The Role of T regulatory Cells (Tregs) in the Development and Prevention of Type 1 Diabetes

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Abstract

In this article, we review the role of T regulatory cells (Tregs) in the development and prevention of type 1 diabetes. We first examine the definition of human Tregs, the generation of Tregs in the thymus and the periphery, their mode of action and their important role in the regulation of the immune response. We then examine the defects in Tregs observed thus far in type 1 diabetes and their role in the development of the disease. Finally, we point to possible clinical applications using Tregs as a therapeutic target for the prevention of type 1 diabetes.

Keywords: Tregs; Type 1 diabetes; Autoimmune regulator; Autoimmune diseases

Abbreviations: AIRE: Autoimmune Regulator; APECED: Autoimmune Polyendocrinopathy Candidiasis Ectodermal Dys trophy; APS1: Autoimmune Polyendocrine Syndrome 1; IPEX Syndrome; Immune Deficiency-Polyendocrinopathy-Enteropathy-X-linked Syndrome; Teffs: CD4+ T effector Cells; Tregs: CD4+ T regulatory Cells

Introduction

Type 1 diabetes results from autoimmune self-destruction of the pancreatic β cells leading to absolute insulin deficiency and requiring life-long insulin treatment. This autoimmune reaction is triggered by the environmental factors in genetically predisposed individuals. Although recent knowledge has contributed to our understanding of the autoimmune pathogenesis of type 1 diabetes, there remains no unifying theory of disease causation. However, it is accepted that autoimmune disease in general results from the dysregulation of the basic processes designed to maintain self tolerance [1,2]. In the few cases where it has been possible to examine the endocrine pancreas of newly-diagnosed type 1 diabetes patients, massive infiltration of mostly CD8+ T lymphocytes was recorded in insulin-containing islets, but not in islets devoid of insulin. CD4+ T cells, monocytes and B lymphocytes were also found in decreasing order [3-6].

Over the past few years, there has been a steadily increasing interest in regulatory T lymphocytes (Tregs) that exhibit several of the properties of the previously studied and so-called suppressor T cells [7,8]. In this review we will examine the generation of Tregs in the thymus and the periphery, their mode of action and importance in the regulation of the immune response. We will then examine the defects in Tregs observed thus far in type 1 diabetes and their role in the development of the disease. Finally, we will point to a few developments that may lead to possible therapeutic applications using Tregs as a therapeutic target for the prevention of type 1 diabetes.

The Definition of Tregs

Nearly 40 years ago immunologists postulated the concept of regulation of the immune response by T suppressor lymphocytes [9]. This was followed by a flurry of activity, identifying several phenotypic markers for the various cell types involved in the suppression of excessive immune responsiveness (CD8+ suppressor effector cells, CD4+ suppressor-inducer cells etc.), as well as several secreted suppressor factors [10,11]. The whole concept eventually fell into disrepute, mostly because of the lack of reproducible assays for these cells and lack of molecular identification of the factors involved [11,12]. The rebirth of the regulatory-suppressor cell originated from the seminal observation that thymectomy of neonatal mice on day 3 resulted in autoimmune gastritis, which could be corrected by transfusion of syngenic CD4+CD25+ T cells, but not their CD4+CD25- counterparts [7].

The phenotypic definition of human Tregs is still under discussion, and has been under continuous evolution. CD4+ Treg cells have been most intensively studied. Nowadays, various phenotype markers are used not only to distinguish Tregs from other CD4+ cells, but also to identify functional (sub) classes of Tregs. The high constitutive surface expression of the IL-2 receptor alpha chain (CD25) is generally considered as a characteristic feature of the vast majority of human Tregs and regulatory activity is enriched in CD4+CD25hi T cells [13-15]. Upon activation of T cells, independently of their regulatory capacity, CD25 can become up-regulated and highly expressed on Teffs as well. This puzzle of distinguishing between bona-fide Tregs and recently activated Teffs has been solved by a very effective utilization of the CD45RA marker (below). A considerable number of other surface markers have been reported to be expressed on human Tregs, such as CTLA-4 (CD152), L-selectin (CD62L), glucocorticoid-induced tumour necrosis factor receptor (GITR), TGF-β, CD95 and PD-L1 [16,17]. Recent studies have demonstrated that down-regulation of the IL-7 receptor α-chain (CD127) distinguishes Treg cells from activated T cells, facilitating the functional characterization of a more representative population [18].

