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TARGETING REGULATORY T CELLS IN THE TREATMENT OF TYPE 1 DIABETES MELLITUS

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Abstract

Type 1 diabetes mellitus (T1DM) is a T cell-mediated autoimmune disease resulting in islet β cell destruction, hypoinsulinemia, and severely altered glucose homeostasis. T1DM has classically been attributed to the pathogenic actions of auto-reactive effector T cells (Teffs) on the β cell. Recent literature now suggests that a failure of a second T cell subtype, known as regulatory T cells (Tregs), plays a critical role in the development of T1DM. During immune homeostasis, Tregs counterbalance the actions of autoreactive Teff cells, thereby participating in peripheral tolerance. An imbalance in the activity between Teff and Tregs may be crucial in the breakdown of peripheral tolerance, leading to the development of T1DM. In this review, we summarize our current understanding of Treg function in health and in T1DM, and examine the effect of experimental therapies for T1DM on Treg cell number and function in both mice and humans.

Keywords

autoimmunity; effector T cells; regulatory T cells; Teffs; Tregs; Type 1 diabetes mellitus

Introduction—Pathogenesis of type 1 diabetes and the role of T lymphocytes

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease characterized largely by T cell-mediated destruction of insulin-producing pancreatic β cells. Individuals become symptomatically hyperglycemic when a critical amount of β cell mass has been lost and the residual β cell mass is unable to match insulin demand. A phenotype similar to human T1DM is seen in the non-obese diabetic (NOD) mouse, and much of our understanding of the pathogenesis of T1DM arises largely from studies of this animal model [1]. The pathogenesis of T1DM is thought to begin when low-level β cell death results in the exposure of β cell antigens, which are then engulfed, processed and presented on the cell surface of antigen presenting cells (APCs) in the context of MHC class II molecules (Fig. 1). It is unclear whether the initial release of β cell autoantigens is prompted by endogenous β cell defects and/or an exogenous trigger, such as a viral infection. In response to antigen presentation and costimulation by APCs, CD4+ T lymphocytes in the local pancreatic lymph nodes proliferate and differentiate into auto-reactive CD4+ effector T cells (Teffs). Teff

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expansion and function is facilitated further by the immune cell-derived complement (C3a and C5a), which is activated locally during this T cell/APC interaction. Within the pancreatic islets, these activated Teffs release a host of cytokines including IFN-γ and IL-2, resulting in recruitment of cytotoxic macrophages and CD8+ T lymphocytes. Cytotoxic inflammatory cells ultimately infiltrate and destroy the islet cells in a process called "insulitis." β cell death ensues, partly as a result of direct perforin/granzyme-mediated toxicity by CD8+ T cells, and partly as a result of the release of pro-inflammatory cytokines (IFN-γ, TNF-α, IL-1β) by macrophages. Chemokines released by injured β-cells promote further mononuclear cell recruitment, and the release of additional auto-antigens allows for expansion and propagation of the autoreactive Teff response [2] (see Fig. 1).

At the earliest stages of T1DM, however, a significant residual mass of β cells exist (perhaps 10–20%) that still produces insulin, but is ineffective at the time of clinical diagnosis owing to hyperglycemia or acidosis [3–5]. This significant residual β cell mass presents a potential therapeutic opportunity to reverse T1DM and is the premise behind a number of immunomodulatory approaches to halt further autoimmune destruction. As such, research into therapeutic interventions in T1DM has long focused on decreasing or blocking Teff activity during the early period following T1DM onset. There is mounting evidence, however, that a second subset of T lymphocytes, known as regulatory T cells (Tregs), is either quantitatively or qualitatively defective during T1DM pathogenesis. Thus, an underlying propensity to develop T1DM may occur when there is an imbalance in the number or function of Treg vs. Teff cells [6–9]. In accordance with this perspective, there has been emergence of therapies that increase the activity of Tregs and restore the balance between the Treg and Teff response. In this review, we will focus on how current T1DM therapies tested in both animal models and humans might be achieving their efficacy via the manipulation of the Treg response.

The Regulatory T cell (Treg) in T1DM

In the 1970s and 1980s, the existence of a population of T cells that could suppress immunity was inferred from studies of neonatally thymectomized mice, which developed multi-organ autoimmunity [10]. The notion of a population of "suppressor cells" capable of inhibiting Teffs fell out of favor in the late 1980s due to concerns that the results were neither reproducible nor generalizable. Despite these questions, the concept of T cells with inhibitory capability was renewed with a seminal study by Sakaguchi in which mice receiving an adoptive transfer of T cells devoid of CD4+CD25+ cells developed multi-organ system autoimmunity [11]. It has been appreciated that this population of T cells, now known as regulatory T cells (Tregs), can keep autoreactive T cells "in check", thereby preventing undesirable immune responses, such as autoimmune diabetes [12]. As such, Tregs may be able to target T cell responses and perhaps antigen-specific responses without broadly immunosuppressive effects.

