Mini Review

Regulatory T-Cells in Treatment of Type-1 Diabetes: Types and Approaches

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ABSTRACT

Regulatory T-cells (Tregs) play important role in regulation of immune responses to self-antigens. Alterations in frequency and function of Tregs have been reported in Type 1 Diabetes (T1D) subjects. Tregs have the potential to prevent destruction of pancreatic beta cells by targeting effector T-cells (Teff) and other immune cells causing inflammation. Therefore, strategies to increase Treg cell numbers and function are being explored as potential immunotherapeutic approaches in treating T1D. Today, several groups are involved in exploring different Treg cell types, sources, induction procedures and experimental systems in pursuit of generation of highly efficacious and stable Tregs for their clinical applications. Various protocols have been developed for the induction and expansion of islet antigen specific Tregs and polyclonal Tregs. Studies have shown that antigen specific Tregs are required at less number and are more efficient than polyclonal Tregs in suppressing autoimmune diabetes and they do not cause generalized immune suppression. Alternatively, generation of colonic Tregs (cTregs) has also gathered attention in recent years as an approach to limit pancreatic inflammation via gut induced tolerance. With a definitive treatment for T1D still elusive, application of Tregs as a part of combination therapy seems promising in treatment of T1D.

KEYWORDS: Type-1 Diabetes; Regulatory T-cells; Autoimmune diabetes; Immune suppression; Antigen specific.

ABBREVIATIONS: Tregs: regulatory T-cells; cTregs: colonic Tregs; Teff: effector T-cells; T1D: Type 1 Diabetes; CTL: Cytotoxic T Lymphocyte; T_H: T Helper; GITR: Glucocorticoid-induced TNF- receptor; APC: Antigen Presenting Cell; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; Nrp-1: Neurphilin 1; FoxP3: Forkhead box P3; PLN: Pancreatic Lymph Nodes; DCs: Dendritic Cells; GALT: Gut Associated Lymphoid Tissue; GILZ: Glucocorticoid-induced leucine zipper; TT: Tetanus Toxoid; ATRA: All Trans Retinoic Acid; nTregs: natural Tregs; iTregs: induced Tregs; ASF: Altered Schaedler Flora; TSDR: Treg Specific Demethylated Region; PDL1: Programmed Death Ligand 1; fHASC: human amniotic fluid stem cells; IFA: Incomplete Freund’s Adjuvant; LAP: Latency- associated peptide; GARP: Glycoprotein A Repetition Predominant; PSA: Polysaccharide A; MLN: Mesenteric Lymph Nodes; SCFA: Short Chain Fatty Acids; Aldh1a: Aldehyde dehydrogenase; Dapl1: death-associated protein like 1; Igfbp4: insulin-like growth factor binding protein.

INTRODUCTION

Type 1 Diabetes (T1D) is mainly a T-cell mediated autoimmune disease characterized by the destruction of pancreatic beta (β) cells leading to insulin deficiency. Regardless of the predisposing factors and environmental triggers, the main pathogenic mechanism leading to T1D is the priming of CD8+ T-cells by the autoreactive CD4+ T-cells. These CD8+ T-cells further recognize and destroy pancreatic β cells by releasing cytoxic granules mainly containing granzymes and perforin molecule.1 Such autoreactive CD8+ T-cells can be easily detected from the peripheral blood of T1D subjects, as they are more differentiated and express central memory markers.2,4 In healthy individuals these autoreactive T-cells are either
eliminated in thymus or suppressed by regulatory T-cells (Tregs) in the peripheral circulation.

