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Alteration of Regulatory T Cells in Type 1 Diabetes Mellitus: A Comprehensive Review

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Abstract Type 1 diabetes mellitus (T1DM) is a T cell-mediated autoimmune disease characterized by the destruction of pancreatic β cells. Numerous studies have demonstrated the key role of $CD4^+CD25^+FoxP3^+$ regulatory T cells (Tregs) in the development of T1DM. However, the changes in Treg expression and function as well as the regulation of these activities are not clearly elucidated. Most studies on the role of Tregs in T1DM were performed on peripheral blood rather than pancreas or pancreatic lymph nodes. Tissue-based studies are more difficult to perform, and there is a lack of histological data to support the role of Tregs in T1DM. In spite of this, strategies to increase Treg cell number and/or function have been viewed as potential therapeutic approaches in treating T1DM, and several clinical trials using these strategies have already emerged. Notably, many trials fail to demonstrate clinical response even when Treg treatment successfully boosts Tregs. In view of this, whether a failure of Tregs does exist and contribute to the development of T1DM and whether more Tregs would be clinically beneficial to patients should be carefully taken into consideration before applying Tregs as treatments in T1DM.

Keywords Regulatory T cells · Type 1 diabetes mellitus · Immunotherapy

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Introduction

The Immune Basis of T1DM

Type 1 diabetes mellitus (T1DM) is a chronic, T cell-mediated autoimmune disease. The immune system attacks the insulin-producing β cells of the pancreatic islet, eventually resulting in insulin deficiency [1, 2]. Under normal conditions, the vast majority of self-reactive T cells are eliminated in the thymus through a mechanism called "central tolerance induction." This is part of the process which ensures immune tolerance to self-antigens. Nevertheless, a few remaining autoreactive T cells escape thymic elimination and are released to the peripheral circulation [3]. Normally, these autoreactive T cells are actively suppressed by regulatory T cells (Tregs). Therefore, impaired thymic deletion and defective Treg function may both contribute to the onset and development of autoimmune T1DM [4].

In animal models of T1DM, islet-reactive T cells are primed in the pancreatic lymph nodes [5] and then infiltrate into islets causing damage to β cells. Abnormal autoreactive T cell responses, together with effect of other immune cells, including macrophages, dendritic cells (DCs), natural killer T (NKT) cells, and B cells, eventually induce the onset of autoimmune diabetes. Specifically, the destruction of β cells is mediated by granzyme and perforin produced by $CD8^+$ T cells, cytokines such as interferon gamma (IFN-γ), and interleukin-17 (IL-17) [6]. Fas/Fas and tumor necrosis factor-α (TNF-α)-dependent pathway also contribute to the pathogenesis of T1DM [3] (Fig. 1).

The Immunological Properties of Tregs

Tregs are a diverse population of lymphocytes that exhibit inhibitory or regulatory effects on immune responses by influencing the activity of other cells. A dysfunction in Treg

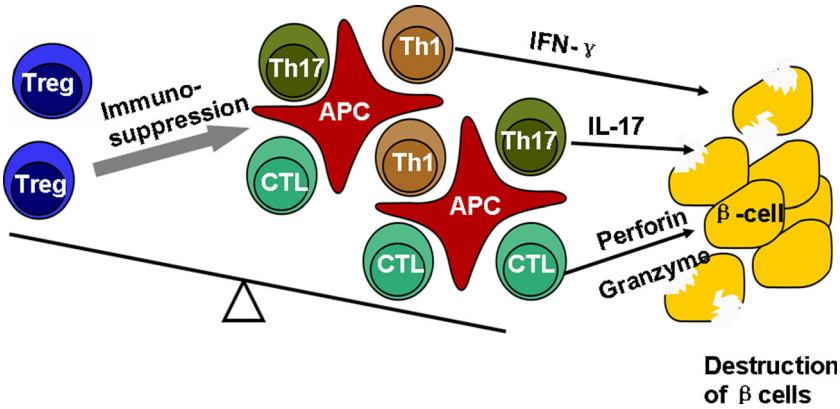

Fig. 1 Immune basis of T1DM Defective Treg failed to regulate autoreactive T cells that target islet β cells, contributing to the development of T1DM