1. Physiologic Treg activity

There are two subpopulations of Tregs, "natural" Tregs (nTregs) and "induced" Tregs (iTregs), both of which participate in the maintenance of peripheral tolerance. nTregs, such as $CD3^+CD4^+CD25^+Foxp3^+$ cells, originate in the thymus during ontogeny, whereas iTregs derive from T cells that are activated by antigen in the periphery. In the organ-specific autoimmunity of T1DM, iTregs likely play a more important role than nTregs owing to their high affinity self-antigen recognition and efficient infiltration of areas with active inflammation [13].

All Tregs constitutively express CD25, the high-affinity receptor for IL-2α, and are defined by their preferential expression of the forkhead winged helix transcription factor *Foxp3* [14–

16]. The absence of *Foxp3*, as in the fatal diseases of scurfy in mice and IPEX syndrome in humans, results in multi-system autoimmunity, highlighting the importance of Tregs in self-tolerance. Interestingly, the most common endocrinopathy encountered in IPEX is T1DM, suggesting that Tregs may be central to the suppression of circulating β cell- specific T cells, and such pre-effector T cells may be in relative abundance compared to other self-reactive T cells [17–19].

Naïve T lymphocytes and T lymphocyte subsets (such as Th1, Th2, Th17, and Tregs), possess a great deal of plasticity in their differentiation, largely dependent on the local microenvironment. The differentiation of T cells into CD4+ iTregs is favored by a microenvironment rich in transforming growth factor (TGF-β) [20, 21 {Luo, 2007 #64] (Figure 1). Active secretion of the potently anti-inflammatory cytokines IL-10 and TGF-β contributes to both the immune suppressive functions and the expansion of iTregs [22–25]. iTregs suppress Teff activity through multiple mechanisms, many of which are still poorly defined and require more study. However, it is known that Tregs can specifically suppress Teff cells via the secretion of the afore-mentioned inhibitory cytokines (TGF-β, IL-10) [26–28], and can directly target Teff for cytolysis via the release of perforin and granzymes [29, 30].

To maintain immune homeostasis, Tregs interplay with Teff cells to achieve a balance between the pro-inflammatory and anti-inflammatory responses. Neither T lymphocyte subset is capable of acting in isolation. In fact, Teff cell activity is required for maintenance of Treg cell function and proliferation. Locally, Teff and Tregs interface through the IL-2/IL-2R pathway, allowing for direct feedback between the two cell types [31–33]. Teffs and Tregs that have the same TCR compete for the same (auto) antigen, and both become activated and proliferate in response to antigen. Tregs possess a higher affinity for self-antigen and are less reliant on costimulation than Teffs, which may be a mechanism that enhances Treg generation in non-inflammatory states, thus promoting self-tolerance [9, 34–36]. Conversely, in pro-inflammatory states, Teffs proliferate more rapidly than Tregs in response to antigen, promoting a Teff/Treg balance favoring pathogenesis. Factors thought to promote inflammatory states and enhance Teff number and function (i.e. TNF and IL2), may paradoxically enhance Treg number and function at sites of inflammation, perhaps mitigating active pathogenic responses or participating in the contraction and resolution of an immune response [37, 38]. Globally, it is clear that Teffs and Tregs are responsive to similar agents (such as antigens and cytokines), but endogenous factors specific to the cell subtype and the local immune microenvironment influence the critical balance of these cells, determining an outcome of either tolerance or pathogenesis. For a therapy to be effective at preventing or reversing human T1DM, Tregs will need to be expanded or spared, while β cell-specific Teffs are deactivated or eliminated.

2. The natural history of Tregs in T1D

The non-obese diabetic (NOD) mouse is arguably the best animal model for T1DM developed to date. Many therapies that have been tested in humans have first shown efficacy in this model [39–43], and NOD mice remain the proving ground for new targets in T1DM. NOD mice develop spontaneous and progressive T-cell infiltration into the islets of Langerhans, resulting in insulitis [1]. The lag time (many weeks) between the initiation of insulitis and the development of overt diabetes in the NOD mouse is suggestive of a progressive breakdown in peripheral regulatory mechanisms, namely Treg cell function. It remains controversial, however, if there is a quantitative vs. qualitative defect in Tregs that predisposes to development of T1DM (reviewed in [44]). In some studies, Treg cell populations were shown to remain constant, if not increased, in affected lymphoid tissues prior to diabetes onset [38, 45]. Yet it is not uncommon in other T cell mediated conditions (i.e. organ rejection) to have an influx of Tregs at sites of inflammation. Nevertheless, the

