

Chapter 16

Addressing the Challenge of Autoimmunity in the Treatment of Diabetes with Stem Cells

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Abstract Type 1 diabetes mellitus is a complex autoimmune disease process encompassing a number of stages, the most significant of which is the loss of immunological tolerance and the initiation of immune dysfunction resulting in the selective destruction of pancreatic β cells. Although exogenous insulin therapy has proven efficacious, it does not address the underlying cause of the disease. A treatment strategy encompassing immunosuppressive and β cell replacement therapy that will promote immunological tolerance, without toxicity or the induction of lymphopenia is required for treatment of patients with hypoglycaemic unawareness. Importantly, this combination strategy must harness a therapy that provides a replacement source of insulin-producing β cells without toxic side effects associated with long-term immunosuppression and induces tolerance to the replacement β cells in order to prevent destruction by allo- and autoreactive T cells. Here, we discuss the current immunosuppressive therapies and potential sources of replacement β cells and review the pitfalls in current combined immunosuppression and islet transplant therapy. Finally, we examine possible combination strategies including stem cells that are likely to succeed in fulfilling the above criteria for the treatment of diabetes in the future.

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16.1 Introduction

Type I diabetes mellitus (T1DM) is an autoimmune disease in which autoreactive T cells target the pancreatic insulin-producing β cells. Destruction of β cells leads to insulin paucity, blood glucose dysregulation and subsequent hyperglycaemia which in turn result in a number of long-term micro and macro-vascular complications [1]. Diabetes is fast becoming a common childhood disease with the incidence set to double in children under the age of 5 by 2020 [2]. On a global scale, the World Health Organisation (WHO) estimate that 220 million people suffer from diabetes worldwide, a number which is set to increase twofold by 2030. The costs of treatment and the clinical management of the associated complications are substantial. The need for a preventative or regenerative therapy is perhaps at its most urgent since the discovery and development of insulin in the 1920s by Banting, Best, Collip and Macleod. Existing therapies include insulin therapy, cell-based therapy and solid organ transplantation as well as immunotherapy [3]. Although insulin therapy revolutionised the treatment of diabetes, there are limitations, particularly in the group of patients with hypoglycaemic unawareness. As insulin therapy does not address the cause of the disease, there is need for a therapy which has the capacity to address the autoimmune response, the replacement of insulin producing β cells and, if necessary, the problem of alloreactivity evoked by the replacement therapy.

A combination of genetic susceptibility [4] and environmental factors trigger changes in the immune system leading to immune dysregulation and subsequent autoimmunity accompanied by the development of islet specific autoantibodies and autoreactive T cells. The disease process starts with genetic susceptibility [4] (stage 1) followed by triggering events (stage 2). Immune dysregulation and environmental triggering (stage 3) are followed by the loss of β cell function detected by abnormal glucose tolerance test (stage 4). At diabetes onset (stage 5) almost 80 % of the beta cells are already lost. The final stage of this process (stage 6) is the total loss of β cells with patients dependent on insulin therapy for survival [5] (Fig. 16.1).

16.2 Current Therapeutic Strategies for Type 1 Diabetes Mellitus

A number of potential therapeutics have been investigated in pre-clinical models of diabetes with many demonstrating efficacy in prevention or reversal of T1DM, however, in the majority of cases, these results do not extrapolate to humans. Factors including variations in genetic predisposition, environmental triggers as well as inter-individual heterogeneity in disease pathogenesis all effect therapeutic outcomes [6–8]. Additionally, a major issue concerning the current replacement therapy for T1DM, namely islet or pancreas transplantation, is the problem of