cells has been implicated in the pathogenesis of many autoimmune diseases [7–9]. Tregs suppress autoreactive T cells and induce immune tolerance, resulting in a dampening of inflammation [10]. Cell-to-cell contact, secretion of immunosuppressive cytokines, killing or modification of antigen-presenting cells (APCs), and competition for growth factors are the four main regulatory mechanisms Tregs exert their regulatory effects on T cells, natural killer (NK) and NKT cells, B cells, and antigen-presenting cells (APCs) [11, 12]. Types of Tregs include $CD4^+CD25^+FoxP3^+$ Tregs, interleukin-10 (IL-10)-secreting TR1 cells, transforming growth factor-β (TGF-β)-secreting T helper 3 (TH3) cells, $CD8^+$ Tregs, $CD8^+$CD28-FoxP3-Tregs, $CD3^+$CD4-CD8-(DN) Tregs, $CD4^+V\alpha14^+$ NKTregs, and γδ-T-cells [10, 13, 14]. A comprehensive review of all the various subsets of Tregs is beyond the scope of this article. $CD4^+CD25^+FoxP3^+$ Treg cells have been one of the more extensively studied types of Treg cells, and the effect of this subset of Treg cells on T1DM is the main focus of this paper.

Among the various characteristic markers of Treg cells, the most specific and important one is foxhead box P3(FoxP3), which is constitutively expressed by Tregs and acts as a master regulator [15, 16]. $CD4^+CD25^+FoxP3^+$ Tregs consist of two major subsets with different origins: thymus-derived natural Treg (nTreg) and peripheral-induced Treg (iTreg). Though there exist some differences between the two subsets, nTreg and iTreg share many similar phenotypes and show comparable suppressive function [17].

Treg Cell Interactions

Animal Models

The existence of suppressor T cells has been recognized for almost two decades [18–21]. However, inconsistent data obtained from different laboratories have delayed our understanding of the mechanism of action of these cells. It was not until 1995 that several laboratories [22–25] demonstrated that a distinct subset of $CD4^+$ T cells expressing CD25 possesses regulatory properties. When $CD4^+$ T cells depleted of this $CD25^+$ population were transferred into the immunodeficient nude mice, various forms of autoimmunity [22, 23] including autoimmune diabetes [26] ensued. Later, FoxP3 [15, 16, 27] was found to be the master regulator of Treg development as well as function [28], directly demonstrated by the observation that FoxP3 point mutations lead to fatal autoimmune disease in mice [27, 28]. Data obtained from animal models of T1DM clearly demonstrate that $CD4^+CD25^+FoxP3^+$ Tregs are crucial for bridling T1DM. Elimination of Tregs in non-obese diabetic (NOD) mice led to the spontaneous development of autoimmune diabetes. In contrast, reconstitution or transfer of Tregs could prevent the development of autoimmune diabetes in murine models [29–31]. As islet-reactive T cell priming takes place in the pancreatic lymph nodes, and then primed effector T (Teff) cells migrate to the islets to destroy β cells, the protective effect of Tregs can occur both in the secondary lymphoid organs and the inflammatory pancreas, respectively.

Treg Cell Effects on T Effector Cells

Tregs regulate the activity of autoreactive T cells via several different mechanisms. In pancreatic lymph nodes (PLNs), Teff proliferation is inhibited by Tregs, demonstrated by the inverse correlation between the level of Tregs and proliferation of Teffs [30]. Attracted to sites of inflammation, Tregs co-localize with Teffs and dampen the activity of Teffs. Direct cell-to-cell contact is required as demonstrated in in vitro experiments [32]. For example, in lymphoid tissues, Tregs impaired the survival of T helper type 1 (Th1) cells via FasL-dependent cytotoxicity [33]. It should be noted that the elimination of Teffs is only one of the many regulatory mechanisms of Treg cells. Van et al. found that Tregs in the PLNs of NOD mice suppress the activation as well as IFN-γ secretion of both $CD4^+$ and $CD8^+$ T cells [34]. In addition, Tregs block Teffs migration to the T–B cell boundary in the lymph node

where antigen-loaded DCs are present [35, 36], thus impinging upon the proper priming of autoreactive T cells. By suppressing CXCR3 expression in Teffs, Tregs reduce their migration to the PLNs [30].

