as evidenced by increased insulin-independent rates achieved in transplant recipients (Table 1).

The Edmonton protocol established a less diabetogenic and corticosteroid-free regimen that utilizes a combination of sirolimus with low-dose tacrolimus as immunosuppression maintenance, with induction achieved using daclizumab, an anti-IL-2 monoclonal antibody[8]. Sirolimus works by blocking T- and B-lymphocyte responses to cytokines that are involved in the recruitment, activation and expansion of T- and B-cells. Tacrolimus shares many of the same intracytoplasmic pathways to inhibit calcineurin. However, tacrolimus is about 10–100 times more potent *in vitro*[37]. The concept of using sirolimus as an effective immunosuppression maintenance drug was introduced in canines[38,39] and further validated in pigs[39] before being introduced in humans.

Achieving a permanent state of recipient tolerance towards an islet allograft remains an important goal. Some islet groups are hoping to eliminate the need for immunosuppression entirely. Cell encapsulation represents a novel method of blocking the immune system of the recipient from destroying the graft by providing a membrane around the islets[40]. The membrane surrounding the islets contains pores that are large enough to allow nutrients and insulin to be exchanged between the host and graft, but small enough to block immune cells from attacking the islets.

Changes to current immunosuppressive regimens represent another route of future improvements. Groups in Minnesota and Miami, USA have experimented with anti-tumour necrosis factor alpha (TNF-α) drugs along with current immunosuppression drugs. It has been shown that TNF-α exhibits negative effects on islet function and engraftment[41]. Etanercept and infliximab are TNF-α-inhibiting drugs that have been proposed. Ten consecutive islet transplants were performed at the University of Alberta using infliximab in addition to the standard regimen, but no positive impact was found when compared with control[42]. Alemtuzumab is used extensively in whole-organ transplants as a monoclonal antibody to CD52. A study conducted at the University of Alberta tested the efficacy of alemtuzumab as an induction agent and compared it with the standard induction approach. Findings suggested that alemtuzumab improves engraftment and insulin independence rate, but requires high doses of concomitant tacrolimus and mycophenolate mofetil[43].

The University of Minnesota group is currently testing the effects of HuOKT3γ1, a humanized anti-CD3-specific antibody, as an induction agent with sirolimus and t777acrolimus maintenance. Anti-CD3 treatment depletes effector T-cells and drives the remaining ones to a TH2 response. The use of anti-CD3 has been reported to induce tolerance in non-autoimmune models of allograft transplant, as well as to slowdown the progression of recent-onset diabetes in humans.

Other potential future immunosuppressive options are also being examined that include anti-thymoglobulin induction, blockade of immunoregulatory pathways, Treg induction, and targeting of dendritic cells. They represent plausible future directions in immunosuppression for allogeneic islet transplantation[44,45].

Table 1. Recently reported immunosuppression protocols in clinical islet transplants

Immunosuppression protocol	No. of patients	Transplant type	Insulin-free	Year/reference
ATG + Dac + Eta/Tac/MMF + Sir	8	ITA	8	2005 (85)
Dac + Inf/Tac/Sir	16	ITA	14	2005 (86)
Dac or Bas/Tac or CsA/Sir or Eve	22	IAK or ITA	15	2005 (87)
Dac/Tac/Sir	65	ITA	44	2005 (88)
ATG or Bas/Tac/Sir or MMF	10	ITA	10	2005 (89)
Dac/Tac/Sir	8	IAK	8	2006 (90)
Dac/Tac/Sir	6	ITA	3	2006 (91)
Dac/Tac/Sir	36	ITA	16	2006 (9)
Dac/Tac/Sir or MMF + Exe	11	ITA	8	2007 (92)
Dac/Tac/Sir	10	ITA	6	2007 (93)
Dac/Tac/Sir or MMF	19	ITA	16	2007 (42)
Dac/Tac/Sir	13	SIK	7	2008 (94)
Dac + Inf + Eta/Pred or Tac/Sir + MMF	7	IAK	6	2008 (95)
ATG + Eta/CyA/Eve + MMF	6	ITA	5	2008 (96)
Dac/Tac/Sir	4	ITA	4	2008 (97)
Dac + Eta/Tac/Sir + Exe	6	ITA	6	2008 (98)
Ale/Tac/Sir + MPA	3	ITA	2	2008 (99)
Dac + Inf/Tac/Sir	6	IBM	3	2008 (100)
Dac/Tac/Sir	14	ITA	14	2009 (101)
Dac or Bas/Tac/Sir	15	IAK	15	2009 (102)
ATG/Sir + MMF + Efa	8	ITA	8	2010 (44)
Dac/Tac/Sir	8	ITA	8	2010 (45)
Dac/Tac/Sir + Efa	4	ITA	4	2010 (45)
ATG + Bela/Sir + MMF	5	ITA	5	2010 (103)
ATG + Efa/Sir + MMF	5	ITA	5	2010 (103)
ATG + ETA + Ana/Tac/MMF	3	ITA	3	2011 (104)
Dac/Tac/Sir	3	ITA	3	2011 (104)
ATG + Tep/Tac or CsA/Sir or Eve ATG + TCDAb + TNFi//Tac or CsA/Sir or Eve	29	ITA	15	2012 (105)
TCDAb + TNFi/Tac or CsA/Sir or Eve	20	ITA	10	2012 (105)
Dac/Tac or CsA/Sir or Eve	177	ITA	35	2012 (105)
ATG + Sir	12	ITA	5	2014 (106)
ATG + Tac + MMF	48	ITA	No data	2014 (107)
ATG or Dac or Bas/Tac/Sir	38	SIK/IAK	4	2015 (108)