persistent development of T1DM in these studies suggests that the Treg response is insufficient to control the marked Teff cell up-regulation. In part, this insufficient Treg response may be related to a decrease in Treg cell potency with age [38, 45, 46]. NOD mice with a genetically engineered disruption of co-stimulatory molecules (such as in CD40, CD28, and B7-1/B7-2) develop diabetes more quickly and at higher rates than wild-type NOD mice, suggesting that these molecules are critical for Treg cell generation and/or maintenance [47–49]. *CD28−/−* mice develop profound lymphoproliferative diseases, presumed secondary to a paucity of Treg cell numbers [50, 51]. Interestingly, *CD154−/−* NOD mice have normal levels of functional Tregs despite CD154 being one of the requisite costimulatory molecules for Teffs [8]. Knock-out models resulting in a reduction in Treg number or function do not clearly demonstrate how or where Tregs are adversely affected. For example, are Tregs affected during thymic generation, homeostatic peripheral maintenance, or during expansion to a specific antigen in pro-inflammation states?

Th17 cells are known to play a role in the pathogenesis of several autoimmune diseases, but a pathogenic role (if any) for Th17 cells in T1DM remains poorly understood. It appears that Th17 cells may act indirectly via their differentiation into Teffs [52, 53]. Th17 cells are also capable of differentiating into Tregs in a milieu rich in TGF-β and TNF [52–54]. Th17 cell function, as measured by IL-17 levels, is known to increase as T1DM progresses [55]. In fact, anti-IL-17 antibody delays the onset of T1DM in NOD mice, concordant with upregulation of Treg populations [56], indicating that Th17 cells play an important role in the Treg/Teff balance.

For at least two reasons, studies of Tregs in humans are more limited than in NOD mice. First, there is yet no marker that appears to be sufficiently reliable for identifying CD4+ Tregs in humans. CD25 is not a specific marker for activated Tregs, as activated Teff cells also transiently express CD25 [57], thereby making it difficult to discern Treg from Teff cell populations. Second, whereas in NOD mice it is straightforward to identify Treg populations relevant to T1DM by analysis of pancreatic lymph nodes or even islets directly, in humans this is not possible, and the interpretation of Treg populations collected in the peripheral blood is likely not analogous to the Treg cell number and function in the inflamed pancreas. Antigen-specific Treg cell assays would be valuable in discerning T1DM-specific Treg cell activity and in monitoring response to therapy, but there is currently no assay with adequate reproducibility [58]. Fortunately, the methylation profile of the *Foxp3* gene is specific to activated Tregs in humans, as activated Tregs show complete demethylation of the *Foxp3* promoter region compared to the partial methylation seen in activated non-Treg cells [59–61].

Despite the limitations inherent to Treg detection in humans, there have been several attempts to study Treg cell frequency and function in people with T1DM [62]. Individuals with T1DM appear to have normal Treg frequencies, but decreased functional responses as demonstrated by reduced levels of iTreg cell-secreted IL-10 [63]. Additionally, children with T1DM have a transient loss of Treg suppressive function (measured by incubation of CD4+CD25+ cells with anti-CD3 or anti-CD3/CD28) during the first 3–6 months from diagnosis with subsequent recovery to that of non-diabetic controls by nine months [64]. It remains largely unknown, however, what is occurring to the Treg/Teff balance in the years or months preceding diagnosis of T1DM in humans.

3. Defects in Treg cell function in T1DM

Based on the aforementioned studies in humans and NOD mice, there is an emerging consensus that Tregs exhibit reduced functionality (perhaps absolute, relative, or both) with respect to Teff cell suppression in T1DM. As described earlier, reduced Treg function results in an imbalance in the Treg/Teff response and a failure to maintain self-tolerance;

this favors the pro-inflammatory Teff response and the resultant Th1-mediated β-cell destruction. A reason for impaired Treg function in T1DM may lie in alterations in the crosstalk between Teffs and Tregs (Figure 1). Defective or decreased IL-2/IL-2R signaling may be one of the main culprits for the altered Treg/Teff cell balance. For example, NOD mice have reduced pancreatic IL-2 levels, conferring reduced stimulation of Tregs [65]. This decline in Treg cell function likely feeds the proliferation of auto-reactive Teff cells. Treg function and number may result from defective signaling from dendritic cells (DCs), but it is unclear if this affects the maintenance of basal homeostatic Tregs or antigen-specific activation of Tregs during a pro-inflammatory response [66]. As with mice, humans may be more prone to development of T1DM because of defective or immature DC signaling. Indeed, individuals with T1DM have decreased circulating DCs compared to healthy controls [67]. Recently, immature gut mucosal DCs in individuals with T1DM were found responsible for reduced differentiation of gastrointestinal CD4+CD25+Foxp3+ Tregs [68]. This defective *de novo* generation of iTregs by gut mucosa may adversely affect the Treg/Teff cell balance, leading to a failure of self-tolerance.