Tregs may also interfere with the interaction between Teffs and antigen-presenting cells (APCs). Tang et al. [37] showed that Tregs and DCs form persistent conjugation, possibly preventing stable associations between Teffs and these APCs. Tregs thus use a variety of mechanisms to restrict DCs activity, consequently suppressing the activation and differentiation of Teffs in the PLNs [38]. Furthermore, it has been proposed that the suppressive function of Tregs involves the ability of Tregs to "educate" other T cells to adopt a regulatory phenotype [39]. In combination with TGF-βand IL-10, Tregs induce $CD4^+CD25^-$ cells to become suppressor cells [39].

Treg Cell Effects on Dendritic Cells (DCs)

It has also been observed that Tregs cluster around DCs in the PLNs of NOD mice and the engagement of Tregs with DCs blocks the activation of Teffs [41]. CLTA4 expressed by Tregs downregulates DC's expression of CD80 and CD86 and thereby inhibits the activation of Teffs [42, 43]. Lee et al. [44] showed that nTreg depletion resulted in accelerated autoimmune diabetes characterized by a large number of DCs infiltrating the pancreas. They further proposed that Tregs can inhibit DC's infiltration by regulating chemotaxis of DCs toward islets-produced CCL19/21 [44].

Cytokines play an important role in the interaction between Treg cells and DCs. Secreted by Tregs, TGF-β and IL-10 affect the function of Teffs and DCs in a number of ways. TGF-β1 reduces the production of inflammatory cytokines from $CD4^+$ T cells [39]. Furthermore, TGF-β1 also induces the production of IL-10 in Th1 cells and hence attenuates Th1 cell function [45]. Reciprocally, IL-10 enhances the response of Teffs to TGF-β1 [45]. Thus, TGF-β and IL-10 work in combination to suppress the function of Teffs. IL-10 also downregulates IL-12 production in DCs and inhibits the function of Th1 cells [46, 47]. IL-2 is a key factor in the activation of T cells. Tregs avidly capture and exhaust IL-2, leading to IL-2 deprivation of Teffs [48]. The inhibition of IL-2-dependent MHC/protein interactions has been postulated to be a potential mechanism by which to shut down autoreactivity and treat autoimmunity [49].

Other Cellular Interactions

Tregs can also regulate pancreatic autoimmune activity through the control of other types of immune cells in addition to autoreactive T cells. Transfer of Tregs resulted in a decrease in the number of macrophages in the pancreas and inhibition of deleterious cytokine production [40]. Natural killer (NK) cells may also be targets of Tregs [50], demonstrated by the observation that NK cells are activated in the islets in Treg-deficient mice [38].

Another NOD-related observation involves the role of a protein CD137, which is a Treg-derived natural immunosuppressive protein. It has been found that NOD mice are deficient in $CD137^+$_Treg cells. An APC independent assay showed that soluble CD137 suppresses highly purified CD4 T cells through its interaction with CD137 ligand on APCs. CD137 is postulated to act via a negative feedback mechanism to suppress hyperactive immune responses. A deficiency in this population of $CD137^+$ Treg cells may play a role in the pathogenesis of NOD mouse T1DM [51].