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Intracellular expression of the FoxP3 transcription factor is the hallmark of Tregs, as the presence of this protein is necessary for their development. FoxP3 is a member of the forkhead or winged helix family of proteins and the respective gene is located on chromosome X [19]. FoxP3-mutant mice have the scurfy phenotype, characterized by massive lymphoproliferation, autoimmunity and death in the second to third month of life. In humans, mutations in the FoxP3 gene lead to the IPEX (Immune deficiency-polyendocrinopathy-enteropathy-X-linked) syndrome. This is characterized by total absence of Tregs, food allergy, enteropathy, eczema and polyendocrinopathy, including neonatal type 1 diabetes and less often autoimmune thyroid disease [20,21]. The FoxP3 protein is also transiently expressed in human activated T effector cells (Teffs), albeit at a lower protein level than in Tregs. However, this transient expression does not confer any regulatory properties on such Teffs. Furthermore, in Tregs the region upstream of the FoxP3 gene is completely demethylated, an indication of persistent and sustained expression of this master switch by Tregs. In contrast, this region is found to be methylated in Teffs. Therefore, DNA demethylation of the 5’ upstream region and the STAT-5 responsive element in the human FoxP3 locus can discriminate Tregs from conventional Teffs, even if the latter transiently express FoxP3 [22-24]. A recent detailed analysis showed that FoxP3+ CD4 T cells are composed of three phenotypically and functionally distinct subpopulations, depending on the expression of the CD45RA molecule: CD25++CD45RA+FoxP3+CD127-/low resting Treg (rTregs) cells (with high intracellular expression of FoxP3, and the low or no expression of CD127 (CD4+CD25highFoxP3+CD127low/- cells). For several tissue-specific proteins their transcription in the thymus is under the control of the transcription factor Autoimmune Regulator (AIRE). Patients with mutations in this gene show defective expression of tissue-specific self-antigens in thymus, leading to autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED), or autoimmune polyendocrine syndrome 1 (APS1) [34]. Such patients have defective suppressive function of their Tregs, most probably due to the significantly decreased expression of FoxP3 protein in these cells, as compared to controls [35].

**Generation of Tregs**

Tregs are generally classified into two categories, natural Tregs (nTregs) and adaptive or induced Tregs (iTregs). Natural Tregs primarily emerge from the thymus, whereas iTregs are generated in the periphery from naive T cells after antigen exposure [25]. Both T cell subsets share a similar phenotype, express intracellularly the transcription factor FoxP3 and possess suppressive capacity. Very recently, it has been shown that nTregs selectively express Helios, an Ikaros-family transcription factor [26]. For several tissue-specific proteins their transcription in the thymus is under the control of the transcription factor Autoimmune Regulator (AIRE). Patients with mutations in this gene show defective expression of tissue-specific self-antigens in thymus, leading to autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED), or autoimmune polyendocrine syndrome 1 (APS1) [34]. Such patients have defective suppressive function of their Tregs, most probably due to the significantly decreased expression of FoxP3 protein in these cells, as compared to controls [35].

Induced Tregs arise from CD4+CD25 precursor cells in peripheral lymphoid organs [32]. It is possible that in the periphery iTregs may develop probably from recent thymic CD4+ T cell emigrants that have high affinity for MHC II+ self-antigens, yet have escaped selection [36,37]. Certainly, dendritic cells synthesize and present self-antigen (including all of the major auto-antigens for type 1 diabetes) to CD4+ T cells [38] and under certain circumstances can be tolerogenic [37], leading to the induction of Tregs, yet the specific mechanisms are under debate. There is now considerable evidence that IL-2 and transforming growth factor (TGF)-β are required for the preservation of iTregs, and that both factors are needed by nTregs and iTregs for the induction and continuous expression of FoxP3 [39-41].
The Functional Role of Tregs

Tregs control the reactivity of self-reactive T effector cells that are not eliminated in the thymus and are thus responsible for maintaining peripheral self tolerance and immunological homeostasis. Initially, Tregs can control T cell activation, expansion and proliferation during lymph node priming. At this stage, Tregs colocalize with dendritic cells at the medullary-cortical junction at the T cell-B cell borders within the proximal lymph nodes [42]. In addition, Tregs can traffic to the site of inflammation and suppress the effector functions of immunocytes within the affected tissue [43].