**Regulatory T-cells**

These cells also called as suppressor T-cells, are a subpopulation of T-cells that play an important role in regulation of exaggerated immune response to self/foreign antigens. They are important in induction and maintenance of self-tolerance. They comprise 1-10% of the T Helper (T_h) cell population in healthy adult humans and mice. These cells express high levels of surface marker CD25, Forkhead box P3 (FoxP3) along with low CD127 which together have been suggested as reliable markers for Tregs. Tregs have the capacity to actively block immune responses, inflammation and tissue destruction by suppressing the functions of various cell types and processes, including classical T_h cells, B-cell antibody production, affinity maturation, CD8+ Cytotoxic T Lymphocyte (CTL) granule release and Antigen Presenting Cell (APC) function and maturation state. Tregs mediate their role mainly by four mechanisms including: 1) production of suppressive cytokines, 2) direct cytolytic activity, 3) cytokine (IL-2) deprivation, and 4) cell contact-induced cell modulation.

Based on acquisition of CD25, Tregs can be divided into two subsets: natural Treg (nTreg) cells and adaptive or induced Treg (iTreg) cells. nTregs acquire expression of CD25 in thymus whereas iTregs acquire CD25 expression in the periphery. However, utility of CD25 as a marker of Tregs is limited because of its expression on activated T-cells as well. iTregs are generated extra-thymically and IL-2 is essential for their generation both in vitro and in vivo. Tr1 and Th3 cells represent other subsets of suppressor T-cells. Tr1 cells do not express FoxP3, but produce high level of immunosuppressive cytokine, IL-10 whereas Th3 cells produce TGF-β, which also has immunosuppressive role. Phenotypically, it is difficult to differentiate nTregs from iTregs as both subsets have similar characteristics and suppressive function. Both Treg subsets express CD25, FoxP3, Glucocorticoid-induced TNF- receptor (GITR) and Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) whereas, nTregs exhibit a higher expression of Programmed cell death-1 (PD-1), Neopterin 1 (Nrp-1) and Helios compared to iTregs. It has been reported that nTregs are generated when there is a need to control inflammatory responses to autoantigens, whereas iTregs are generated in response to stimulation with foreign antigens such as intestinal flora and food allergens.

### Table 1: Characteristic features of natural and induced Tregs.

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<thead>
<tr>
<th>Characteristics</th>
<th>Natural Tregs</th>
<th>Induced Tregs</th>
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<tbody>
<tr>
<td><strong>Site of induction</strong></td>
<td>Thymus</td>
<td>Secondary lymphoid organs, inflamed tissues</td>
</tr>
<tr>
<td><strong>Co stimulation requirement</strong></td>
<td>CD27,28</td>
<td>CD28,29</td>
</tr>
<tr>
<td><strong>Cytokines requirement</strong></td>
<td>IL-2,28,29</td>
<td>TGF-β,29 IL-2</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>Predominantly self-antigens21,23</td>
<td>Intestinal flora and environmental, food allergens</td>
</tr>
<tr>
<td><strong>Common markers</strong></td>
<td>CD25, FoxP3, GITR and CTLA4,CD127 low</td>
<td>CD25, FoxP3, GITR and CTLA4, CD127 low</td>
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<tr>
<td><strong>Specific Markers</strong></td>
<td>Higher expression of PD1,22 neuropilin 1,21,23 Helios26,27 and CD7328</td>
<td>Dap1, Igbp4,27,35</td>
</tr>
<tr>
<td><strong>Methylation status of TSDR of FoxP3 promoter</strong></td>
<td>Demethylated/low TSDR methylation26,42</td>
<td>Intermediate TSDR methylation26</td>
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</tbody>
</table>

### Alteration in the Frequency and Function of Tregs in T1D

Several groups have reported alterations in the frequency, function and phenotype of Tregs in patients with T1D. Subjects with T1D may harbor lower frequency of Tregs in the peripheral blood. Ryba-Stanislawowska, et al. showed that patients with T1D had a decreased percentage of circulating CD4+CD25hi Tregs and elevated levels of serum IL-12 and IL-18 in comparison to their healthy controls. However, a few studies have also reported no alteration in the frequency of the Tregs in peripheral blood of T1D subjects. A recent study by Xufré, et al. reported that the frequency of peripheral CD4+CD25hi Treg cells are similar between T1D subjects and healthy controls. However, the yield of sorted Treg cells was found to be significantly lower in T1D subjects than in controls. Again, upon comparison of Treg cell phenotype between the two groups, the only difference observed was the low expression of GITR in T1D subjects. Zoka, et al. studied the expression of CD25 on CD4+FoxP3+ cells and reported that T1D subjects have higher proportion of CD25-/FoxP3+ cells among CD4+/FoxP3+ Treg cells. Willcox, et al. analyzed postmortem pancreatic samples from T1D subjects. FoxP3+ Tregs were only found in islets from a single subject, suggesting that the lack of local Treg cells might be important in the pathogenesis of T1D.