Alteration of Peripheral Tregs in T1DM Patients

Methods

Because of the important regulatory role that Tregs play in the pathogenesis of T1DM, the number and/or function of Tregs in T1DM patients have been investigated in the recent years. We searched the PubMed for all potentially relevant articles. The key search terms were "diabetes mellitus, type 1"; "diabetes mellitus"; "T-lymphocytes, regulatory"; and 'T-lymphocytes." We focused on studies which investigated the number/frequency and/or function of Tregs in patients with type 1 diabetes. The search was restricted to cross-sectional or longitudinal studies conducted in humans. In addition, we manually reviewed references from important review articles for relevant articles.

Results

The search identified 24 relevant references that investigated the frequency and/or number of Tregs in T1DM patients. Sixteen studies reported the frequency of Tregs in T1DM patients. Twelve studies investigated the suppressive function of Tregs in patients with type 1 diabetes. The main results of the included studies are summarized in Table 1.

As shown in Table 1, conflicting results have been obtained thus far. Peripheral Treg number and frequency have been shown to be slightly increased, significantly decreased, or normal in T1DM patients (Table 1). Functional assessments have also demonstrated lower or normal suppressive function of Tregs in T1DM patients.

The lack of a general consensus on the role of Treg cells is not unexpected for the reasons outlined below.

1. Currently, there is no precise definition of Tregs. Different experiments use different markers to define Tregs, making comparison of the results almost impossible. Most work [52–63] utilized $CD4^+CD25^+$/high to define Tregs, but

Table 1 Treg frequency and function in T1DM

Surface markers used to identify Treg	Frequency/function of Treg in PBMC	Reference
$CD4^+CD25^+$	Decreased Treg frequency	[52]
$CD4^+CD25^+CD127^-$	Decreased Treg frequency	[71]
$CD4^+CD25^{high}$	Decreased Treg frequency	[53]
$CD4^+FoxP3^+$	Decreased Treg frequency	[64]
iTreg: $CD4^+CD25^+CD127^{lo/-}FoxP3^+$	Increased iTreg frequency	[65]
$CD4^+FoxP3^+$	Normal Treg frequency; Increased frequency of $CD45RO^+$ Treg; Decreased frequency of $CD45RA^+$ Treg; Increased frequency of IFN^+ Treg; Increased frequency of TNF^+ Treg; Normal Treg function	[66]
$CD4^+CD25^{high}$	Normal frequency of Treg; Decreased suppressive function	[54]
$CD4^+CD25^{high}$	Normal Treg frequency; Normal suppressive function	[55]
$CD4^+CD25^+CD127^{lo/-}FoxP3^+$	Normal Treg frequency	[67]
rTreg: $CD45RA^+CD25^+FoxP3^{low}$ aTreg: $CD45RA^-CD25^+FoxP3^{high}$	Normal frequency of rTreg and aTreg	[68]
$CD4^+CD25^{high}CD127^-$	Normal Treg frequency; Normal suppressive function	[72]
$CD4^+CD25^{high}$	Normal Treg frequency	[56]
$CD4^+CD25^{high}$	Normal Treg frequency	[57]
$CD4^+CD25^+$	Normal Treg frequency; Decreased suppressive function	[58]
$CD4^+Foxp3^+$	Normal Treg frequency	[69]
aTreg:$CD45RA^-FoxP3^{high}$	Increased frequency of aTreg; Decreased suppressive function of aTreg	[70]
$CD4^+CD25^{high}$	Normal suppressive function	[59]
$CD4^+CD25^+CD127^{lo/-}$	Normal suppressive function	[73]
$CD4^+CD25^{high}$	Decreased suppressive function	[60]
$CD4^+CD25^{high}$	Decreased suppressive function	[61]
$CD4^+CD25^+FoxP3^+$	Transient decrease of Treg function during 3–6 months from diagnosis, restored by 9 months	[62]
$CD4^+CD25^{high}$	Increased suppressive function of iTreg	[63]

after the identification of FoxP3, researchers [64–70] preferred to use this more specific marker to differentiate Tregs from other T cells. More recently, studies by Badami et al. [71], Ferraro et al. [72], and Liu et al. [73] exploited lower expression of CD127 in combination with the expression of CD4 and CD25 as more precise markers for live Tregs, allowing for flow cytometry-based cell sorting.