Similar to CD4+ Tregs, CD8+ Treg induction may be limited by impaired DC presentation. The induction of potent CD8+ Tregs requires activation by the heat shock protein Hsp60sp, which is bound to the MHC class II HLA-E. Many individuals with T1DM have CD8+ T cells defective in their ability to recognize HLA-E/Hsp60sp [69], offering a potential mechanism for T1DM pathogenesis. Interestingly, this CD8+ defect can be corrected by administration *in vitro* of autologous immature DCs loaded with Hsp60sp peptide [69].

Tregs as a therapeutic target in T1DM

Given that T1DM is a T cell mediated process, strategies to increase Treg cell number and/or function have been viewed as potential therapeutic approaches. In this respect, there is an ongoing clinical trial of intravenous infusions of autologous Tregs expanded *ex vivo* in individuals newly diagnosed with T1DM (currently enrolling, NTC01210664). Similarly, human cord blood stem cells, which are rich in Tregs and also may be capable of favoring differentiation of naïve T lymphocytes to Tregs, are an active area of research in diabetes [70, 71]. Given the expense and technical difficulty in infusing antigen-specific Tregs or harvesting cord blood stem cells, however, therapies that target endogenous Treg cell function are clearly desirable. The discussion below will focus on approaches to increase endogenous Treg cell production and/or function (summarized in Table).

1. Modification of dendritic cell function to induce Tregs

Treg cell maintenance during immune quiescence is linked to the direct interaction with DCs [72, 73], the only major APC subset involved in activating the T lymphocyte response to self-antigen [74, 75]. In the NOD mouse, Tregs expanded by antigen-loaded DC offered protection and restored euglycemia in overt diabetes [76]. Additionally, iTregs can be induced when naïve T cells are treated with β-islet antigen pulsed DC in a TGF-β-rich environment [77]. These iTregs have proven protective in preventing islet graft rejection, suggesting tolerogenic DCs may have a therapeutic role in the destructive autoimmunity of T1DM [76, 78]. G-CSF, a hematopoietic growth factor of the myeloid lineage, has also been shown to increase tolerogenic DCs and Tregs in the peripheral blood of healthy human subjects. In NOD mice, G-CSF caused recruitment of immature tolerogenic DCs, which subsequently led to recruitment of CD4+ Tregs [79, 80].

The complement system, which links the innate and adaptive immune response, is another potential therapeutic target in modifying DC function. Cognate T cell/DC signaling results in activation of the complement system, particularly C3a and C5a fragments, which is implicated in Th1-mediated autoimmune disease pathogenesis [81, 82]. Activated

complement fragments amplify the pro-inflammatory Th17 and Th1 response [82]. Conversely, inhibition of complement results in Treg expansion and function [83–85]. Interestingly, complement C3-deficient mice remain insulitis- and diabetes-free in response to STZ but do have an expanded TGF-β-dependent Treg population [83]. Complement appears requisite for diabetes development [86], perhaps by shifting the Treg/Teff balance towards pro-inflammatory Teffs.

2. IL-10 therapy

Tregs secrete high levels of IL-10, a potent anti-inflammatory cytokine that promotes Treg differentiation and function. IL-10 allows antigen-activated Tregs to suppress in a non-antigen-specific manner, permitting broader suppressive activity [22]. Clinically, IL-10-producing Tregs naturally regulate tolerance in the setting of bone marrow and solid organ transplantation, as the incidence of graft-versus-host disease is inversely related to endogenous IL-10 production [87–90]. In fact, it is this “non-specific” immune suppressive activity that may be of concern in clinical translation as alterations to the Teff/Treg balance may adversely affect critical and desirable protective T cell immune responses.

IL-10-secreting islet antigen-specific T cell responses, markers of Treg function, are present but reduced in individuals with T1DM compared to healthy, antibody-negative, controls [63, 91–93]. This deficit in Treg function is amplified by an increased Teff function, as reflected by increased antigen-specific IFN-γ secretion [63, 91, 92]. Higher frequencies of IL-10-secreting Tregs at diagnosis were associated with improved glycemic control at 3 months of disease [93].

