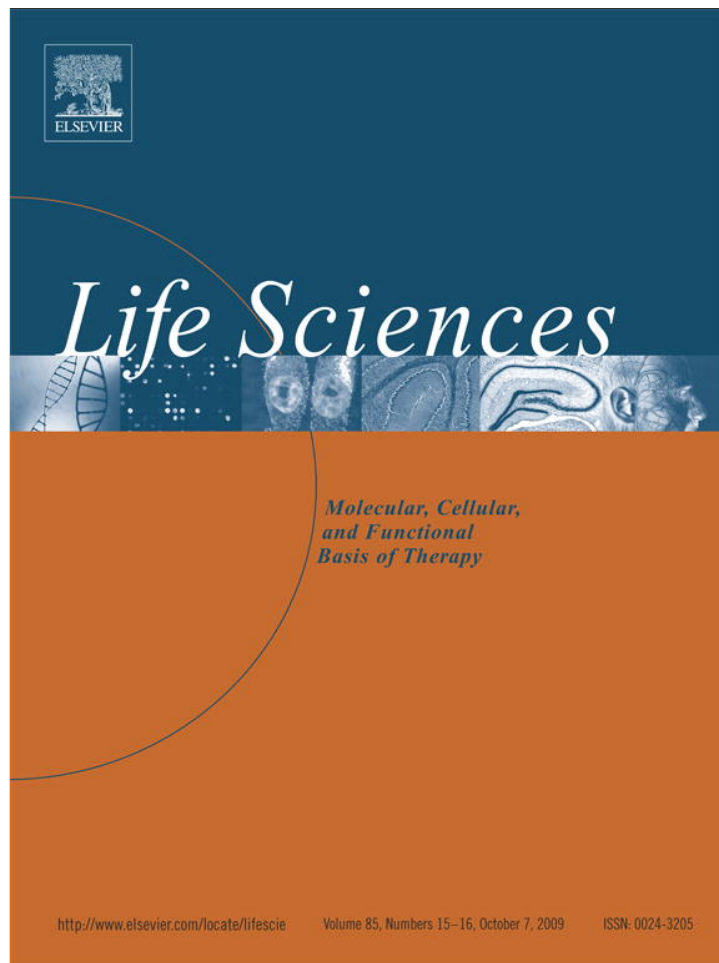


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Minireview

Do immunotherapy and β cell replacement play a synergistic role in the treatment of type 1 diabetes?Dong-Sheng Li^a, Garth L. Warnock^b, Han-Jun Tu^a, Ziliang Ao^b, Zehua He^b, Hong Lu^b, Long-Jun Dai^{a,b,*}^a Hubei Key Laboratory of Embryonic Stem Cell Research, Tai-He Hospital, Yunyang Medical College, Shiyan, Hubei, China^b Ike Barber Human Islet Transplant Laboratory, Department of Surgery, University of British Columbia, 400-828 West 10th Avenue, Vancouver, BC, Canada V5Z 1L8

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ABSTRACT

Type 1 diabetes (T1D) is the result of the autoimmune response against pancreatic insulin-producing β -cells. Its ultimate consequence is β -cell insufficiency-mediated dysregulation of blood glucose control. In terms of T1D treatment, immunotherapy addresses the cause of T1D, mainly through re-setting the balance between autoimmunity and regulatory mechanisms. Regulatory T cells play an important role in this immune intervention. An alternative T1D treatment is β -cell replacement, which can reverse the consequence of the disease by replacing destroyed β -cells in the diabetic pancreas. The applicable insulin-producing cells can be directly obtained from islet transplantation or generated from other cell sources such as autologous adult stem cells, embryonic stem cells, and induced pluripotent stem cells. In this review, we summarize the recent research progress and analyze the possible advantages and disadvantages of these two therapeutic options especially focusing on the potential synergistic effect on T1D treatment. Exploring the optimal combination of immunotherapy and β -cell replacement will pave the way to the most effective cure for this devastating disease.

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Introduction

Type 1 diabetes (T1D) is a disease resulting from a cell-mediated autoimmune attack against pancreatic insulin-producing β -cells. It is characterized by insulin insufficiency, blood glucose dysregulation, persistent hyperglycemia and long-term complications. According to its process of development, islet-specific autoimmunity leads to β -cell

* Corresponding author. Ike Barber Human Islet Transplant Laboratory, Department of Surgery, University of British Columbia, 400-828 West 10th Avenue, Vancouver, BC, Canada V5Z 1L8. Tel.: +1 604 875 4111x62501; fax: +1 604 875 4376.