2. Nearly all of the studies in Table 1 used Tregs from peripheral blood. Most of their conclusions were based on the hypothesis that T cell population in the peripheral at least partly parallels the one infiltrating various tissues including the pancreas. However, it has been suggested that Tregs exert their function within the target organ undergoing autoimmune attack as well as in draining lymph nodes (DLN) [35, 37, 38, 40, 44]. Consequently, it is possible that the frequency or function of Treg population in the local immune sites does not parallel to the one in the peripheral blood. Preferably, Tregs in T1DM patients should be assessed in the context of the organ where the autoimmune process takes place in addition to the peripheral blood.
3. Though most Tregs retained FoxP3 expression after adoptive transfer under physiological conditions [74, 75], a minority of Tregs were found to have lost FoxP3 expression following transfer into lymphopenic hosts in animal models [74]. Likewise, diminished maintenance of FOXP3 expression in Tregs has been shown to occur in T1DM patients [76]. Thus, under particular inflammatory conditions, the

environment may impact on the frequency and/or function of Treg cells.

4. The discrepancies between various studies may also result from the complexity of the in vitro systems. Since tools for the identification of Tregs in vivo are limited, in vitro assays are widely used, despite the fact that they are of limited relevance to the physiological or pathological conditions in vivo. Furthermore, different systems may clamp cell behavior to different artificial environment.
5. The disparities of characteristics between patients and healthy subjects recruited by each study should also be taken into consideration. Various factors including age, gender, and disease duration can all influence the immune status of the patients and possibly change Treg number or function to some extent. For example, the majority of studies [53, 54, 62, 65, 66, 68, 72] in Table 1 used age-matched controls to avoid the potential confounding effect of age. Moreover, in several studies [52, 61, 63, 65, 67], the researchers divided T1DM patients into two subgroups, namely the new-onset T1DM patients and established T1DM patients (6).

Recently, it has been reported that the effector T cell population in T1DM can resist the regulatory activity of Tregs [57, 59]. Thereby, it is tempting to contemplate that instead of defects in Tregs, the refractory nature of hyperactivated effector T cells to the control of Tregs mimics the defect in Treg function. Taken collectively, current published data suggests that the frequency of Tregs in the peripheral blood of T1DM patients may appear normal. However, it is still unclear whether Treg cells from T1DM patients have intrinsic defective function or whether the responder T cells are resistant to suppression. To answer this question, with certainty, further studies are urgently needed.

Tregs in the Pancreas and Peripheral Lymph Nodes

The characterization of Treg population in the pancreas and PLNs remains poorly addressed in clinical settings. One study mentioned in Table 1 compared PLNs-derived Tregs from T1DM patients and controls, reporting that PLN-derived Treg functions were impaired in T1DM subjects [72]. Willcox [77] analyzed postmortem pancreatic samples from 29 T1DM patients. FoxP3$^+$ Tregs were only found in islets from a single patient, suggesting that the lack of Treg cells may play a role in autoimmune pathogenesis in T1DM patients.

Compared to the void of studies in human subjects, several animal studies have focused on Tregs in the pancreas and PLNs. Tonkin [40] generated TGF-β-induced islet-specific Tregs and demonstrated their ability to suppress the transfer of diabetes into NOD.scid mice using diabetic spleen cells. Infiltration of both Teffs and Tregs were observed in the pancreas, suggesting the active role that Tregs play in the inflammatory site. By the induction of hemopoietic chimerism, antea-diabetic mice were restored to adequate pancreatic islet function even after they had been rendered hyperglycemic. Compared to the antea-diabetic mice, the numbers of Tregs in the PLNS were significantly decreased in NOD mice, indicating that Tregs in the PLNs had a potential role in ameliorating disease progression in the model [78]. The accumulation of Treg cells that was observed in the islets and PLNs in mice models likely played a significant role in controlling anti-islet inflammation [79].