As a therapeutic angle, IL-10 has been found to reduce diabetes development in the murine model through up-regulation of Tregs [94, 95]. IL-10 has also been found capable of inducing tolerogenic DCs. IL-10-conditioned dendritic cells are capable of delaying and preventing diabetes onset in NOD and HLA transgenic animal models, a protective effect also transferrable via adoptive transfer and associated with increased CD4+CD25+Foxp3+ Treg cell populations [96]. Interestingly, transgenic expression of IL-10 in the islet β cells of NOD mice actually caused acceleration of diabetes, suggesting over-expression may play a role in β cell stress [95, 97]. As such, there again appears to be a critical balance between promoting Treg number and function and increasing β cell antigenicity and destruction by Teffs. It is possible that unbridled Treg activity as a therapeutic intervention would likely be harmful; therefore, striking a delicate balance between Teff and Treg function remains the goal.

3. Approaches to increasing TGF-β

As discussed previously, TGF-β is critical in promoting Treg differentiation, expansion, and function. Therefore, therapies that increase TGF-β, especially early in the T cell response, may be beneficial in altering Treg/Teff cell interplay and mitigating β cell destruction. Supporting the valuable role for increased endogenous TGF-β is the finding of reduced TGF-β levels preceding diabetes development in NOD mice and in individuals with a positive family history [98, 99]. NOD mice genetically engineered to constitutively express islet-specific TGF-β from birth have reduced diabetes incidence [100]. TGF-β appears to alter APC signaling [101] and induce apoptosis of infiltrating lymphocytes [100]. Unfortunately, constitutive expression of TGF-β was associated with the undesirable outcome of extensive pancreatic fibrosis [100].

Dipeptidyl-peptidase IV (DPP-IV) inhibitors promote Treg development through their positive effects on endogenous TGF-β1. DPP-IV is a ubiquitous serine-type protease that degrades the incretin hormones of glucagon-like peptide-1 (GLP-1) and glucose-dependent

insulinotropic polypeptide (GIP). DPP-IV inhibitors prevent this degradation, thereby prolonging endogenous incretin activity. As reviewed by Drucker, incretin hormones also have direct β cell effects including increased insulin synthesis and secretion, as well as increased β cell mass by enhanced proliferation and suppressed apoptosis [102]. The ability of DPP-IV inhibitors to directly preserve β cell mass and function have made them an attractive therapy in T1DM. In fact, GLP-1 stimulation delays diabetes development in NOD mice [103, 104] with the effect magnified when the GLP-1 receptor agonist is combined with the immunosuppressant therapies, such as anti-CD3 monoclonal antibody [105].

Apart from these direct effects on the β cell, however, DPP-IV inhibitors are also immunomodulatory, a property perhaps more relevant to T1DM pathogenesis. Their immunomodulatory effects occur through CD26, a lymphocytic cell surface peptidase with intrinsic DPP-IV activity [106]. CD26 contributes to T cell development, maturation, and migration, as well as cytokine production and T cell-dependent antibody production (reviewed in [107]). As such, CD26/DPP-IV inhibition results in T cell and cytokine suppression and TGF-β up-regulation [108–110]. It is unclear if the enhanced TGF-β levels are a cause or effect of Teff cell suppression. Regardless, DPP-IV inhibition results in reversal of diabetes in NOD mice, albeit non-sustained with treatment cessation [111]. Diabetes remission in this study was associated with increased TGF-β levels and CD4+CD25+Foxp3+ T cells, especially in the inflamed pancreas [111]. Interestingly, re-occurrence of disease was paralleled by reductions in Treg populations and TGF-β1 levels. Currently, a clinical trial is underway using a DPP-IV inhibitor (sitagliptin) combined with a proton-pump inhibitor (lansoprazole) in children and adults with newly diagnosed T1DM (NCT01155284).

4. Anti-CD3 monoclonal antibody

Anti-CD3 monoclonal antibody (anti-CD3 mAb) has shown promise as a therapeutic approach in T1DM through its ability to directly block antigen-dependent T cell activation [112–114]. A short treatment course with anti-CD3 mAb resulted in diabetes remission in NOD mice [39, 40]. These findings in mice has led to human trials using two modified formations of the hOKT3 monoclonal antibodies, ChAglyCD3 (otelixizumab) and hOKT3γ1(Ala-Ala) (teplizumab). In children with newly diagnosed T1DM, a limited course of hOKT3γ1 attenuated the loss of insulin production in patients (as evidenced by reduced HbA1c and total daily insulin dose), but did not reverse the need for exogenous insulin [42, 115]. Similarly in a trial of ChAglyCD3, β cell function was improved and insulin requirement was lower in drug-treated recipients [116]. Some data suggests that in addition to depleting Teffs, such antiCD3 therapies enhance Treg numbers and perhaps function. Anti-CD3 mAb causes increased expression and function of human and murine CD4+CD25+Foxp3+ Tregs [41], but may have even more profound effects on CD8+ Tregs. hOKT3γ1(Ala-Ala) has been found to increase functional circulating iCD8+ Tregs in individuals with T1DM [117]. The induction of iCD8+ Tregs by hOKT3γ1(Ala-Ala) requires TNF-α, with action mediated by the NF-κB cascade [117]. It is possible that TNF-α secreted in the pro-inflammatory milieu of T1DM stimulates these potent CD8+ Tregs.