E-mail addresses: lj dai@interchange.ubc.ca, longjundai@gmail.com (L.-J. Dai).

damage which in turn results in insulin insufficiency. There are three conventional therapeutic options used for treating T1D which are currently available: insulin therapy, cell-based therapy and immunotherapy (Powers 2008). Insulin therapy is typically the most common and widely used therapy to treat T1D. The discovery of insulin revolutionized T1D therapy and made this incurable and debilitating disease manageable, saving thousands of lives since its discovery nearly one century ago. However, insulin therapy for treatment of T1D is passive in nature and does not directly address the cause of the disease. Successful results using insulin therapy remain limited as patients still struggle with both hyper- and hypoglycemia. As a result of the stringent maintenance required to maintain strict glycemic control using insulin replacement therapy, diabetic complications are inevitable and are often life-threatening. Although a higher percentage of diabetic patients are classified as type 2 diabetics, the increase in incidence of T1D has been surprisingly high in the recent decade (Wild et al. 2004; Onkamo et al. 1999). In some countries, such as the UK and China, the increase in the incidence rate has reached over 70% per year in children aged less than 14 years (Devendra et al. 2004), pushing for more fundamentally curative solutions to be intensively investigated, including cell-based therapy and immunotherapy. The present review highlights the current progress of these two therapies and the potential significance of their combination.

Immunotherapy

T1D is a chronic autoimmune disorder associated with the generation and activation of autoreactive T cells recognizing pancreatic β -cell autoantigens in genetically susceptible individuals. Self-reactive CD4⁺ and CD8⁺ effector T lymphocytes (T_{eff}) infiltrate the pancreas and selectively destroy the insulin-producing β -cells in the islets. The β -cell destructive process occurs progressively at any stage of life and continues after initial clinical presentation. The ultimate goal of therapeutic intervention is the prevention or reversal of the disease by the arrest of autoimmunity and by the preservation or restoration of β -cell mass and function. Ideally, immune-based therapies should be applied before the onset of T1D or shortly afterwards when a reasonable β -cell mass is still retained in the pancreas. Maintenance of immune tolerance in the periphery can be envisioned as a balance between autoreactive lymphocytes and regulatory mechanisms (Valencia and Lipsky 2007). In the past decade, an overwhelming body of literature showed that CD4⁺CD25⁺FoxP3⁺ regulatory T cells (T_{regs}) are a dominant mechanism regulating the decision fate of different immunological outcomes and their dysregulation implicates various autoimmune disorders including T1D (Sakaguchi et al. 2008). Theoretically, any means aimed at correction of the balance between autoimmunity and regulatory mechanisms could lead to therapeutic approach to treat T1D. Based on the examination of pancreas biopsies and serum C-peptide levels, it has become evident that over 80% of T1D patients maintain islet β -cell function for many years after they became dependent on insulin therapy (Meier et al. 2005, 2006; Pechhold et al. 2009). C-peptide is cosecreted with insulin by the β -cells as a by-product of the enzymatic cleavage of proinsulin to insulin. The measurement of C-peptide provides a validated means of quantifying endogenous insulin secretion being closely related to the amount of functioning β -cell mass. The constant existence of β -cells in diabetic pancreas broadens the spectrum of immunological intervention on T1D.

Evidence of T_{reg} function in the control of T1D

Non-obese diabetic (NOD) mice are susceptible to spontaneous T1D and most female mice will inevitably go on to develop destructive insulinitis leading to overt diabetes within a few months. It is widely accepted that the defect of T_{regs} in NOD mice results in the activation

of autoaggressive T cells and diabetes progression. The regulatory function of T_{regs} in NOD mice was further demonstrated in adoptive transfer studies with NOD.SCID recipients (Szanya et al. 2002). Because NOD.SCID mice do not have any T cells (including T_{regs}), the transfer of purified CD4⁺ or CD8⁺ T cells from diabetic mice resulted in T1D (Sgouroudis and Piccirillo 2009; Wicker et al. 1986). In contrast, cotransfer of autoaggressive T cells with T_{regs} from pre-diabetic NOD mice prevented the induction of diabetes in NOD.SCID recipients. Furthermore, adoptive transfer is more effective when the transferred T_{regs} are specific for an islet antigen (Tang et al. 2004). FoxP3 (forkhead box protein 3) is essential for the self-tolerance function of T_{regs} . FoxP3^{-/-} NOD mice display an increased incidence and early onset of T1D compared with wild type NOD mice although it is unclear whether the infusion of T_{regs} compensates for the primary deficit in T_{regs} (Chen et al. 2005).

In humans, defects in polyclonal T_{regs} have been proposed as one mechanism by which individuals develop T1D. The defect of T_{regs} appears to be a function as compared with the number of T_{regs} (Brusko et al. 2005; Lindley et al. 2005; Tritt et al. 2008; Brusko et al. 2007). Conflicting data in human T1D patients show the decrease in T_{reg} cell frequency (Kukreja et al. 2002), unchanged T_{reg} cell frequency with marked decrease in suppressive activity in vitro (Lindley et al. 2005) and no differences at all compared to healthy controls (Putnam et al. 2005). These differences can be attributed to several details including the accuracy of the method of T_{reg} isolation and purification, and the lack of functional assay on islet-specific T_{regs} in the peripheral blood. In addition, studies in murine models suggest that T_{regs} exert their function within the targeted organ rather than solely in lymph node draining sites (Sgouroudis et al. 2008). Thus, subtle functional differences in the T_{reg} cell pool within sites of inflammation may not be adequately reflected in the peripheral blood.