The mechanisms used by Tregs to suppress immune responses are still unresolved, yet the prevailing view is that cell contact between Treg and Teff is obligatory [12-17,26-29]. This however, does not prevent subsequent bystander suppression as well, in the milieu generated by the thus activated Tregs [25,42]. In general, the activation of Tregs in vivo follows that of Teffs, and while capable of division, their functional programme consists of deactivation of the pathways found in Teffs, and up-regulation of pathways accumulating suppressive molecules that may interact with Tregs are depicted as well as most candidate interaction molecules. It is now evident that DCs are very important for the maintenance of the regulatory function of Tregs [39,40], and there is evidence that CD8 T regulatory cells as well as B regulatory cells may play a role in the immune circuit [103,104]; neither population is shown here.

For another possible pathway, the transcription programme of Tregs includes diminution of TCR-induced downstream signaling and maintenance of a suppressive phenotype [32,33,47]. Specifically, the gene for the cAMP degrading enzyme phosphodiesterase 4 (pde4) is suppressed, while that of IL-7Ra is downregulated [31-33]. This leads to a considerable build-up of cAMP in Tregs, and up-regulation of pathways accumulating suppressive molecules in the cytoplasm, on their cell membrane and extracellularly (cAMP, cGMP, IL-2, IL-2Rαβγ [31,32,34,44] (Figure 2). In vitro activated human Tregs may directly kill activated CD4+ and CD8+ T cells in a perforin- or granzyme-dependent manner [45]. Although evidence for such cytotoxicity is lacking in vivo, patients with mutations in the perforin gene suffer from haemophagocytic lymphohistiocytosis (HLH), indicating a key involvement of perforin in immune regulation, perhaps via Tregs [46].

For another possible pathway, the transcription programme of Tregs includes diminution of TCR-induced downstream signaling and maintenance of a suppressive phenotype [32,33,47]. Specifically, the gene for the cAMP degrading enzyme phosphodiesterase 4 (pde4) is suppressed, while that of IL-7Ra is downregulated [31-33]. This leads to a considerable build-up of cAMP in Tregs. Upon proper Teff contact, Tregs establish communication with Teffs via gap junctions, transferring cAMP and rendering the latter cells inactive [48]. In addition, the CD39 and CD73 ectonucleases on the surface of Tregs use extracellular ATP to generate adenosine, which in turn activates the suppressive adenosine receptors on neighboring Teff cells [49]. CTLA-4, a membrane molecule whose gene locus is already linked to type 1 diabetes and autoimmunity (IDDM12) [50], binds with 20X higher affinity than CD28 to CD80/CD86 (B7 family) receptors, located on antigen-presenting cells (APCs) [3]. The CD28-CD80/86 interaction may function as the second signal to TCR—MHC II-peptide recognition. Thus, CTLA-4+ Tregs by tightly binding to CD80/86 receptors on APCs block the APC-Teff interaction necessary for activation and also send negative signals, preventing such activation. It has recently been shown that TGFβ, in its immature or mature form, is found on the surface of human Treg cells, bound to the membrane protein GARP (Glycoprotein A-repetitions predominant protein) [51]. There, immature TGFβ may be converted to its mature form by a variety of proteins, such as furin, thrombospordin and certain Arg-Gly-Asp—recognizing integrins [52,53]. The importance of TGFβ to the generation and maintenance of Tregs has long been demonstrated, and with this finding another potential mechanism of action of Tregs is revealed (Figure 2). The role of HLA-DR on the surface of CD4+CD25+ Tregs is worth mentioning, as it is the HLA-DR+ fraction of such cells that exhibits the most potent regulatory activity [54]. As in most autoimmune diseases, specific HLA-DR/DQ alleles are associated with susceptibility to type 1 diabetes [55].