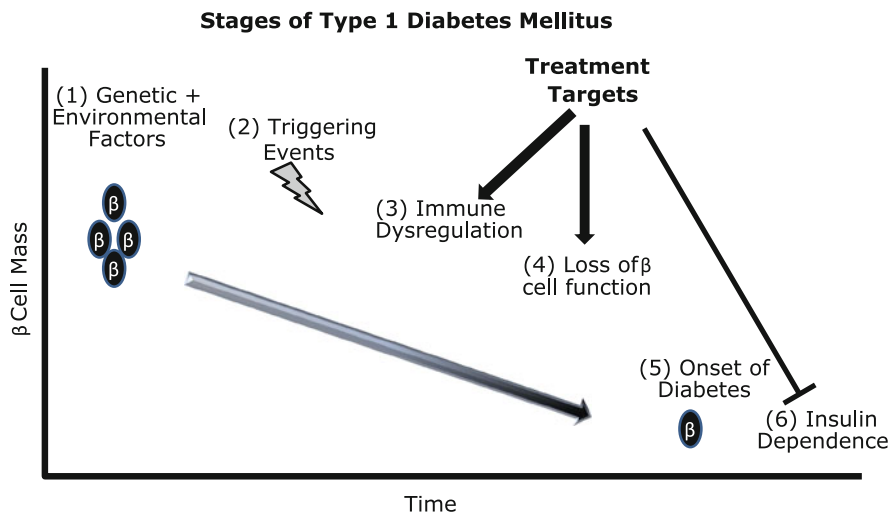


Fig. 16.1 Stages of Type 1 Diabetes. Diabetes is thought to be initiated by interactions between genetic susceptibility and environmental factors (1). Evidence suggests that triggering events such as enterovirus infection contribute to the pathogenesis of T1DM (2). Loss of immunological tolerance coincides with immune dysregulation (3) resulting in the activation of autoreactive T cells and subsequent destruction of β cells leading to loss of β cell function (4) and significantly decreased β cell mass signifying the onset of diabetes (5) which inevitably results in insulin dependence (6). Treatments are specifically targeted to stage (3) using immunosuppressive tolerance induction strategies and importantly stage (4/5) utilising β cell replacement therapy in an attempt to prevent insulin dependence

recurrent autoreactive T cells [9] which seem to be resistant to suppression by conventional immunosuppressive drugs. Here, we discuss the immunotherapies and potential sources of β cells for the treatment of diabetes patients focussing on the possibility of combination therapies of immunosuppressive agents that will effectively address autoimmunity and alloreactivity (Fig. 16.2).

16.3 Immunotherapy

16.3.1 Global Immunosuppression

Randomised trials initiated in the 1980s tested the effects of global immunosuppressive drugs in modulating autoimmune diabetes, including cyclosporine [10, 11], azathioprine alone [12], or in combination with prednisone [13], anti-thymocyte globulin and prednisone [14] and rituximab (humanised monoclonal antibody (mAb) specific for CD20) [15]. All of these strategies led to improved endogenous β cell function and a decrease in insulin requirements in patients with new onset diabetes. However, the beneficial effects were limited to the duration of the treatment and the