Recent large Phase 3 industry-supported trials, including DEFEND-2 (using ChAglyCD3 (otelixizumab)) [118] and the Protégé trial (hOKT3γ1(Ala-Ala) (teplizumab)) [119], in newly diagnosed T1DM failed to meet their primary endpoints. Similarly, a Phase 2 trial sponsored by the Immune Tolerance Network again showed that one course of hOKT3γ1(Ala-Ala) (teplizumab) slowed β cell decline [120], but there was no added benefit of a second course one year later despite more adverse events.

5. Manipulation of the IL-2/IL-2R pathway

IL-2, a cytokine secreted by activated Teff cells, binds to the constitutively active trimeric IL-2R (CD25) of Tregs. Through this IL-2/IL-2R interaction, IL-2 acts as an important stimulatory signal for the expansion and function of Tregs [33, 121, 122]. IL-2, and the resultant Treg expansion, is critical for maintenance of self-tolerance, as evidenced by the severe multi-organ autoimmune disease that develops in mice deficient in IL-2 or IL-2R [123, 124]. In both NOD mice and humans, expression of genes that contribute to aberrant IL-2/IL-2R signaling, such as *CD25* and *PTPN 2*, are associated with increased T1DM incidence [57, 65, 125]. In children with T1D, abnormal IL-2R signaling in the CD4+ T lymphocytes was found to contribute to decreased *Foxp3* expression and, therefore, a reduction in Tregs [126].

Given these observations, therapies that increase activity of the IL-2/IL-2R pathway, such as IL-2 agonists, may be helpful in preventing T1D. Low-dose IL-2 was found to increase Treg cell populations and largely prevented the development of T1DM in young NOD mice [65]. In overtly diabetic NOD mice, 60% developed long-lasting remission after receiving low-dose IL-2, associated with a concomitant increase in Tregs [38]. Diabetes remission was likely due to expansion of Treg activity, as Treg-deficient *CD28*$^{-/-}$ NOD mice did not have diabetes remission [37]. Additionally, there was increased expression of Treg-associated molecules (*CD25*, *Foxp3*, *CTLA-4*, and glucocorticoid-induced TNF receptor (*GITR*)). Notably, low-dose IL-2 treatment minimized the effect of IL-2 on NK and Teff cells, reducing the incidence of cytokine storm seen with higher dose IL-2 agonists [65, 127, 128]. A Phase 1 trial in newly diagnosed T1DM evaluating IL-2 and rapamycin supported by NIAID's Immune Tolerance network recently completed enrollment, with results pending (NCT00525889).

6. Rapamycin therapy

Rapamycin, a non-calcineurin inhibitor, blocks signaling in response to cytokines and favors differentiation of tolerogenic dendritic cells, perhaps indirectly resulting in increased Treg activity [129]. Rapamycin promotes the differentiation of functional Tregs when added to murine CD4+ lymphocytes *in vitro*. [130]. Likewise, administration of rapamycin *in vivo* prevents diabetes onset in NOD mice and allows for expansion of Tregs [131]. Unfortunately, a clinical trial of rapamycin in adults with T1DM did not alter the frequency or proliferation of Tregs [132]. Rapamycin may perform better as an adjunct when used in combination with IL-10 to increase Tregs [133].

7. GAD-alum

Glutamic acid decarboxylase 65 (GAD65) is a major autoantigen in T1DM [134]. In the NOD mouse, introduction of a GAD65 peptide results in immune tolerization and halts further T cell mediated β cell destruction [43]. Inherent to this tolerization, GAD-alum has been found to induce a GAD65-specific Treg response [135–139]. In Phase II clinical trials, alum formulated GAD (GAD-alum), given early in the course of T1DM, resulted in a modest preservation of residual insulin secretion, as reflected by increased fasting C-peptide levels [140]. Nevertheless, recent results from the phase 3 trial of GAD-alum did not show any impact on β cell loss in those with newly diagnosed T1DM. [141].