Findings for Tregs in Type 1 Diabetes

Experimental animal studies

In the NOD mouse model of type 1 diabetes various defects have been noted in the Treg (CD4+CD25+) compartment. It appears that such cells are defective in suppressing the proliferation of Teff cells [56,57]. Remarkably, Tregs from 4 weeks old NOD mice are capable of suppressing T cell proliferation, yet Teffs from older NOD mice are refractory to such suppression [58]. Tregs can affect Teffs at several levels (proximal lymph nodes, sites of tissue inflammation), by controlling T cell trafficking to tissues as well as their reactivation whenever the first line of protection in the draining lymph node fails [42,43,58], and the islet micro-environment takes on characteristics of the lymphoid system [59]. Experimental studies in mice have shown that diabetes progression depends on a delicate balance between effector Tγδ cells and Tregs in the pancreatic lymph nodes and within the inflamed pancreas [60,61]. After the onset of diabetes, autoimmunity progresses as the ratio between effector Tγδ cells and Tregs within the inflamed pancreas continuously increases [62]. On the other hand, TGF-β may also induce Tregs directly through the induction of FoxP3 and/or Treg proliferation, even at the site of tissue damage [41]. Interestingly, a transient pulse of TGF-β in islet cells of NOD mice during the priming phase of diabetes is sufficient to inhibit disease onset and stimulate expansion of intra-islet Tregs [63].

In many experimental models for type 1 diabetes immune tolerance was obtained following treatment with immunosuppressants, including old studies with polyclonal anti- T cell antibodies [64,65] or monoclonal antibodies targeting specific receptors or pathways (such as CD3, CD4, CD8, ICOS, CTLA-4g etc) [66-69]. In most of these cases the immune tolerance relied on the expansion of Tregs rather than on deletion or anergy of effector T cells [69]. Also, in studies with NOD mice where possible β-cell autoantigens were administrated, it was pointed again the major role of Tregs in the induction of self tolerance, even the nature of Tregs was not very precise [70,71]. Long-term survival of pancreatic islet allografts induced by a soluble fusion protein composed of CTLA-4-Ig in mice depends on toleropathyc catabolism.
by dendritic cells, via the enzyme indolamine 2, 3–dioxygenase (IDO) [72,73], a known participant in the tolerogenic and Treg-inducing function of immature dendritic cells. Of great interest, CD4+CD25+ Tregs that adoptively transferred in NOD mice, effectively prevented or even reversed the disease [74].

**Human studies**

Unfortunately studies of Tregs in type 1 diabetes suffered from the lack of an acceptable criterion for the definition of Tregs. The first work to deal with Tregs in human type 1 diabetes showed a significantly decreased percentage of the CD4+CD25+ T cell fraction in young newly-diagnosed patients compared to older controls [75]. Subsequent works in newly-diagnosed patients as well as in patients with long-standing diabetes could not find such differences, even when distinguishing between CD4+CD25+ (activated Teffs) and CD4+CD25high (Tregs); some of these works found a deceased regulatory function in type 1 diabetes patients, while others did not. As there is a continuum of CD25 intensities from CD25+ to CD25+++ (CD25high), the distinction between these two populations was of necessity artificial. The Treg definition and separation outlined in [24], which is subsequent to all works estimating the percent of Tregs in type 1 diabetes patients, effectively solves this problem. Remarkably, the average values for these percentages differed considerably from one study to the other [76-78], with one study showing that there was an age-dependent increase in Tregs in controls [78]. Each of these works used essentially a different definition of Tregs. In a mini-meta-analysis comparing these studies, the differences in testing for suppressor activity were pointed out and it was recommended that expression of FoxP3 should be used as a criterion, even though activated Teffs also express transiently lower levels of FoxP3 [79]. Yet, another study that grouped together all FoxP3-expressing cells (i.e. including those Teffs transiently expressing the protein), reported no differences in the frequency of such cells between type 1 diabetes patients and controls [80]. A different group showed that CD4+CD25high Tregs from newly-diagnosed type 1 diabetes patients and autoantibody-positive at risk subjects had a higher tendency for apoptosis, compared to such cells from age-matched controls or long-standing type 1 diabetes patients [81]. Two subsequent studies also pointed to refractoriness of Teff cells to the action of Tregs as one reason for the defective regulatory function observed in type 1 diabetes patients [82,83]. Interestingly, Tregs in one of these works [82], appear as CD4+CD25high cells, as also documented in a just published study by the same group and by us in a previous communication [84]. There has been no study enumerating Tregs after the seminal work by Miyara et al. in which issues regarding Foxp3 expression by Teffs and Tregs were settled, and active and resting Tregs were unequivocally defined [24]. Importantly, a histological study of pancreases from persons who died of type 1 diabetes as long as 6 months after clinical disease onset reported that FoxP3+ cells were rarely found in CD4+ T cell-infiltrated insulin-expressing islets, suggesting an inadequate presence of these cells at the site of inflammation and autoimmune attack [85].