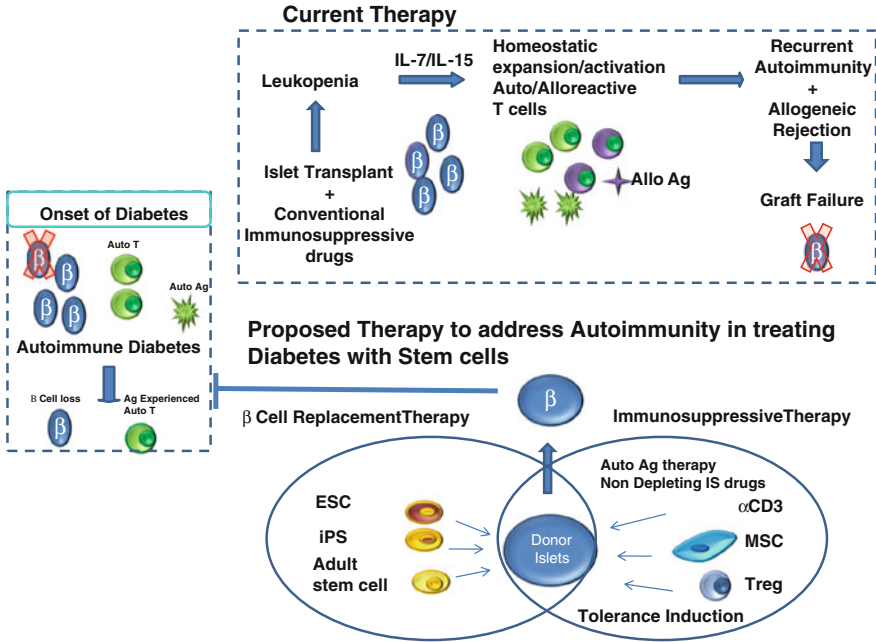


Fig. 16.2 Strategies of combination therapy for T1DM. The onset of diabetes is thought to be initiated by multiple factors involving genetic and environmental factors as well as triggering events which induce β cell death, initiating the process of autoimmunity—in which autoantigen and autoreactive T cells play a key role. Current therapy consisting of donor allogeneic islet transplant and conventional immunosuppressive drugs induce a state of leukopenia and promotes the homeostatic expansion and activation of pre-existing autoreactive T cells that target the transplanted islets for destruction and result in graft failure. Future therapy involving an ES cell, iPS cell or adult stem cell-derived β cell source in conjunction with a combination immunotherapy consisting of anti-CD3, MSCs, Treg, autoantigen therapy or non-depleting immunosuppressive drugs may provide a more successful outcome through avoidance of leukopenia and induction of tolerance

side effects associated with cyclosporine in particular [16] suggested that global immunosuppressive therapy alone was unsuitable for treatment of T1DM.

The standard immunosuppression used in whole pancreas transplantation can vary between different centres, however, a protocol involving thymoglobulin as induction, with tacrolimus, mycophenolate mofetil (MMF) and prednisone for maintenance therapy, results in 80–85 % of grafts maintaining function after 1 year [17]. In contrast, utilisation of the Edmonton immunosuppressive protocol (optimised over a number of years) in conjunction with transplantation of allogeneic human islets has proven successful in rendering 100 % of patients ($n = 7$) with T1DM insulin independent for at least 1 year and provides a prime example of the potential of immunosuppressive drugs (discussed in more detail below) [18].

16.3.2 Antigen-Specific Therapies

A number of diabetes related autoantigens including insulin, pro-insulin, insulin peptides, glutamic acid decarboxylase (GAD) and hsp60 and hsp peptides p277 have been utilised in tolerance induction strategies. The NOD mouse has provided a useful platform to test the efficacy of antigen-specific therapy in diabetes. Studies have demonstrated the ability of exogenous insulin, pro-insulin or insulin peptides delivered orally, subcutaneously, or intranasally to prevent or suppress diabetes [19–23]. Delivery of GAD [24–27] or hsp60 and hsp60 p277 peptide [28, 29] through similar routes, also revealed promising results in NOD mice. Extrapolation of these antigen-specific therapies from the NOD mouse into human clinical trials has, however, proven difficult. Trials performed in new onset diabetes patients using oral insulin or an altered peptide ligand of the 9–23 insulin B chain peptide (NBI-6,024) demonstrated no effect [30, 31].

16.3.3 T cell and Co-Stimulation Targeting Agents

A number of monoclonal antibodies targeting CD3, CD4, CD8 and $\alpha\beta$ T cells as well as major histocompatibility complex (MHC) class II, CD28 and CD154 have been examined for their capacity to prevent or reverse T1DM [32–38]. However, among these; anti-CD3 therapy has prevailed as the most promising so far.

16.3.4 Anti-CD3 Therapy

Anti-CD3 mAb therapy has successfully induced a permanent state of disease remission in a rodent model of T1DM. In these studies antigen-specific tolerance was induced with mice regaining full immune competence after a few weeks of treatment [33, 39]. Clinical trials tested the efficacy of two humanised Fc engineered monoclonal anti-CD3 antibodies called teplizumab (Hokt3 γ 1 (Ala-Ala)) [40, 41] and otelexizumab (ChAglyCD3) [42, 43]. A multi-centre, Phase II placebo-controlled trial consisting of 80 patients with new onset T1DM receiving a 6-day treatment of either otelexizumab or placebo was carried out. This trial demonstrated that the anti-CD3 antibody preserved β cell function. Patients receiving the antibody maintained significantly higher levels of endogenous insulin secretion than placebo controls at 6, 12, 18 and 48 months after treatment [42, 43]. However, after 24 months, the beneficial effect diminished over time with a decline in β cell function and increase in insulin dependence [43]. Similar results were observed in the teplizumab trial which entailed a 12 or 14 day mAb treatment with 2–4 days of incremental dose escalation to 10 days of a full dose of drug mAb. There were significant improvements in C-peptide responses accompanied by reduced haemoglobin A_{1c} (HbA_{1c}) and

insulin requirements in patients receiving the mAb, however, the effects waned after 2 years [40]. Furthermore a small open labelled phase IIb clinical trial demonstrated that a higher dose (40 % higher) of teplizumab resulted in increased adverse events without an improved efficacy of the drug [44].