Besides numbers, many studies have reported that Tregs isolated from peripheral blood of the T1D patients are defective in suppressive function. Ferraro, et al. showed that Tregs from peripheral blood of T1D subjects have normal suppressive activity but Tregs isolated from Pancreatic Lymph Nodes (PLN) of same subjects are functionally defective. It has also been reported that Tregs are unstable in T1D subjects since they lose the expression of FoxP3 due to defect in IL-2R signalling.
Another study showed that T1D subjects harbor substantial percentage of cells with transient or unstable expression of FoxP3. These exFoxP3 cells produce inflammatory cytokines, indicative of a high degree of plasticity in Treg phenotype.\textsuperscript{41,54} It has also been reported that the Teff cell population in T1D subjects are resistant to suppression by Tregs.\textsuperscript{55,58} Thus, it is still unclear whether Treg cells from T1D patients have intrinsic defective function or whether the responder T-cells are resistant to suppression, warranting the need for additional studies. Moreover, studies on the role of Tregs in T1D were performed on peripheral blood rather than pancreas or PLN, therefore the defects in local Tregs are not well known.

### Potential of Tregs in Treatment of T1D

There are many evidences which show that Tregs have the potential to prevent destruction of pancreatic islets, thereby protecting from T1D. Hence, strategies to increase Treg cell numbers and/or function are being explored as potential therapeutic approaches in treating T1D. Most of the treatment regimens to reverse diabetes in NOD mice worked via induction of Tregs or proliferation of Tregs.\textsuperscript{77,62} Therapy of T1D subjects with Tregs has been shown to prolong survival of pancreatic islets.\textsuperscript{63} At the same time, the knowledge on use of different type of Tregs for their clinical applications has increased tremendously. Today, several groups are engaged in exploring different Treg cell types, sources, induction procedures and experimental systems in pursuit of generation of highly efficacious and stable Tregs for immunotherapy of T1D.

#### Approaches used for in vitro Induction of Tregs

Clinical use of Tregs is hindered by their low frequency in peripheral blood.\textsuperscript{64} Therefore several methods have been developed for induction and expansion of Tregs (Table 2), few of which have led to trials in T1D subjects with varying success rates (Table 3). Generation of iTregs from CD25- T-cells in vitro is still not fully understood. However it has been established that it requires TCR stimulation, IL-2 and TGF-β both in vitro and in vivo. Supplementation of other compounds such as rapamycin and All Trans Retinoic Acid (ATRA)\textsuperscript{25,65} increase the yield and purity of Tregs.\textsuperscript{66} Addition of TGF-β induces transcription of FoxP3 by a mechanism that involves transcription factors STAT3 and NFAT at the FoxP3 gene enhancer element.\textsuperscript{67,68} Tregs induced in the presence of rapamycin/TGF-β are more stable than ATRA/TGF-β iTregs.\textsuperscript{69} However, upon re-stimulation the expression of FoxP3 decreases in both the iTregs; which in turn may lead to loss of suppressive activity.\textsuperscript{66} nTregs expanded in presence of rapamycin maintain FoxP3 expression and are highly suppressive than IL-2 expanded nTregs. Tregs expanded with anti CD3 and anti CD28 infused into T1D subjects have been shown to prolong the survival of pancreatic islets.\textsuperscript{63} Recently Lu, et al. reported that iTregs induced from CD39+ naive T-cells demonstrated enhanced proliferative and suppressive ability.\textsuperscript{69} However, expanded nTregs are shown to be superior to fresh nTregs since in vitro expansion improves their in vivo regulation.\textsuperscript{7}