Mechanisms of T_{reg} function in T1D

T_{regs} are produced in the thymus as a functionally mature subpopulation of T cells and are known as natural T_{regs} (nT_{regs}). They can also be induced from naïve T cells in the periphery known as adaptive or induced T_{regs} (iT_{regs}). The full extent of differences and similarities between nT_{regs} and iT_{regs} remains to be defined. It is speculated that the development of iT_{regs} is driven by the need to maintain a noninflammation environment, to suppress immune responses, and to decrease chronic inflammation, whereas nT_{regs} prevent autoimmunity and raise the activation threshold for all immune responses (Curotto de Lafaille and Lafaille 2009). T_{regs} suppress the proliferation and cytokine responses of several immune cell subsets including differentiated CD4⁺ and CD8⁺ T cells, dendritic cells (DC), NK, NK-T, B cells and macrophages. Adoptive transfer systems have demonstrated an inverse correlation between the proliferation of T_{eff} cells and the amount of T_{reg} cells present in draining pancreatic sites, and the infiltration of T_{eff} cells in the target organ was markedly enhanced in the absence of T_{reg} cells (Tritt et al. 2008; Sarween et al. 2004).

T_{regs} are specialized for immune suppression and investigating the mechanism of T_{reg} -mediated suppression is a key issue of current research on T_{regs} (Sakaguchi et al. 2008). Several mechanisms of T_{reg} -mediated suppression have been proposed, and these include secretion of immunosuppressive cytokines, cell-contact-dependent suppression, and functional modification or killing of antigen-presenting cells (APC). In a model of T_{reg} -mediated suppression described by Sakaguchi et al., there exist at least three possible mechanisms of T_{reg} -mediated immune suppression, and more than one mechanism of T_{reg} -mediated suppression may be operational for control of a particular immune response in a synergistic and sequential manner (Sakaguchi et al. 2008). First, upon antigenic stimulation, antigen-specific T_{regs} are swiftly recruited via chemokines to APCs, especially DCs, and out-compete antigen-specific naïve

T cells in aggregating around the DCs. Second, antigen-activated T_{regs} contact DCs then downregulate DC function, thereby hindering the activation of other T cells that are recruited to DCs. Finally, T_{regs} may then further differentiate to secrete granzyme/perforin and other immunosuppressive cytokines (such as IL-10, IL-35, TGF- β and CTLA-4) (Wing et al. 2008) to kill or inactivate responder T cells depending on the strength and duration of antigenic stimulation.

Therapeutic potential of T_{regs} on T1D

The autoimmune destruction of the insulin-producing β -cells is a dynamic process involving multiple players such as diabetogenic T_{eff} , DC and T_{reg} . The loss of frequencies and/or functions of T_{reg} leads to pathologic imbalance between T_{reg} and T_{eff} , thereby inducing pancreatic insulinitis. Therefore, restoration of the balance, i.e. reconstitution of immunological tolerance to pancreatic autoantigens should pave the way for a reasonable therapy for T1D. Manipulation of immunity, possibly through the induction of T_{regs} , can be achieved either by non-antigen-specific treatments or antigen-based therapies. Current evidence indicates that antigen-specific induction of potent regulatory mechanisms is influenced by the systemic milieu, suggesting that systemic modulation might be an essential prerequisite for antigen-based therapy and the successful maintenance or reestablishment of tolerance. Most treatments that prevent autoimmune diabetes in NOD mice require intervention at early pathogenic stages. However, T_{regs} could treat diabetes at later stages of the disease, when most of insulin-producing islet β -cells had been destroyed by infiltrating lymphocytes. Tarbell et al. (2007) expanded T_{regs} from BDC2.5 T cell receptor (TCR) transgenic mice with antigen-pulsed DCs and IL-2 and then applied to NOD mice. A single dose of as few as 5×10^4 of these islet-specific T_{regs} blocked diabetes development in pre-diabetic 13-week-old NOD mice. T_{regs} also induced long-lasting reversal of hyperglycemia in 50% of mice that developed overt diabetes (Tarbell et al. 2007). To translate this to a clinical application as a cell therapy for autoimmune T1D, the induced T_{regs} must meet following minimum criteria: functional T_{regs} must be produced in adequate numbers; the cells must escape rejection by the recipient's immune system, and their regulatory effects must be focused on the immunopathology without causing generalized immune suppression.