It seems likely that autoreactivity was only transiently suppressed in these trials leading to the subsequent loss of additional β cells and the increased requirement for exogenous insulin with time after cessation of the therapy. A study carried out by Albamunits et al. clearly demonstrated that β cell replication is reduced after immune therapy using an anti-CD3 mAb resulting in progressive loss of β cell mass [45], similar to the functional decline observed in humans after treatment with anti-CD3 [43]. The mode of action of the anti-CD3 mAb remains unclear but alteration of lymphocyte migration or trafficking, rather than depletion, has been suggested [44].

Although this therapy has been the most promising to date, it is likely that a combination of immunomodulatory agents coupled with a β cell replacement strategy (or β cell regeneration strategy in patients with a small but sufficient β cell mass) will be more efficacious in addressing the multiple factors associated with current allogeneic islet transplantation and future β cell replacement providing a more successful outcome.

16.3.5 Regulatory T cell Therapy

Autoimmune diabetes manifests from the loss of immunological tolerance. T1DM patients as well as NOD mice, exhibit a decreased frequency of regulatory T cells (Treg) with alterations in function [46–49] and IL-2/IL-2R signalling pathway [50]. There is evidence to suggest that NOD mice have Treg that prevent diabetes development early on, but the functional capacity of the Treg is lost over time [51], allowing dysregulated autoimmune attack of insulin producing β cells [52, 53]. Similarly, Treg cells taken from peripheral blood of T1DM patients also exhibit defective suppressive functions in vitro [54].

Although it seems likely that endogenous naturally occurring Treg are functionally defective in diabetic mice, adaptive Treg present in NOD mice have been shown to suppress autoreactive T cells mediated by TGF- β [51] and therefore represent a possible target for tolerance induction in vivo. Treatment with anti-CD3 ϵ antibody induced tolerance in NOD mice through the activation of adaptive Treg in a TGF- β -dependent manner [55], highlighting the possibility of driving a tolerance induction pathway through mAbs. TGF- β -producing Treg have also been implicated in tolerance induction strategies involving immunisation with plasmid DNA encoding GAD65, IL-4 and IL-10 [56] in a NOD mouse model.

The other option available involving Treg therapy, is the selective ex vivo expansion of polyclonal or antigen-specific Treg [57]. Tang et al. [58] have demonstrated that in vitro expanded antigen-specific Treg cells suppress autoimmune diabetes in the NOD mouse with greater potency than expanded

polyclonal Treg. Utilising a humanised mouse model, our lab has demonstrated the capacity of ex vivo expanded human Treg to prevent rejection of a life sustaining human islet allograft in streptozotocin induced diabetic mice (Wu, Wieckiewicz and Wood unpublished results). The data from animal models of diabetes supports the use of human Treg in the treatment of autoimmune diabetes, however, the major discrepancy is the ability to generate human antigen-specific Treg directed against autoantigen. Additionally, although Treg have proven efficacious in the NOD mouse, extrapolation to the clinic will not be without difficulty and it is likely that a combination therapy of Treg and a short-acting immunosuppressive drug like anti-CD3 may be efficacious in breaking autoimmunity or preventing islet/stem cell-derived β cell rejection.

16.3.6 Mesenchymal Stem Cells

Mesenchymal Stem Cells (MSCs) are adult stem cells found within the bone marrow (as well as many other tissues and organs) whose primary functions are to provide stromal support for hematopoietic stem cells and to act as a reservoir for the continuous turnover of mesenchymal lineages under regular conditions. Moreover, MSCs possess immunosuppressive characteristics which make these cells an attractive source for cellular immunotherapy both in transplantation and autoimmunity settings.