<table>
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<tr>
<th>Methods</th>
<th>Tregs specificity</th>
<th>Reference</th>
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<tr>
<td>IL-10</td>
<td>Antigen specific</td>
<td>\textsuperscript{71}</td>
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<tr>
<td>Immature DC</td>
<td>Antigen specific</td>
<td>\textsuperscript{72,73}</td>
</tr>
<tr>
<td>Anti-CD3,anti-CD28+TGF-β</td>
<td>Polyclonal</td>
<td>\textsuperscript{74}</td>
</tr>
<tr>
<td>Peptide +Irradiated PBMCs</td>
<td>Antigen specific</td>
<td>\textsuperscript{75}</td>
</tr>
<tr>
<td>Anti-CD3,anti-CD28+IL-2+TGF-β</td>
<td>Antigen specific</td>
<td>\textsuperscript{76,77}</td>
</tr>
<tr>
<td>Plasmacytoid DCs</td>
<td>Antigen Specific</td>
<td>\textsuperscript{78}</td>
</tr>
<tr>
<td>Glucocorticoid induced leucine zipper expressing (GILZ) expressing DCs</td>
<td>Antigen specific</td>
<td>\textsuperscript{79}</td>
</tr>
<tr>
<td>Mature DC+antigen</td>
<td>Antigen specific</td>
<td>\textsuperscript{80}</td>
</tr>
<tr>
<td>Anti-CD3 and autologous APC</td>
<td>Polyclonal</td>
<td>\textsuperscript{81}</td>
</tr>
<tr>
<td>PBMCs+ mesenchymal stem cells</td>
<td>Polyclonal</td>
<td>\textsuperscript{82}</td>
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<tr>
<td>Programmed death ligand 1 (PDL1) coated beads</td>
<td>Polyclonal</td>
<td>\textsuperscript{83}</td>
</tr>
<tr>
<td>IL-2 + irradiated APC + peptide</td>
<td>Antigen specific</td>
<td>\textsuperscript{84}</td>
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<tr>
<td>Lentivirus T cell receptor gene transfer in nTregs</td>
<td>Antigen specific</td>
<td>\textsuperscript{85}</td>
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<tr>
<td>CD40 activated B cells+antigen</td>
<td>Antigen specific</td>
<td>\textsuperscript{86}</td>
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<tr>
<td>IL-2+TGF-β+APC</td>
<td>Polyclonal</td>
<td>\textsuperscript{77}</td>
</tr>
<tr>
<td>Delta like 1 ligand (notch signaling)+ memory CD4+T cells</td>
<td>Polyclonal</td>
<td>\textsuperscript{87}</td>
</tr>
<tr>
<td>IL-2+TGF-β+All trans retinoic acid (ATRA)</td>
<td>Polyclonal</td>
<td>\textsuperscript{88}</td>
</tr>
<tr>
<td>IL-2+TGF-β + rapamycin</td>
<td>Polyclonal</td>
<td>\textsuperscript{89}</td>
</tr>
<tr>
<td>PBMCs + human amniotic fluid stem cells (FHASC)</td>
<td>Polyclonal</td>
<td>\textsuperscript{88}</td>
</tr>
<tr>
<td>Lentiviral insulin (B,9-23) epitope expression in hepatocytes</td>
<td>Antigen specific</td>
<td>\textsuperscript{89}</td>
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*Table 2: Approaches used for in vitro induction of Tregs.*
Antigen Specific Tregs are More Potent than Polyclonal Tregs