The induction of T_{regs} in vivo

The polyclonal activation and expansion of T_{regs} can be induced by non-antigen-specific stimulation. Complete Freund's adjuvant (CFA) has been used to prevent the onset of diabetes in NOD mice (Qin et al. 1993; McInerney et al. 1991). Immunotherapy with CFA is effective in not only preventing spontaneous autoimmune diabetes, but also restoring self-tolerance to islet autoantigens. Recent studies conducted by Tian et al. (2009) demonstrated that CFA treatment ameliorates autoimmunity in diabetic NOD mice by up-regulating T_{regs} and increasing TGF- β 1 production. The percentage of T_{regs} in the pancreatic lymph nodes of CFA-treated NOD mice was significantly increased at 1, 5, and 15 to 17 weeks after CFA treatment. In a preliminary trial, the analogous reagent to CFA, Bacille Calmette-Guerin (BCG), exhibited clinical remission in 11 out of 17 newly diagnosed T1D patients (Shehadeh et al. 1994). Nonmitogenic anti-CD3 mAb had been reported to be a potent inducer of anergy and tolerance and had been able to treat T1D in NOD mice (Smith et al. 1997). Treatment with modified anti-CD3 mAb ameliorated the disease process in human patients with T1D (Herold et al. 2002). A European trial reported that administration for 6 consecutive days of an aglycosylated form of anti-CD3 to patients with new-onset T1D was able to maintain β -cell function and insulin production for at least 18 months (Keymeulen et al. 2005). Anti-CD3 mAb-induced tolerance was demonstrated to be related to the activation of iT_{regs} (Bisikirska et al. 2005; You et al. 2007). In addition, hematopoietic stem cells and

mesenchymal stem cells from various origins could modulate T_{reg} function in autoimmune-caused T1D presumably through releasing immunosuppressive cytokines such as IL-10 and TGF- β (Zhao et al. 2009; Dai et al. 2009).

Antigen-specific T_{regs} can be induced by immunization with self-antigens. Specific activation of T_{regs} by vaccinating T1D patients with low-dose autoantigen, such as insulin and glutamic acid decarboxylase (GAD), can be expected to be a possible therapeutic strategy. The expansion of islet-specific T_{regs} can also be achieved by controlling the route of antigen administration such as oral or sublingual routes. T_{regs} specific for islet antigen were revealed to be more potent in suppressing diabetes in NOD mice than polyclonally activated T_{regs} (Tang et al. 2004; Masteller et al. 2005). In addition to the antigen- or TCR-based approach to T_{reg} cell expansion, control of activation, proliferation, and death of T_{regs} and/or T_{eff} cells by using cytokines or drugs (such as exenatide) (Xue et al. 2008) is favourable to establishing T_{reg} -mediated dominant self-tolerance and immune homeostasis.

The induction of T_{regs} in vitro

Horwitz and colleagues were the first to demonstrate that T_{regs} can be developed from naive human $CD4^+$ T cells in the presence of TGF- β and IL-10 (Yamagiwa et al. 2001). The subsequent work showed that naive mouse $CD4^+CD25^-$ T cells can be converted to T_{regs} by stimulation via TCR in the presence of TGF- β (Chen et al. 2005; Luo et al. 2007). These TGF- β -induced iT_{regs} exhibited cell-contact-dependent suppression. Recently, Long et al. (2009) demonstrated that functional islet-specific T_{regs} can be generated from $CD4^+CD25^-$ T cells of T1D patients, indicating the feasibility of using patients' own cell source to treat T1D. In addition to $CD4^+$ T cells as the origin to develop iT_{regs} , some adult stem cells, such as hematopoietic stem cells and mesenchymal stem cells, can also upregulate the T_{reg} population indirectly through various growth factors or directly transdifferentiate into T_{regs} under certain conditions (Hutton et al. 2009; Abdi et al. 2008; Uccelli et al. 2008). However, for future clinical application of iT_{regs} , it is necessary to determine the culture conditions that effectively lead to the expansion of a homogeneous population of T_{regs} stably expressing very high levels of FoxP3. Further studies are required to verify in vivo functional stability of iT_{regs} and their fate after the transfer to the host.

β cell replacement

β -cell insufficiency-mediated dysregulation of glucose metabolism is the ultimate consequence of T1D. Islet transplantation complements β -cells directly, and insulin-producing cells generated from other sources can also be used to replace β -cells in the diabetic pancreas. However, it is worth noting that the replaced or regenerated β -cells still encounter the islet-specific immune attack if the aggressive autoimmune environment is actively retained.

Islet transplantation

Islet transplantation has become a very progressive research field in the last few decades. It took almost 30 years from the first islet transplantation in rats to the first successful islet allotransplantation in patients with type 1 diabetes (Ballinger and Lacy 1972; Shapiro et al. 2000). Both whole organ pancreas and islet transplantation are currently regarded as acceptable therapeutic options for patients with T1D, and the Edmonton protocol is currently accepted as providing the standard guidelines for human islet transplantation (Sutherland et al. 2004). Clinical islet transplantation performed under the Edmonton protocol represented a groundbreaking innovation in this emerging field of new therapies (Shapiro et al. 2000). Following islet transplantation, insulin independence with tight glycemic control