In the context of autoimmunity, MSCs have been shown to be efficacious in suppressing autoreactive T cell responses in mouse models of colitis [59], experimental autoimmune encephalomyelitis (EAE) [60] and collagen induced arthritis (CIA) [61] among others.

Furthermore, MSCs have proven therapeutically beneficial in a mouse model of T1DM [62, 63]. Administration of MSCs derived from BALB/c or non-obese resistant strain but not non-obese diabetic (NOD) mice, delayed diabetes onset in pre-diabetic recipients. This delay in disease onset was thought to be associated with the expression of PD-L1 by MSCs and the promotion of a Th2 type response in treated NOD mice [62]. Importantly, this study highlights the important fact that MSCs isolated from diabetic patients may not have the same immunosuppressive capacity as MSCs from healthy patients and, therefore, use of allogeneic MSCs may need to be considered in this case.

Considerable evidence supports the ability of MSCs to prevent allogeneic graft rejection [64]. A number of in vivo studies demonstrate the ability of MSCs to suppress alloreactive responses both in skin and heart allograft models [65, 66]. In a fully MHC-mismatched baboon skin transplant model, a single dose of donor MSCs administered intravenously resulted in prolonged skin graft survival (11.3 ± 0.3 days compared to 7.0 days in untreated controls). However, this effect was non-specific as third party grafts were also prolonged in a similar time scale [65]. Casiraghi and colleagues report that both donor and recipient derived MSCs have the capacity to prolong cardiac allograft survival in a semi-allogeneic but not

a fully MHC-mismatched cardiac allograft model. Protection of cardiac allografts was associated with the expansion of Treg cells and the abrogation of anti-donor Th1 activity. Significantly, this study highlighted the differences between donor and recipient derived MSCs and shows that pre-transplant intraportal administration of a single dose of MSCs proved more efficacious than the intravenous route [66]. Unfortunately, this is not something that we can test in human subjects and therefore we must learn what we can from the successful use of MSCs in steroid refractory graft versus host disease (GvHD) patients, which involves intravenous administration of MSCs of autologous or allogeneic origin [67].

The capacity of MSCs to prevent donor allogeneic islet graft rejection was investigated utilising a life-preserving mouse islet allograft model. This streptozotocin induced diabetes model allowed us to examine the effect of MSCs in suppressing an alloreactive effector T cell attack on transplanted allogeneic islets in an immunodeficient mouse. In the absence of MSCs, donor allogeneic islet grafts were rejected with a mean survival time of 30 days, however, in the presence of MSCs islet grafts were maintained long term with stable normoglycemia. The ability of MSCs to prevent rejection in this study is likely an attribute of co-localisation of MSCs with the islet graft associated with the provision of a local immunosuppressive milieu by MSCs in the locality of the islet graft. In this local microenvironment MSCs produce soluble factors, in particular MMP-2 and 9 which impair alloreactive T cell activation and expansion [68].

In addition to suppression of autoreactivity, MSCs also potentially modulate alloantigen specific responses and thus provide a promising therapeutic strategy for the treatment of type 1 diabetes. MSCs are unique as a cellular therapy in that they have the capacity to address both the problem of autoimmunity and alloreactivity against a cell replacement therapy for diabetes patients. However, although MSC therapy has shown much promise in controlling both allo- and autoreactivity, there is little doubt that MSCS therapy alone will not be sufficient and will likely be more efficacious as a combination therapy; with a single dose of, for example, anti-CD3.

16.4 β Cell Replacement Therapy

16.4.1 *Islet Transplantation*

By far the most forward moving of the cell replacement therapies is allogeneic islet therapy, which involves the transplantation of deceased donor-derived islets percutaneously into the portal vein of the liver, combined with immunosuppressive drugs. Islet therapy has recently improved in efficacy through introduction of the Edmonton protocol [18]. The success of the Edmonton team involved an improved islet isolation technique combined with infusion of large numbers of freshly isolated islets. In addition, an altered immunosuppressive regimen avoiding steroids

(glucocorticoid therapy), and combining a reduced dose of tacrolimus (a calcineurin inhibitor which can inhibit insulin secretion) with the addition of sirolimus (mTOR inhibitor) [18] was implemented. Under this regimen, 68 % of patients receiving islet allografts maintained insulin independence at year 1. Unfortunately, insulin independence was not sustained long term with less than 10 % of patients remaining insulin independent at year 5. However, 80 % of patients had measurable levels of C-peptide indicating the continuous low level production of insulin [69], which allows improved glycemic control. The benefits associated with islet transplantation although small, supports the utility of this procedure in a select group of patients experiencing hypoglycaemic unawareness (patients who have lost significant β cell mass). Problems associated with this therapy are the inefficient isolation procedure and the requirement of at least two pancreatic donors, for which there are simply not enough donors available. This lack of islet tissue has led to the proposed use of adult, embryonic or induced pluripotent stem (iPS) cells for replacement therapy.