Harnessing Tregs is a promising approach for treating autoimmune disease. Administration of polyclonal Tregs may be associated with significant off-target effects, including a global immunosuppression that may compromise beneficial immune responses to infections and cancer cells. Therefore, the objective of research in recent times has shifted to antigen specific therapeutic approaches that can reverse the disease by selectively halting the harmful immune response without requiring lifelong immune suppression. Adoptive transfer studies suggest that antigen specificity is required by Tregs for trafficking and maintenance in inflammatory tissues such as the pancreas in T1D.\textsuperscript{85,100,101} Previous studies have shown that small numbers of antigen specific Tregs are sufficient to reverse TID in comparison to large numbers of polyclonal Tregs.\textsuperscript{100} Antigen specific Tregs have been reported to exhibit a much lower threshold for activation and may be activated by a broad range of loosely-defined analogs of their cognate antigen; normally it is conceivable that the polyclonal Tregs may have received sufficient signaling within the pancreas to become suppressive.\textsuperscript{100} Besides, the site specific mode of action, antigen specific Tregs have the ability to act as bystander suppressor locally in the organ under attack. It has also been shown in mice that antigen-specific Tregs treat autoimmunity without compromising antibacterial immune response.\textsuperscript{100} However, isolation of sufficient number of antigen specific Tregs is a major challenge, particularly when sampling is limited to peripheral blood. Moreover, success in inducing antigen-specific tolerance has been hampered by the inability to identify peptides triggering the diabetogenic versus the regulatory response.

Generation of Antigen Specific Tregs

Several protocols have been established to induce antigen specific Tregs. Groux, et al. described induction of antigen specific Tregs by stimulating CD4+ T-cells with antigen and IL-10 \textit{in vitro}. This resulted in generation of antigen specific IL-10 producing Tr1 cells.\textsuperscript{71} Immature Dendritic Cells (DCs) as well as plasmacytoid DCs exhibit regulatory functions.\textsuperscript{78,104-106} Therefore, these DCs, have been used to induce antigen-specific CD4+ Tregs from CD4+CD25-T-cells.\textsuperscript{78,79} Walker, et al. used the mature DCs loaded with hemagglutinin (306-319, PKYVKQNTLKLAT) to generate Influenza hemagglutinin epitope specific Tregs from CD4+CD25-T-cells.\textsuperscript{75} CD40 activated B cells are more potent than immature DCs for the induction of antigen specific Tregs.\textsuperscript{96,107} Wenwei, et al. developed a method for expansion of alloantigen specific Tregs using CD40 activated B cells as APCs.\textsuperscript{107} Alice, et al. generated the islet antigen specific Tregs from CD4+CD25- T-cells by growing them in presence of GAD65 and IL-2 and observed that GAD65 derived epitope specific Tregs exhibit bystander suppression in the presence of antigen. In the suppression assay these epitope specific Tregs suppressed not only proliferation of GAD specific Teff cells but also of Tetanus Toxoid (TT) specific Teff cells in the presence of GAD. However, this bystander suppression was not observed in absence of GAD65 peptides or when TT was present alone.\textsuperscript{90} Therefore these observations indicate that it might be possible to reverse autoimmune diabetes by small number of epitope specific Tregs rather than having Tregs specific for all the diabetes associated antigens. Brusko, et al. used lentiviral T-cell Receptor (TCR) gene transfer system to generate antigen specific Tregs from murine nTregs.\textsuperscript{80} Tregs generated using this approach effectively blocked antigen-specific Teff cell activity. Also, DCs treated with glucocorticoids, upregulate Glucocorticoid-induced