8. LFA3-Ig/Alefacept

Ideally, a therapeutic agent in T1DM would alter the Teff/Treg balance by targeting Teffs while sparing Tregs. The biologic agent alefacept, FDA-approved for the treatment of plaque psoriasis, now shows promise as an immunomodulatory therapy in T1DM. In psoriasis, alefacept results in prolonged clinical tolerance with remission lasting over 2 years

[142, 143]. Alefacept is a fusion protein containing the “head” of human-LFA3 and an IgG “tail” [144, 145]. In humans, LFA3 is found on a variety of antigen presenting cells, and interacts with its cognate ligand *CD2* on T cells for adhesion and costimulation. Alefacept appears to both interfere with T cell activation and facilitates NK cell-mediated deletion of T cells [145, 146]. During studies of alefacept in psoriasis, it was recognized that naïve T cells (Tn; *i.e.*, CD3+CD45RO−, CCR7+) were relatively spared, central memory cells (Tcm; *i.e.*, CD3+CD45RO+, CCR7+) were depleted intermediately, and effector memory T cells (Tem; *i.e.*, CD3+CD45RO+CCR7−) were depleted to the greatest extent [147, 148]. Although *CD2* is expressed on all T cells, the highest expression is on effector memory T cells and lowest on naïve T cells. Recent data from non-human primates suggest that highly activated and “armed” effector memory T cells involved with active immunopathogenic responses express the highest levels of *CD2* [149]. This suggests that T cells with the highest level of expression of *CD2* are most susceptible to alefacept. In preliminary studies, *CD2* expression on human Tregs is at low levels, nearly equivalent to those of naïve T cells (Rigby, MR personal communication). It is thus plausible that like naïve T cells, Tregs would be relatively spared by alefacept.

Currently, there is an actively enrolling NIAID/Immune Tolerance Network clinical trial (T1DAL, or Type 1 Diabetes with Alefacept) evaluating if alefacept can induce remission in new onset T1DM (NCT00965458). This agent may fit the “model” profile of an immunotherapy in T1DM by targeting the most highly pathogenic diabetes-causing Teffs, while leaving both the naïve protective T cell repertoire and Tregs intact. Thus, alefacept may be a prime example of how to tilt the Teff/Treg balance to favor β cell protection. The development of similar specific therapeutic agents will be facilitated by advances in understanding of the unique surface makers, activation requirements, and unique pathways utilized by both Tregs and Teffs.

Conclusion

To date, no immunomodulatory therapeutic trial has induced long-lasting or significant β cell protection in humans with T1D. Despite a number of clinical trials and decades of research, no clinically beneficial effect is seen or a period of β cell protection is followed by subsequent rate of decline in β cell function equal to that of controls. In no studies had insulin freedom been achieved, yet some studies suggest better glycemic control with early immunomodulatory intervention. The long-term impact for mild successes (i.e. antiCD3) remains debatable. The reasons for this lack of success are unclear, but perhaps therapeutic interventions geared to reduce the Teff cell response also adversely affects Treg function. For example, therapies that block the requisite costimulatory signals may also inhibit Treg differentiation and proliferation. Recently, a cytotoxic T lymphocyte associated molecule-4 (CTLA-4) antagonist, which blocks the CD28:B7 costimulatory interaction, failed to provide long-lasting β-cell protection in recently diagnosed individuals with T1DM [150] This study was performed in spite of preclinical data suggesting that CTLA4Ig therapies affect active pathogenic Teff function little, and affected Treg activation little [9] (Rigby MR, unpublished data}. In addition, the interdependence between Tregs and Teff cells [37] may mean that any therapy that blocks Teff cell stimulation and expansion also leads to reduced Treg population and function. Ideally, immunomodulation in T1DM would specifically inhibit Teff activation while also preserving or even promoting Treg function. The currently ongoing T1DAL trial is investigating that premise. In the evolving field of T cell biology, it is becoming clear that Teff and Tregs are not mutually exclusive and there is a significant interdependence their numbers and function. In T1DM, the resultant balance for a number of processes acting in concert will promote self-tolerance and protecting the β cells or shift to facilitating β cell destruction and T1DM. Therefore, the goal of restoring Teff/Treg homeostasis remains an exciting and novel approach to T1DM management.