was achieved in patients for up to 1 year. The longest (over 11 years) case of insulin independence after allogeneic islet transplantation was recently reported (Berney et al. 2009), and several such cases in the near future will reach the symbolic 10-year mark. However, the overall long-term outcomes were less encouraging. As indicated in a five-year follow-up study, only 31% and 10% of patients remained insulin independent at 2 years and 5 years respectively (Ryan et al. 2005). But it should be noted that C-peptide secretion, a marker for endogenous insulin, was maintained at relatively high levels in 80% of patients up to 5 years post-islet transplantation. This phenomenon was also observed in our own clinical trials (Warnock et al. 2005). The hypoglycemic score, lability index and HbA_{1c} improved significantly in those who retained reasonable C-peptide. The benefits of long-term C-peptide secretion need to be determined. The major obstacle for islet transplantation to be used as a standard remedy for T1D is attributed to both insufficient source of human islets and the toxicity of currently used immunosuppressive reagents. Our recent *in vitro* study with perfusion techniques demonstrated that tacrolimus is toxic to freshly isolated human islets compared with other two currently used immunosuppressive reagents, sirolimus and mycophenolate mofetil (Johnson et al. *in press*). At the present time, the most optimal clinical situations for islet transplantation might be autotransplantation under certain circumstances, allotransplantation from donors with perfect HLA matching, and combined allotransplantation with other solid organs where anti-rejection drugs are required. The limitation of allogeneic islet transplantation has turned the attention of researchers towards finding alternative sources of insulin-producing cells.

Autologous adult stem cell-derived β -cell replacement

Hematopoietic stem cells (HSC) and mesenchymal stem cells (MSC) are the major components of stem cells in the bone marrow. Since the bone marrow-derived stem cells were found to have trans-differentiative plasticity, there has been great interest in their potential therapeutic application (Pittenger et al. 1999; Jiang et al. 2002). The feature of self-origin and readily *ex vivo* expansion renders these stem cells a practical approach to avoiding the use of anti-rejection drugs. A number of studies demonstrated that bone marrow-derived stem cells could be differentiated into β -cell-like insulin-producing cells both *in vitro* and *in vivo* (Chen et al. 2004; Chang et al. 2008). The differentiated cells possessed the ability to control blood glucose level in streptozotocin (STZ)-induced diabetic animals. Compared to bone marrow-derived MSCs, MSCs isolated from other origins, such as adipose tissue and umbilical cord blood, have been found to have the same morphology, phenotype, *in vitro* differentiation ability and similar gene expression profile (Lee et al. 2004; Tsai et al. 2007; Li et al. 2009). Sun and colleagues used bone marrow-derived MSCs from diabetic patients to differentiate into functional insulin-producing cells, suggesting the feasibility of using diabetic patients' own MSCs as a source of autologous insulin-producing cells for β -cell replacement (Sun et al. 2007).

The first clinical attempt using autologous stem cells to treat T1D was performed by Voltarelli's group (Voltarelli et al. 2007; Couri et al. 2009). They transplanted autologous nonmyeloablative HSCs into newly diagnosed T1D patients following the use of high-dose immunosuppression. After a mean follow-up of 29.8 months following transplantation, C-peptide increased significantly and the majority of patients achieved insulin independence with good glycemic control. The rationale was to preserve residual β -cell mass and facilitate endogenous mechanisms of β -cell regeneration. HSCs and MSCs probably do not have the capacity to differentiate *in vivo* into reasonable numbers of β -cells, and therefore HSCs were used to reestablish β -cell tolerance through immunosuppression and the regeneration of T_{regs}. The exact mechanism of action operating in this treatment is still unclear (Voltarelli et al. 2008). In our clinical trial

with autologous MSCs, T1D patients showed temporary (3–6 months) C-peptide elevation and partial insulin independence after each autotransplantation of bone marrow-derived MSCs without pre-immunosuppression (Dai et al., unpublished observation). The combination of MSCs and differentiated β -cells is expected to improve the outcomes of the treatment. MSCs isolated from adipose and umbilical cord blood possess similar characteristics to those from bone marrow. Haller et al. (2008) initiated a clinical study with autologous umbilical cord blood infusion for T1D patients. This is the most advantageous circumstance for the use of umbilical cord-derived MSCs in patients who have pre-banked umbilical cord blood.