A most interesting development has been the remarkable result of about 50% lower insulin requirement and higher C-peptide, found in responding type 1 diabetes patients, 4 years after brief anti-CD3 (Otelixizumab®) treatment upon diagnosis [86]. These responders started with an initially higher C-peptide level. A thorough investigation of the effect of this treatment on T cells did not reveal any preferential sparing of any category of T cells, even though in an abstract it was claimed that the antibody had a sparing effect on Tregs [87,88]. As this is the most promising immune intervention in type 1 diabetes thus far, it deserves further attention, especially regarding the possible enhancement of Treg function and/or percent of cells.

While several studies have mapped all the genes associated with the pathogenesis of type 1 diabetes, it has been very difficult thus far to decipher a possible mechanism of action for any of them that would include their role in the generation and function of Tregs. Tregs from type 1 diabetes patients have been shown to be defective in their IL-2R signaling, compared to controls [89]. Just recently, a detailed study has shown that polymorphisms in the CD25 gene associated with susceptibility or with resistance to type 1 diabetes, could be linked to the level of expression of CD25 on Tregs and Teffs. Indeed using healthy controls it was shown that the disease-susceptible SNIPS were associated with significantly lower levels of expression of CD25 in aTregs, rTregs, and Teffs, and diminished IL-2 responsiveness in antigen-expressing CD4 T cells, and also associated with lower FoxP3 levels and lower levels of suppression of the proliferation of autologous Teffs [90]. These two studies make physiological sense, because Tregs cannot synthesize IL-2, rather they may obtain it from activated Teffs, after the latter have satisfied their own needs in the cytokine [43]. A lower level of CD25 in the membrane of Tregs would mean less efficient capture of IL-2 by the IL-2Rαβγc complex [91].

Also of interest for type 1 diabetes, is the observed emergence of host Tregs specific for the grafted tissue, and donor Tregs, specific for components of the host in transplantations [92]. There are no reports regarding Tregs after islet transplantation in humans, but the implications are obvious. Apparently, cyclosporine suppresses induced Treg generation from Teffs, while rapamycin supports it [93]. Interestingly, bone marrow transplant for correction of the IPEX syndrome in young males (whose symptoms included type 1 diabetes) resulted either in cure of type 1 diabetes via normal insulin secretion, and elimination of GAD antibody levels (4-month old child) or in a diminution of GADA levels and the daily insulin dose in another patient (1.5 years old); both patients evidenced appearance of sufficient Tregs after engraftment, that restored proper immune function [94,95].

**Prospects in the Prevention of Type 1 Diabetes**

Tregs are now well established as a new tool, not only for understanding type 1 diabetes pathogenesis, but also for giving new prospects in the prevention and treatment of the disease.

The defects of Tregs found in type 1 diabetes patients explain the loss of immune tolerance in these patients [75-83]. As the pathways for suppression by Tregs are elucidated and the roles of different molecules already found in Tregs become clearer, our understanding of their role in type 1 diabetes is expected to increase. The fact that bone marrow transplantation is accompanied by the appearance of functional Tregs at levels comparable to those in controls [94,95] offers hope of inducing tolerance via re-induction of a proper Treg repertoire. Methods for ex-vivo large scale production of antigen-non-specific or antigen-specific Tregs are already in place [96,97]; these could become a good starting place for the lasting blockade of β-cell destruction and/or successful islet transplantation, with optimized sorting strategies that could dramatically improve the isolation of highly potent Tregs [98]. The use of autologous Tregs cultured ex-vivo could of course lead to a re-appearance of disease after a temporary relapse, in a fashion that may be a re-enactment of what has been observed in a number of idiopathic juvenile rheumatoid arthritis patients, considering that type 1 diabetes has a very potent immune memory [99,100].
The journey towards prevention and cure of type 1 diabetes has been a long one and Tregs promise of being part of the solution [101]. The recent discovery of a transposition genetic element that is essential prior to their introduction into patients. After all, we are still watching for possible application in many autoimmune diseases [102]. Of course, the transition of such cell therapies from animal studies to human clinical trials is a real challenge and knowledge of the purity and stability of cell therapy products is essential prior to their introduction into patients. After all, we are still under the Hippocratic dictum of doing no harm.

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References


The effector T cells of diabetic subjects are resistant to regulation via CD4+ FOXP3+ regulatory T cells. J Immunol 181: 7350-7355.


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