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<th>Results/Impact</th>
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<tr>
<td>Anti CD3 treatment</td>
<td>increased iTregs, preserved residual endogenous β-cell mass</td>
<td>80</td>
</tr>
<tr>
<td>Insulin B-chain in incomplete Freund’s adjuvant (IFA)</td>
<td>Induction of Tregs</td>
<td>81</td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>Increase in percentage of Tregs, no differences in fasting C-peptide levels</td>
<td>82</td>
</tr>
<tr>
<td>GAD-Alum</td>
<td>Preservation of residual insulin secretion and induction of antigen specific Tregs</td>
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<td>Administration of ex vivo expanded Tregs in children</td>
<td>Increase in the percentage of Tregs in peripheral blood, preservation of β cells</td>
<td>84</td>
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<tr>
<td>Rapamycin/IL-2 combination therapy</td>
<td>Increase in Tregs with transient β cell dysfunction</td>
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<tr>
<td>Ex vivo expanded Tregs infusion in adults</td>
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<tr>
<td>Anti-thymocyte globulin/G-CSF</td>
<td>Relative preservation of Tregs and β cells</td>
<td>87</td>
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<tr>
<td>Low-dose IL-2</td>
<td>Expansion of Tregs</td>
<td>88</td>
</tr>
<tr>
<td>Oral insulin</td>
<td>Increase in Tregs, decrease in hypoglycemic events</td>
<td>89</td>
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Table 3: Immunotherapeutic approaches involving induction/use of Tregs in T1D subjects.
leucine zipper (GILZ). GILZ expressing DCs in the presence of IL-10 induce antigen specific CD28hi CTLA4+ Tregs.79 Recently Akbarpour, et al. transferred an immunodominant insulin epitope (B9-23) expressing lentivirus vector in hepatocytes of NOD mice. The therapy induced insulin specific Tregs that inhibited immune cell infiltration in the pancreatic islets and halted diabetes development.80 While induction of antigen specific Tregs is difficult, analysis of their characteristics is also technically challenging. Following induction, either ex or in vivo, antigen specific Tregs can be sorted using MHC class II tetramers loaded with peptide of interest.84 Latency-associated peptide (LAP) and Glycoprotein A Repetitions Predominant (GARP) protein have also been reported as markers to identify human antigen-specific Tregs.108

Stability of Tregs

Clinical usage of Treg cells is hindered due to their instability. Tregs have been shown to lose FoxP3 expression under inflammatory environment.54,109-113 Proinflammatory environment may abrogate the suppressive activity of Tregs114-116 or cause Teff cells resistant to suppression.117 There are certain reports that show that plasticity of Tregs might play important role in pathogenesis of autoimmune diseases. Indeed, increased frequency of IFN-γ+FoxP3+ cells has been reported in subjects with T1D.118 Th17 cells originating from FoxP3+ T-cells have shown to play a key role in the pathogenesis of autoimmune arthritis.119 Stable Tregs can be distinguished from the unstable ones on the basis of epigenetic modifications in the CpG-rich TSDR of the FoxP3 locus.66 Demethylation of the TSDR region correlates with the stability of FoxP3 gene. Strong methylation in the TSDR of FoxP3 promoter may be associated with unstable phenotype of Tregs. Analyzing the demethylation status of the TSDR in the FoxP3 may aid in distinguishing the stable Tregs from unstable Tregs.36

Role of Colonic Tregs

The gut immune system plays an important role in autoimmune diabetes. One of the most influential environmental factors that influences gut immune system is the gut microbiota. The development of clinical diabetes is preceded by intestinal alterations such as an aberrant intestinal microbiota, a leaky intestinal mucosal barrier and an altered mucosal immune system.119 Therefore, a hygiene hypothesis has been postulated which suggested a reduction in childhood exposure to infections leading to the accelerated development of T1D.120 The gut microbiota shape the mucosal immune system by controlling many types of T-cells including the colonic regulatory T-cells (cTregs) which are a type of induced Tregs. It has been proposed that pathogenic microbes promote T1D development by enhancing self-reactive T-cells,119 while many microbial species such as Clostridia species has been shown to be potent inducers of cTregs. The gut microbiota modulates local immune system by acting on various immune cells including DCs. These lamina propria CD103+ CD11c+ DCs direct the antigens that cross the epithelial barrier to the Gut Associated Lymphoid Tissue (GALT), and enable the differentiation of naïve CD4+ T-cells to cTregs via TGF-β and retinoic acid.104,112 These Tregs control inflammation via anti-inflammatory agents such as IL-10 and TGF-β.122 Studies in T1D subjects have reported low frequency of FoxP3+ Tregs and an impaired differentiation of FoxP3+ Treg cells by intestinal CD103+CD11c+DCs.31 Due to the immunological connection between the GALT and the PLN, the immunological changes taking place in the gut are reflected in the pancreas. Thus the impaired generation of Tregs in the gut alters the Teff/Treg cell balance in PLN and islets thereby promoting Teff cell responses against pancreatic self-antigens.123,124 This leads to failure of self-tolerance and development of autoimmunity.