Mechanistically, local production of CCL22 in islets recruits Tregs to the islets and leads to protection from T1DM in the NOD model [80]. In parallel, pDCs [81] was shown to take a critical role in recruiting Tregs to the pancreas and preventing the progression of T1DM. After entering the pancreas and PLNs, Tregs function to prevent islet destruction via a variety of mechanisms, including the blocking of interactions between Teffs and DCs [37]. Further studies are needed to establish whether or not clinical effects in human subjects parallel those results seen in animal models of T1DM.

Antigen-Specific Tregs in T1DM

Studies on the impairment in the function of Tregs specific to islet antigen are sparse. In most of these studies, the Tregs under investigation are not truly antigen-specific Tregs but are polycolonal in nature. However, it has been demonstrated that antigen-specific Tregs are much more potent in suppressing autoimmunity in T1DM than polyclonal Tregs [29]. In the NOD model, β cell peptide-pulsed DCs can lead to the differentiation of islet-specific Tregs, which are capable of preventing the development of diabetes when co-transferred with diabetogenic cells [82, 83]. NKG2D, an immune-activating receptor found on NK cells and CD8 cells, has been shown to be increased in virus-induced T1DM. Treatment with antibody to NKG2D in combination with antigen-specific Treg cells was able to prevent the development of T1DM in 75 % of mice belonging to a rat insulin promotor (RIP) lymphocytic choriomeningitis virus (LCMV) mouse model. On the other hand, NKG2D blockade by itself was unable to prevent the development of T1DM in a NOD mouse model, even in the presence of the downregulation of NKG2D in NK and CD8$^+$ T cells. The authors concluded that while NKG2D can help maintain Treg cell functionality during ongoing inflammation, it is insufficient by itself to protect against T1DM in the face of strong inflammatory signals [84].

Similarly, in several clinical trials, islet-specific Tregs have been generated and deemed as one of the mechanism contributing to the control of disease progression. For example, in a phase I clinical trial [85], insulin B-chain immunotherapy

induced the generation of insulin B-chain-specific Tregs. GAD65-specific Tregs are also generated during treatment with a GAD-alum moiety [86]. However, the existence of naturally occurring islet-specific has not yet been investigated. It is also possible that even if levels of polyclonal Tregs are unaltered in T1DM patients, islet-specific Tregs may be deficient in number or function in these subjects with T1DM.

Treg Cell-Based Therapy for T1DM

Several clinical trials aiming to re-establish immune tolerance via Treg induction or direct infusion of Treg cells have emerged (Table 2), including anti-CD3 therapy [87, 88], glutamic acid decarboxylase (GAD) injection [89, 90], hematopoietic stem cell transplantation (HSCT) [91, 92], autologous umbilical cord blood transfusion [93–95], and stem cell educator therapy [96]. Some of these therapies have shown efficacy, as demonstrated by increased C-peptide levels and decreased daily dose of insulin requirement, while the others failed in meeting their primary goals.

Anti-CD3 Therapy

Anti-CD3 monoclonal antibody effectively blocks T effector cell activation and, hence, inhibits the development of T1DM in animal models [97, 98]. In addition, anti-CD3 therapies lead to the depletion of pathogenic T cells, but preserve or even boost Treg cell numbers [99]. In general, clinical trials of anti-CD3 therapy have shown improvement in islet function in patients with T1DM [88, 100, 101].

Antigen-Specific Therapy

Administration of GAD [89, 102, 103], insulin [104, 105], and DiaPep277 [106] all fall within the realm of antigen-specific therapy. The mechanism of these therapies involves the induction of antigen-specific Tregs [107]. Such Tregs then potently regulate autoimmune responses. However, there are