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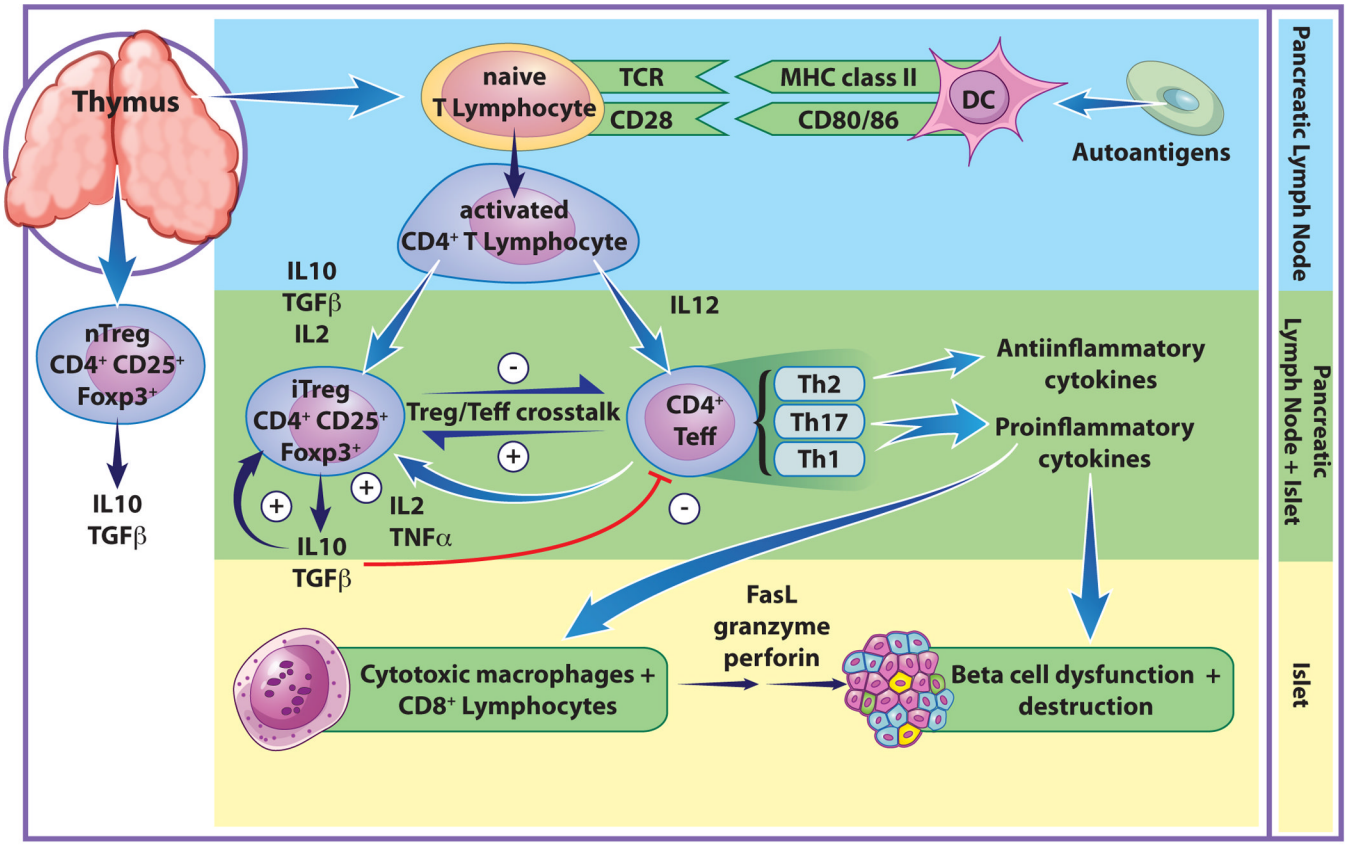

Figure. Pathogenesis of type 1 diabetes

The figure shows the general events from antigen presentation at the pancreatic lymph node (top) to immune cell-mediated destruction of beta cells (bottom), focusing on key events and cell types involved in the processes. Details are provided in the text. MHC class II – major histocompatibility complex class II, DC – dendritic cell, TCR – T cell receptor, IL10 – interleukin-10, TGFβ – transforming growth factor-beta, IL2 – interleukin-2, TNFα – tumor necrosis factor-alpha, iTreg – induced regulatory T cell, nTreg – natural regulatory T cell, Teff – effector T cells, FasL – Fas ligand, Th2 – T helper lymphocyte 2, Th17 – T helper lymphocyte 17, Th1 – T helper lymphocyte 1.

Table

Therapeutic approaches to increase endogenous regulatory T cell populations and function as demonstrated by murine studies and clinical trials

		Reference	
		Murine Studies	**Human Studies**
B lymphocyte blockade			
	Anti-CD20 mAb		[151]
Enhanced Treg differentiation			
	Foxp3 demethylation	[152]	
Improved Treg activation			
Dendritic cell presentation			
	G-CSF	[79, 80]	NCT01106157 NCT00662519
	Antigen-loaded DC	[76, 78]	NCT00445913
	Flt3	[153–155]	
	Rapamycin	[129, 130]	[131–133] NCT00525889
	GAD-alum	[43, 135–137]	[138–140]
	Complement inhibition	[83]	
Co-stimulatory molecules			
	CD28 and/or *CD154* antagonism	[9]	
Increased Treg proliferation/expansion			
	DPP-IV inhibitors	[103–105, 111]	NCT01155284
	IL-10 agonists	[94–96]	
	IL-17 antibody	[56]	
Improved Teff-Treg interaction			
	IL-2 agonists	[38, 65]	NCT00525889
	Anti-CD3 mAb		[151]
Selective targeting of highly diabetogenic Teffs while sparing Tregs			
	LFA3Ig (putative)		NCT00965458