SIK, Simultaneous islet kidney transplantation; Sir, Sirolimus; Tac, Tacrolimus; TCDAb, T-cell depleting antibodies; TNFi, Tumor necrosis factor-α inhibition; Ale, Alemtuzumab; ATG, Antithymocyte globulin; Ana, Anakinra; Bas, Basiliximab; Bela, Belatacept; CsA, Cyclosporine A; Dac, Daclizumab; Efa, Efalizumab; Eta, Etanercept; Eve, Everolimus; Exe, Exenatide; IAK, Islet after kidney transplantation; Inf, Infliximab; ITA, Islet transplantation alone; MMF, Mycophenolic mofetil; MPA, Mycophenolic acid; Pred, prednisone.

Biomarkers to monitor islet graft function

During the islet tansplantation there is significant loss in the islet mass and function due to innate immunity, drug toxicity or metabolic exhaustion. Thus some clinically relevant, high-resolution techniques are required to monitor islet graft function and survival to give suitable therapy[46]. There are few traditional biomarkers to assess the islet graft function such as blood glucose level, insulin and C-peptide. Further, these markers are influenced by physiological changes or released by beta-cell stimulation. The fundamental qualities of good biological markers are the stability or increased half-life, and it must to be independent of physiological changes or metabolic stimulation.

Previously, antibodies raised towards islet autoantigens were used as damage markers. Obviously, it is impossible to prevent their endogenous presence during peritransplant[47]. The islet-enriched proteins, namely glutamate decarboxylase-65, doublecortin, protein phosphatase 1, regulatory (inhibitor) subunit 1A (PPP1R1A), ubiquitin C-terminal hydrolase-L1 and the high-mobility group box-1 protein (HMGB1) were widely studied to predict the islet damage. However, the short half-life of these proteins restricts them to become reliable biomarker to predict the islet damage[45,48–50].

In recent years, experiments on nucleic acid biomarkers such as miRNAs, circulating cell-free DNA (cfDNA), and unmethylated/methylated insulin DNA ratio have increased drastically. MicroRNA-375 has been recognized as a highly expressed, islet-specific miRNA and it has also been confirmed that its endogenous expression is crucial for maintaining the regular function of islets[51,52]. Additionally, the absolute and relative quantification of miRNA-375 correlated to the islet damage *in vitro* and in the plasma of patients undergoing islet

transplantation[53]. Importantly, the previous clinical study results indicated that miRNA-375 detected in high resolution during and after islet infusion promotes its ability to monitor beta-cell damage[53].

The donor-specific circulating cfDNA was also detected at high levels in the patients immediately after infusion and also correlated to the insulin dependency after infusion[54]. However, the lack of tissue specificity in cfDNA limits its advantage in predicting the islet damage during islet transplantation followed by total pancreatectomy.

Another recent nucleic acid biomarker unmethylated/methylated insulin DNA ratio attracted the islet transplantation field as an indicator for islet cell destruction and development of T1DM or for new-onset T1DM[55,56]. Clinically, there was significant elevation in unmethylated/methylated insulin DNA ratio in patients who underwent total pancreatectomy followed islet transplantation compared to normal healthy individuals, during infusion and remained elevated up to 14 days after infusion[57,58]. This elevated unmethylated/methylated insulin DNA ratio also correlated with fasting blood glucose level and glucose tolerance test results in streptozotocin-induced diabeteic NOD mice[55]. cfDNA is not a reliable biomarker to predict beta-cell damage compared to this unmethylated/methylated insulin DNA due to its short half-life, approximately 30 min to 2 h (refs 58, 59). In addition, due to the association with membrane-bound vesicles like exosomes, the miRNA biomarkers retained stability and were also detected with high resolution in the plasma samples stored over years at –80°C temperature[53].