Table 2 Clinical approaches for T1DM affecting T reg number or function

Type	Agent	Main mechanisms	Efficacy
Anti-CD3 therapy	ChAglyCD3 [88]	1. Prevention of Teff activation 2. Boosting Treg numbers	Residual beta-cell function was better maintained
	hOKT3gamma1(Ala-Ala) [87]		Improved C-peptide responses to a mixed meal, reduced HbA1c and insulin requirements
	ChAglyCD3 [101]		Suppression of the rise in insulin requirements of recent-onset T1DM patients over 48 months
	Teplizumab [100]		Reduced decline in C-peptide at 2 years in some patients with new-onset T1D
	Teplizumab [99]		Better C-peptide responses but no improvement in HbA1c
	Otelixizumab [110]		No significant effect on C-peptide perseveration
Antigen-specific therapy (GAD vaccination)	Alum-formulated GAD / GAD-alum(Diamyd) [89]	Induction of antigen-specific Treg	Preservation of residual insulin secretion over 30 months but no reduction in insulin needs
	GAD-alum(Diamyd) [102]		No significant reduction in loss of stimulated C-peptide
	GAD-alum(Diamyd) [103]		No alteration of the course of loss of insulin secretion during 1 year
Antigen-specific therapy (insulin vaccination)	Oral insulin [104]		No significant effect on C-peptide perseveration
	Oral insulin [105]		Improved C-peptide responses in patients diagnosed at ages greater than 20 years
Antigen-specific therapy (heat shock protein vaccination)	DiaPep277 [111]		no beneficial effect in preserving islet function
	DiaPep277 [112]		Increase of C-peptide after diagnosis in the low-risk HLA genotype subgroup
Stem cell therapy	HSCT [91]	1. Direct infusion of Tregs 2. Inhibition of immune activation by APCs by stem cells 3. Recruitment of Tregs	Increase of C-peptide and insulin independence achieved for the majority of patients
	HSCT [113]		Insulin independence achieved for the majority of patients
	Autologous cord blood infusion [94]		No preservation of C-peptide
	Autologous cord blood infusion [95]		No change in C-peptide preservation

pitfalls to the success of antigen-specific therapies in the clinical settings, and most trials brought about no clinical benefit.

Direct Infusion of Treg Cells

Direct infusion of Tregs is also a promising strategy in the treatment of T1DM. Stem cells from bone marrow and umbilical cord blood are abundant with Tregs and may be utilized for Treg cell transplant. In addition, stem cells have been shown to inhibit immune activation triggered by antigen-presenting cells (APCs), recruit immunosuppressive cells including Tregs, and support islet function and regeneration. Though proven to be effective in reversing autoimmunity in NOD mice [108] and reducing the levels of islet autoantibodies as well as the level of blood glucose in a clinical trial [109], most stem cell therapies did not bring about improvement in metabolic parameters. The exceptions to this include two HSCT therapy trials [91, 92].

Conclusions

The evidence supports a defect in the regulation of effector T cell activity by Tregs, either due to intrinsic defects of Treg function or resistance of Teff to Treg modulation. Nevertheless, a definitive answer to the question as to whether the frequency/function of Tregs is different between T1DM patients and healthy controls needs to be further explored, perhaps by larger clinical surveys.

Animal models clearly demonstrate that $CD4^+CD25^+FoxP3^+$ Treg cells play a pivotal role in modulating the outcome of autoimmunity. Early clinical trials affecting the number of function of Treg cells have produced encouraging results that indicate the development of Treg cell-based treatments should be a strategy that should be pursued. However, the lack of specific markers, imperfect systems for testing Treg function, the plasticity of Tregs as well as varying clinical manifestations across studies as a function of age or disease duration all contribute to the mixed results reported from these clinical studies. Future studies of Treg number and function in local sites of inflammation and the effects of antigen-specific Tregs in T1DM patients will bring new insights into the precise role of Treg cells in T1DM. Moreover, the definition of the specific defects in Treg regulation in autoimmune diabetes will lead to improved diagnosis as well as possible cure for the disease. Ultimately, a more thorough understanding of T1DM and the role of Tregs will contribute to the development of safe and effective novel treatment strategies of T1DM.

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Conflict of Interest All authors declare that they have no conflict of interest.

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