Embryonic stem cell-derived β -cell replacement

Embryonic stem cells (ESC) are highly proliferative and pluripotent cells which have recently received much more attention in the field of cell-based therapy. Derived from the inner cell mass of the early developing embryo, ESCs are capable of undergoing multi-lineage differentiation into highly specialized cells representing all three germinal layers (Raikwar and Zavazava 2009). Owing to their properties of self-renewal and pluripotency, ESCs hold great potential to be an unlimited source for targeted therapies and regenerative medicine especially for T1D. Soria et al. (2000) reported the first successful generation of insulin-producing cells from mouse ESCs in 2000. They developed an insulin-secreting cell clone from undifferentiated ESCs using a cell-trapping system and found that these cells were able to restore normoglycemia and normal body weight following implantation in STZ-induced diabetic mice. In the following year, Lumelsky et al. (2001) described the generation of insulin-secreting structures similar to pancreatic islets from mouse ESCs through a five-step protocol. During last few years, the procedures for β -cell-like differentiation from ESCs *in vitro* were getting more feasible and controllable, and *in vitro* derivations of functional insulin-producing cells from human ESCs had been reported from many research institutes including our own laboratory (D'Amour et al. 2006; Jiang et al. 2007; Kroon et al. 2008). Despite reports that ESCs can be differentiated into cells capable of insulin expression, there is considerable controversy as to whether the data is sufficiently robust to infer successful β -cell differentiation. It is worth noting that many of the insulin-positive cells in ESC cultures may be the product of insulin uptake from culture medium instead of endogenous synthesis (Rajagopal et al. 2003; Hansson et al. 2004). Nevertheless, even with success in differentiating ESCs into β -cells, a persistent concern of ESC transplantation is the oncogenic potential of the undifferentiated ESCs, and the risks of malignant transformation must be carefully considered prior to its clinical application. In addition, the issue of the immunogenicity of β -cells differentiated from allogeneic ESCs remains unresolved.

Recent efforts on somatic cell reprogramming opened a novel avenue towards more practical regenerative medicine or cell-based therapy. Since the cloning of Dolly demonstrated that nuclei from mammalian differentiated cells can be reprogrammed to an undifferentiated state by trans-acting factors present in the oocyte (Wilmot et al. 1997), many groups have been searching for factors that could mediate similar reprogramming without somatic cell nuclear transfer. In addition to dedifferentiation being triggered by placing the nucleus of a differentiated cell in the cytoplasmic milieu of an egg cell, a small number of transcription factors can reprogram cultured adult cells to pluripotent stem cells termed induced pluripotent stem cells (iPSC). iPSCs were successfully developed from animal and human somatic cells in different institutes (Takahashi and Yamanaka 2006; Takahashi et al. 2007; Yu et al. 2007; Park et al. 2008). The related studies point to the possibility of regenerating mammalian tissues by first reverting skin or other adult cells to iPSCs and then redifferentiating these cells into various cell types. More recently, Zhou et al. (2008) describe an approach whereby differentiated adult cells of one type can be

directly and efficiently converted into functional cells of another type within an organism and without activation of dedifferentiation, which is described as transdifferentiation. By using a strategy of re-expressing key developmental regulators *in vivo*, they identified a specific combination of three transcription factors (*Ngn3*, *Pdx1* and *Mafa*) that reprograms differentiated pancreatic exocrine cells in adult mice into cells that closely resemble β -cells. The induced β -cells express genes that are essential for β -cell function and can ameliorate hyperglycemia by remodeling local vasculature and secreting insulin. This approach has a potentially lower risk of tumor formation than one involving the induction of a self-renewable pluripotent stem cell type (Heimberg 2008). However, several hurdles must be cleared before the reprogramming approach can be applied to diabetic patients, such as the potential risks involving genetic manipulation during *in vivo* treatment, viral carriers associated with insertional mutagenesis and hence tumor initiation, and transdifferentiation control corresponding to the physiological requirement. A recent study conducted by Yu et al. (2009) demonstrated that reprogramming human somatic cells does not require genomic integration or the continued presence of exogenous reprogramming factors and removes one obstacle to the clinical application of human iPSCs.

Advantages and disadvantages of immunotherapy and β -cell replacement

Based on the pathogenesis of T1D, both immunotherapy and β -cell replacement are active treatments for T1D. T_{reg} -induced re-set of immunoregulation in new-onset T1D subjects is able to halt the destruction of remaining β -cells, perhaps allowing these residual β -cells to recover function and hopefully lessen the severity of clinical manifestations and disease progression. The damaged or destroyed β -cells can be complemented by β -cell replacement. However, the replaced or regenerated β -cells may still encounter autoimmune attack if islet-specific T_{eff} cells remain active. The possible advantages and disadvantages of these two therapeutic options are summarized in Table 1.

The potential benefits of combined therapy on the treatment of type 1 diabetes

As described above, immune intervention could block autoimmunity against pancreatic islets thereby preserving residual β -cell function. However, it is unable to restore or replace the already destroyed β -cells, which is especially crucial to most on-going T1D subjects. β -cell-based therapy is able to complement the destroyed β -cells, but islet-specific autoimmune destruction still exists and the newly replaced β -cells also encounter the same attack. Therefore, the most beneficial treatment of T1D could be established through employing the combination of the two therapeutic options, immunotherapy and β -cell replacement. The multiple goals of controlling autoimmunity, protecting complemented β -cells and promoting stable regeneration of insulin-producing β -cells should be considered as cornerstones of the successful development of a cure for T1D.