Alternative beta-cell replacement approaches – human stem cells

Different cell types can be differentiated *in vitro* into functioning beta-cells that respond to glucose stimulation by secreting insulin. Beta-cells generated from such cell sources could be transplanted in T1DM patients, which makes these cells a potential alternative source to exogenous insulin treatment.

Embryonic stem cells

Research studies have demonstrated that embryonic stem cells can be differentiated into insulin-producing cells[60–62]. Such differentiated insulin-producing cells can be cryopreserved until needed, easily expanded and differentiated *in vitro*. The advantage of these cells is the unlimited capacity for self-renewal. However, there are major limitations; besides ethical considerations, patients need immunosuppression to prevent rejection, as these cells would be foreign to the immune system of the recipients. The side effects of high blood glucose levels would be replaced by those of immunosuppression. Moreover, cells would be rejected after some time, even with immunosuppression. Due to these potential drawbacks as well as ethical considerations, these cells seem, to be suboptimal candidates for future beta-cell replacement therapies[33].

Induced pluripotent stem cells

The limitations of embryonic stem cells, including the ethical considerations, can be overcome using induced pluripotent stem cells, even though these cells can be generated from somatic cells and share properties with embryonic stem cells. Moreover, induced pluripotent stem cells would be available for the future recipients, and there is no need for immunosuppression after transplantation. Alipio *et al.*[63] demonstrated the reversal of hyperglycaemic conditions in an *in vivo* diabetic animal model using these cells. After reprogramming skin fibroblasts to become induced pluripotent stem cells, the cells were differentiated using an established protocol for embryonic stem cell differentiation. The generated cells produced insulin in response to glucose stimulation *in vitro*, and in a mouse model of type-2 diabetes mellitus (T2DM) transplantation of these cells ameliorated hyperglycaemia. In another model, blood glucose levels were normalized after cell transplantation in mice with streptozotocin-induced diabetes.

Mesenchymal stem cells

Mesenchymal stem cells can easily be extracted from various tissues (e.g. bone marrow and adipose tissue, among other sources) and depending on cytokines and cell–cell interactions, can differentiate into various cell types that form bone, cartilage, adipose tissue, and hepatocytes. Phadnis *et al.*[64] demonstrated that human bone marrow-derived mesenchymal stem cells can differentiate into endocrine pancreatic cells. *In vivo*, secretion of human C-peptide was present after transplantation of these cells into pancreatectomized and streptozotocin-induced diabetic mice; using transplantation of human bone marrow-derived mesenchymal stem cells, normoglycaemia could be maintained. In recent clinical trials, the potential of these cells in treating T1DM and T2DM diabetes was demonstrated. Trivedi *et al.*[65] isolated mesenchymal stem cells from adipose tissue, differentiated them into insulin-producing cells and performed cotransplantation with cultured bone marrow cells in patients with T1DM. HbA1c levels decreased, less insulin was needed and C-peptide serum levels increased in these patients. In another clinical trial in patients with T2DM, transplantation of placenta-derived mesenchymal stem cells led to increased C-peptide levels as well as a decreased need for insulin[66]. The easy availability of mesenchymal stem cells, the

successful early clinical trials, and the promising *in vitro* and *in vivo* experiments render these cells a promising candidate for transplantation-based therapies to overcome diabetes.

Liver cells

Studies have demonstrated that liver cells can also be used to generate beta-cells. Yang *et al.*[67] showed that cultures of mouse embryo liver cells generated insulin-positive cells when transduced with an adenoviral vector encoding three genes: *Pdx1*, *Ngn3* and *MafA*. Sapir *et al.*[68] demonstrated that PDX-1-treated human liver cells expressed insulin, stored it in defined granules and secreted the hormone in a glucose-regulated manner. When these cells were transplanted under the renal capsule of diabetic immunodeficient mice, they became normoglycaemic for prolonged periods of time.

Pancreatic acinar cells, alpha cells and duct cells

Studies have shown that it is possible to generate beta-cells from adult human pancreatic cells. Recent work demonstrated that differentiated cell types in adult organs, including the mouse pancreas, can be experimentally 'reprogrammed' into progeny resembling islet cells, suggesting a new strategy for beta-cell replacement[40]. For example, adult mouse pancreatic acinar cells can be converted into insulin-producing cells *in vitro* and *in vivo*[69,70]. The islet alpha cell is another closely related cell type that has been studied for reprogramming into insulin-producing cells. Recently, Thorel *et al.*[71] demonstrated by lineage tracing in a mouse model of beta-cell ablation, that a large fraction of regenerated beta-cells originated from alpha cells.