16.4.2 Embryonic Stem Cells

Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of the blastocyst with the capacity for unlimited self-renewal in an undifferentiated state, and the ability to undergo induced differentiation into all three germ layers in vitro and in vivo. The ability of mouse ES cells to differentiate in vitro into functional insulin producing cells or islet like clusters which can recover and maintain normoglycemia in streptozotocin-induced diabetic mice has been demonstrated by a number of groups [70, 71]. Human ES cell differentiation into insulin producing cells has proven more difficult due to an inability of ES cells to generate definitive endoderm. However, Baetge's group have generated a protocol that promotes differentiation of human ES cell derived endodermal cells into cells expressing pancreatic markers [72]. Although this protocol did not lead to glucose responsive insulin producing cells in vitro, implantation of human ESC derived—endodermal derived—insulin positive cells in immunodeficient mice resulted in the production of insulin in response to glucose several months after transplantation [73]. This study was the first of its kind to demonstrate that insulin producing cells derived from human ES cells could maintain normoglycemia in a mouse model of streptozotocin-induced diabetes. However, this study also highlights the fact that further research is required in order to fully optimise the differentiation protocol and to ensure phenotypically and functionally stable insulin producing cells are induced.

Importantly, the issues that these studies do not address is that of autoimmune and alloreactive attack. In the case of alloreactivity against ES cell-derived tissue, it is likely that these cells/tissues will be recognised as foreign and subsequently rejected by the immune response. The immune response directed against fully differentiated insulin producing tissue derived from mouse ES cells was examined using a mouse model of streptozotocin-induced diabetes. In this study, functional

insulin producing cells were rejected in immunocompetent hosts and this response was mediated primarily by T cells [74] with evidence for involvement of both innate and adaptive components of the immune system [75–77] .

16.4.3 Induced Pluripotent Stem cells

Ground breaking research describing the factors required to reprogram adult cells back into iPS cells in 2006 [78] has paved the way for tissue engineering and therapeutic application. Initial problems concerning homogeneity and efficiency of the reprogramming process are now being addressed promptly by a number of groups. However, clinical application of iPS cell-derived tissue remains a long way off with safety concerns regarding the stability of the cells in vivo and the possibility of tumour formation. Recently Tateishi et al. reported that human iPS cells derived from skin fibroblasts could be differentiated into islet like clusters, which expressed insulin. Of four iPS cell lines, two differentiated into islet like cell clusters and one of these released low levels of C-peptide in response to glucose stimulation [79]. iPS cells have also been derived from adult cells from patients with disease and one group have reported the differentiation of skin biopsy-derived iPS cells into insulin producing cells. This study was more convincing with evidence that cells released human C-peptide (5 fold increase) in response to glucose stimulation, suggesting that insulin producing cells were functional [80].

16.4.4 Adult Stem Cells

Bone marrow-derived cells can differentiate into a number of lineages and have been demonstrated to play a role in regeneration and repair and therefore represent an attractive source for tissue engineering in T1DM. Transplantation of bone marrow-derived cells into streptozotocin-treated mice led to the instigation of endogenous pancreatic tissue regeneration resulting in insulin production and improved survival [81]. In contrast, a number of studies have suggested that bone marrow-derived cells differentiated into insulin positive cells, however, the stem cell community is still not convinced that this lineage switch is possible. Nonetheless, two studies in particular have demonstrated that bone marrow-derived islet-like clusters transplanted into streptozotocin-induced diabetic rodents have the capacity to control blood glucose and maintain normoglycemia which was reversed after removal of the graft [82, 83].