Apparently, immune intervention and β -cell replacement play a synergistic role in the treatment of T1D. This viewpoint is proposed considering the following observations. First, β -cell transplantation can complement the destroyed β -cells in the diabetic pancreas, and the replaced β -cells can be protected from immune attack by immunotherapy while it abrogates the β -cell-directed autoimmunity found in T1D. This point is supported by a recent transplantation study conducted by Solari et al. (2009). Long-term islet allograft survival and sustained normoglycemia were obtained after allogeneic islets were co-transplanted with *in vitro*-expanded syngeneic MSCs in a rat model of STZ-induced T1D, although no significant differences in the frequencies of T_{regs} in the blood or mesenteric lymph nodes were observed in long-term surviving graft recipients and normal rats. In a

Table 1
Advantages and disadvantages of immunotherapy and β -cell replacement.

Advantages	Disadvantages
<i>Immunotherapy</i>	
<ul style="list-style-type: none"> The treatment addresses directly the cause of T1D. Normoglycemia can be achieved if applied early enough. Autologous islet-specific T_{regs} can be generated from subject's own cell source, such as $CD4^+$ T cells, MSCs and HSCs <i>in vitro</i>, excluding the use of anti-rejection drugs. 	<ul style="list-style-type: none"> Uncontrolled induction of T_{regs} may awaken the host defense and lead to chronic complications such as cancer and viral infection. Any single intervention is unlikely to mediate successful T1D therapy. Suitable markers for T_{regs} in human peripheral blood need to be precisely defined. Islet-specific Teff cell assays need to be standardized to track immune responses after immunization. The life cycle and stability of transplanted iT_{regs} are not clear.
<i>β cell replacement</i>	
<ul style="list-style-type: none"> Islet Tx is able to restore blood glucose control and limit complication in T1D patients. HSC- and MSC-derived β-cells can be autotransplanted without involving anti-rejection drugs and ethical concern. ESC-derived β-cells can act as an unlimited cell source for T1D. iPSC-derived β-cells can be used autologously without involving anti-rejection drugs and ethical consideration. 	<ul style="list-style-type: none"> Islet Tx is limited by donor islet tissue, and requires life-long anti-rejection drugs. The long-term outcome is not encouraging. Transdifferentiation efficiency needs to be improved and <i>in vivo</i> stability has not been verified. There exist the oncogenic potential and possibility of rejection. Potential risks of genetic manipulation and viral carrier-associated insertional mutagenesis need to be solved.

HSC: hematopoietic stem cell; iPSC: induced pluripotent stem cell; MSC: mesenchymal stem cell; Tx: transplantation.

clinical case report, a high count of circulating T_{regs} was observed in the longest insulin-free case after allogeneic islet transplantation (Berney et al. 2009). The involved mechanisms need to be carefully elucidated. Secondly, some immune interventions possess a twofold effect on T1D treatment. For example, autotransplanted HSCs or MSCs exert their influence on the balance between T_{eff} and T_{reg} cells through cytokine-mediated process, and to some degree, they can also replace destroyed β -cells through transdifferentiation into insulin-producing cells. This could be a partial, if not complete, explanation for satisfactory outcomes after HSC or MSC implantation in some new-onset T1D patients. Finally, immune interventions may enhance functional β -cell number indirectly through protecting newly regenerated β -cells. It is believed that both destructive process and β -cell regeneration co-exist during the entire course of T1D. As described earlier, a considerable number of T1D patients have proven the persistent existence of β -cells (Meier et al. 2005, 2006; Pechhold et al. 2009), and a similar observation was also obtained from a T1D subject who held an allogeneic islet graft for 2 years (Smith et al. 2008). The continuously regenerated β -cells protected from further immune attack will undoubtedly contribute to the restoration of β -cell mass in the diabetic pancreas.

It is worth noting that the possible disadvantages or side effects of immunotherapy and β -cell replacement (Table 1) may also affect the outcomes of the combinational therapy. The optimal combination should maximize the advantages and minimize the disadvantages of each approach in arriving at the most effective treatment of T1D. Presumably, the combinational therapy is most suitable for subjects with long-term T1D. Along with the immunointervention approaches, strategies of direct β -cell mass restoration should be performed as part of the treatment because much of β -cell mass had been destroyed at the time of intervention (Couri and Voltarelli 2008). Elaborately designed pre-clinical studies are definitely required