Pancreatic ducts constitute 30–40% of the human pancreas and have been proposed as a potential source of replacement beta-cells[72,73]. During pancreas development, foetal endocrine cells derive from embryonic ductal epithelium[74,75]. In addition, some studies have suggested that in adult mice, beta-cells may be produced from pancreatic ductal epithelium[76]. In humans, previous studies have suggested that adult human primary ductal cells in heterogeneous cell mixtures may harbour the potential to generate endocrine-like progeny[77], but interpretation in these studies was limited by the probability of islet cell contamination. Therefore, the potential for conversion of pancreatic ductal cells towards an endocrine cells remains unclear. Moreover, previous studies have revealed only a limited proliferative capacity of primary human pancreatic ductal cells in culture[78]. Thus, despite their relative abundance, multiple practical issues have prevented the development of human pancreatic ductal cells as a source of replacement beta-cells.

Autologous islet transplantation

Chronic pancreatitis (CP) is a disease characterized as advanced inflammatory disorder leading to irreversible destruction of the pancreas, and manifested by chronic intolerable pain and permanent loss of exocrine or endocrine function. The exact mechanism(s) for CP is still unclear; however, the main causes have been proposed as alcohol consumption, autoimmunity and also genetic mutations[79]. In most of the cases, the standard treatments for CP such as celiac blocks and administration of anti-inflammatory drugs have been unsuccessful, and whereas removal of entire pancreas by total pancreatectomy leads to brittle diabetes or SHEs that cause substantial morbidity and mortality due to the loss of counter-regulatory pancreatic glucagon[80]. The alternative treatment is total pancreatectomy with islet transplantation (TPIAT). Patients with refractory chronic pancreatitis undergo total or partial pancreatectomy to alleviate pain and also autologous islet transplantation to retain pancreatic endocrine function after surgery. The isolated islets are infused to the liver via intra-portal vein. Although some patients remain insulin-independent after the transplant, other patients with less optimal outcome require exogenous insulin to maintain normoglycaemia and also have less chance for the occurrence of severe hypoglycaemic events[81,82]. In TPIAT, the need of immunosuppression is not required because the tissue is taken from the same patient. Moreover, the same strategies typically used in allogenic islet transplantation were utilized to assess the graft function in TPIAT. For example, the SUITO index is used in autologous islet transplantation to predict the graft function or insulin independence[83].

In most of the cases of the TPIAT, the post-transplant function is shown to be much better than allogenic islet transplantation, with less IEQ/kg of islets transplanted compared to allogenic transplants. In allogenic transplants, more than 10,000 IEQ/kg of islets is considered as a factor of insulin independence, whereas in TPIAT 5000 IEQ/kg of islets is considered as a successful factor[84]. In TPIAT, most of the patients attains good quality of life because of significant relief from pain and have better long-term survival of graft after achieving insulin-independent status.

Conclusion

Islet transplantation has been shown to be a promising treatment that could result in freedom from requirement of exogenous insulin in T1DM patients. One of the major advantages of islet transplantation is the minimally invasive nature of the procedure when compared to whole-organ pancreas transplantation. Despite its widespread use at several major transplant centres, the volume of patients receiving islet transplants remains low when

compared to the number of 'brittle' T1DM patients eligible for this procedure. Recently, impressive gains have been made in the improvement of post-transplant islet function. This is primarily due to the use of T-cell depleting immunosuppression during induction phase after transplant followed by the use of tacrolimus, rapamycin and/or mycophenolic mofetil during the maintenance phase. In addition, to several advances made in donor selection, pancreas procurement, enzymatic digestion, islet purification and islet culture seem to have contributed to this success. Recent completion of a large-scale phase-III clinical trial sponsored by the NIH has given hope that soon this procedure may be approved for clinical use. In light of these advances, there is optimism that the remaining hurdles could be overcome to improve the long-term function of the transplanted islets. The number of patients with diabetes in India has shown a sharp increase in recent decades. Enormous amount of resources is being mobilized to treat diabetes in India. While the prevalence of T2DM far outweighs the occurrence of T1DM, patients with T1DM stand to gain from transplantation of islets by achieving freedom from life-threatening episodes of severe hypoglycaemia. However, no clinical islet transplants have been performed thus far in medical centres in India. Practical steps aimed at the introduction of clinical islet transplants will undoubtedly mark a significant milestone in the treatment of diabetic patients in India.

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doi: 10.18520/cs/v113/i07/1267-1276