Umbilical cord blood has stimulated interest both as an immunomodulatory therapy and as a potential source of insulin-producing cells for use in T1DM. Islet-like clusters derived from human Wharton's jelly MSCs in umbilical cord matrix produced low levels of insulin and have some effect in controlling blood glucose and maintaining normoglycemia in vivo [84]. Overall, it seems likely that adult

stem cells may have the capacity to differentiate into insulin producing islet like cells, however, the process of trans-differentiation is as yet an unproven phenomenon and therefore it is unlikely that adult cells will provide the most useful/optimal source of replacement β cells.

16.5 Strategies to Address Autoimmunity in β Cell Replacement Therapy

The prevailing issues that need to be addressed in the treatment of T1DM with β cell replacement therapy are the presence of autoreactive T and B cells specific for islet cell antigens (autoantigens), as well as lymphocytes with the capacity to respond to mismatched MHC antigens (alloantigens) of the β cell donor. Additionally, depending on the source of β cells, there may be other antigens, as in the case of ES cell-derived β cells which could evoke immune activation; for example ES and iPS cells express surface antigens that disappear at later stages of development and which are not expressed by adult cells [85, 86]. Graft failure has been reported in a minority of transplant patients and has been attributed to chronic rejection. However, another reason for graft failure is the recurrence of autoimmunity which was initially described in patients receiving pancreas graft from HLA-identical siblings with no or reduced immunosuppression [87, 88]. A large study examining 100 grafts described autoimmune diabetes recurrence in $\sim 10\%$ of patients receiving donor grafts with immunosuppression [89]. More recently, the recurrence of T1DM after simultaneous pancreas-kidney transplantation, despite the use of immunosuppressive drugs, was reported to be associated with both autoantibodies and autoreactive T cells [9]. Furthermore, conventional immunosuppressive drugs currently used in allogeneic donor islet transplantation prevent rejection through the depletion of leukocytes. The immune system responds to this through production of common γ chain cytokines IL-7 and IL-15 which stimulate the expansion of any remaining lymphocytes in an effort to restore homeostasis [90–92]. This effect also results in the expansion of pre-existing autoreactive cells and can lead to destruction of transplanted islets [93, 94]. Indeed, the production of IL-7 was shown to promote the expansion of autoreactive T cells in response to a lymphopenic environment [93]. Moreover, examination of the serum from T1DM patients after islet transplant, revealed the presence of increased concentrations of IL-7 and IL-15 [95]. There is some evidence to suggest that certain immunosuppressive drugs will promote homeostatic proliferation while others may inhibit it. Two patients receiving MMF plus tacrolimus in place of sirolimus plus tacrolimus (due to sirolimus intolerance) displayed reduced proliferation with no change in IL-7 expression, indicative of the capacity of MMF, but not sirolimus, to block cell proliferation [95]. This phenomenon was also observed in 3 patients who received kidney-pancreas transplant with anti-thymocyte globulin induction therapy followed by MMF plus

cyclosporine A (CyA) or FK506 maintenance therapy [95], demonstrating that the immunosuppressive therapy utilised may impact on the outcome of islet or β cell replacement therapy [96].

A poor clinical outcome has also been associated with the presence of islet specific autoantibodies [97] indicating that assessment of autoantibodies present before transplant of replacement β cells will be important in the choice of (patient tailored) immunosuppressive therapy.

16.6 Conclusion

Although significant progress has been made in the field of immunotherapy to halt autoimmune T1DM, the development for a combination therapy encompassing both immunotherapy and β cell replacement therapy (currently donor islet transplantation but stem cell-derived β cell tissue in the future) has been somewhat elusive. Encouragingly, there are already a number of promising therapies available targeted at autoimmune diabetes, which, if combined, could provide a very successful therapy, the key to which is undoubtedly a tolerance induction strategy.

In summary, the issues which need to be addressed in the quest for this optimal therapy are (1) evaluation of the presence and scale of pre-existing autoreactive T cells in patients before transplant of replacement β cells; (2) examination of the effect of new immunosuppressive regimens on the activation and expansion of pre-existing autoreactive T cells after transplant and (3) development of new therapeutic agents that have the capacity to prevent rejection and regulate the pre-existing autoimmune response without inducing profound leukopenia.

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