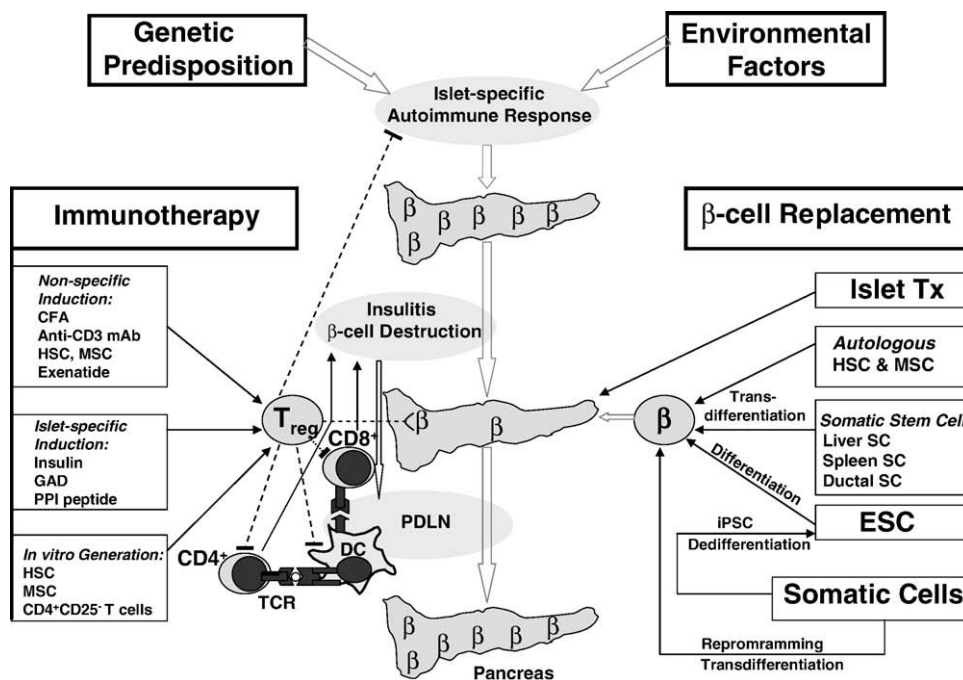


Fig. 1. The mutual effects of immunotherapy and β cell replacement on T1D. Islet-specific autoimmune response is the direct cause of insulinitis, which is initiated by various environmental factors on the genetically predisposed individuals. In the insulinitic islets, dendritic cells (DC) are activated by β cell peptide and proinsulin (PPI) peptide through MHC class II and HLA-A2 respectively. Then, activated DCs migrate to the pancreatic draining lymph node (PDLN), where they present β cell peptide to $CD4^+$ T cells and PPI peptide to $CD8^+$ T cells through T cell receptors (TCR). The activated $CD4^+$ and $CD8^+$ T cells migrate to the islets and destroy insulin-producing β cells. The ultimate consequence is β -cell insufficiency-mediated dysregulation of glucose metabolism. Immune intervention addresses the cause of T1D through up-regulating regulatory T cells (T_{reg}) thereby blocking autoimmune attack to the islets (indicated by the dashed lines). The circle around β cell indicates the protection of T_{reg} s on the remaining and/or regenerated β cells. T_{reg} s can be up-regulated by both non-specific induction and islet antigen-specific induction, and they can also be generated in vitro through transdifferentiation from other cell types. The use of insulin-producing β cells is able to reverse the consequence of the disease by replacing destroyed β cells in the diabetic pancreas. Islet transplantation (Tx) can complement β cells directly, even though the actual islets are not implanted into pancreas. The required insulin-producing β cells can also be generated from many other sources, such as autologous adult stem cells, embryonic stem cells (ESC) and somatic cells through reprogramming or induced pluripotent stem cells (iPSC).

before rendering this tentative therapeutic strategy clinically meaningful and practicable.

Summary and conclusion

As illustrated in Fig. 1, insulinitis-mediated β -cell destruction is directly caused by islet-specific autoimmune attack, which is initiated through the combined effect of autoimmune-related genetic dysfunction and environmental factors. The dysregulation of blood glucose control reflects the insufficiency of functional insulin-producing β -cells. Immune intervention and β -cell replacement are two therapeutic options targeting different stages of T1D progression. The main approach of immune intervention is to enhance T_{reg} effect quantitatively or functionally. Up-regulated T_{reg} s are able to inhibit T_{eff} -mediated autoimmune response directly or indirectly through releasing cytokines. The major targeted cell types include β -cell-specific $CD4^+$ T cell, PPI peptide-specific $CD8^+$ T cell and dendritic cell. β -cell replacement is able to compensate for destroyed β -cells during the course of T1D. However, the application of allogeneic source of β -cells (such as ESC-derived β -cells and islet transplantation) requires anti-rejection immunosuppression which causes harmful side effects. The benefit of using autologous stem cell-derived β -cells is the avoidance of immunosuppressive drugs, but some practical problems need to be resolved before routinely applying to the patients, especially for iPSC-derived β -cells.

In conclusion, immune intervention and β -cell therapy address the direct cause and consequence of T1D respectively. Immunotherapy exerts therapeutic effect on T1D mainly through re-setting the balance between autoimmunity and regulatory mechanisms, and T_{reg} s play an important role in this immune intervention. β -cell replacement is able to reverse the consequence of T1D by replacing destroyed β -cells in the diabetic pancreas. Obviously, a synergistic

effect on T1D treatment could be obtained when these two therapeutic options properly apply to the T1D subjects. Through searching for more optimal combinations in astutely designed pre-clinical studies, it is possible to achieve a more efficient therapy for T1D.

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