Strategies to Induce/Increase Abundance of cTregs

cTregs play a critical role in limiting the intestinal inflammation. They are constitutively present in the intestinal mucosa as well as the GALT and thus maintain immune homeostasis. However the breakdown of gut immune system leads many autoimmune diseases including T1D. Hence various strategies have been developed to generate cTregs.

Animal studies have shown that intestinal colonization with commensal bacteria activate and expand Treg cells, as well as de novo generate cTregs. The colonization of germ free mice with Altered Schaedler Flora (ASF) species resulted in the generation of Tregs in colonic lamina propria. These Tregs limited the proliferation of Th1 and Th17 cells.125 Furthermore, a defined cocktail of 17 strains of clostridium species within the cluster IV, XIVa and XVIII of Clostridia strains has been shown to trigger the expression of TGF-β in the intestinal epithelial cells, thereby promoting the accumulation of FoxP3+ Tregs.126 Also Polysaccharide A (PSA) secreted by Bacteroides fragilis has been shown to act via TLR2 expressed on CD4+ T-cells which enable their conversion to FoxP3+ T-cells that produce IL-10.10 The specific Bifidobacterium strains present in healthy microbiota provides protection against pathogens; accordingly the early administration of Bifidobacterium infantis to mice attenuated the severity of colitis by the induction of Tregs in the Mesenteric Lymph Nodes (MLN).127 With encouraging reports, several groups have come up with probiotics, live beneficial microorganisms that when administered continuously can induce gut immunity. In an important study, oral administration of probiotic VSL#3 to NOD mice during the early stages of life showed a delay in the progression of diabetes. This prevention was associated with the generation of IL-10 producing Tregs in the GALT.28 Autoantigen specific therapies also hold great promise in the reversal of T1D by induction of oral tolerance. One such approach involved the administration of Lactococcus lactis for controlled secretion of GAD65 and IL10 in the gut, which favored the induction of Tregs.129
Besides bacteria, their metabolic products such as, Short Chain Fatty Acids (SCFA) have been shown to affect the colonic health as they can penetrate the intestinal epithelium and restore intestinal immune responses. The administration of SCFA such as acetate, propionate and butyrate enabled the restoration of cTregs in germ free mice and significantly increased the expression of IL-10 and TGF-β in cTregs.\textsuperscript{130} Among the SCFA, butyrate has received a lot of attention due to its effect on colonic function. The dietary administration of butyrylated high amylase maize starches to mice showed an increase in the frequency of cTregs.\textsuperscript{131} Butyrate is also well known to epigenetically modify the FoxP3 gene by inhibiting the class I and IIa of histone deacetylases, thereby increasing the FoxP3 expression and differentiation of Treg cells.\textsuperscript{132-134} The colonic DCs and macrophages express the cell surface receptor Gpr109a.\textsuperscript{135,136} Butyrate acts via these receptors and induces the expression of anti-inflammatory molecules such as IL-10 and aldehyde dehydrogenase (Aldh1a), thereby supporting the differentiation of cTregs.\textsuperscript{137,138} Additionally, intervention strategies such as dietary supplementation with 1,2 dihydroxy-vitamin D (1,25(OH)2D3), an active form of vitamin D promotes the development of FoxP3+ Treg cells and inhibits the differentiation of Th1 and Th17 cells.\textsuperscript{139} High doses of vitamin D3 safely reduced diabetes development by preventing insulitis and differentiation of Th1 and Th17 cells.\textsuperscript{139} High doses of vitamin D3 safely reduced diabetes development by preventing insulitis and preserving β cell mass in NOD mice.\textsuperscript{140} Also the deficiency of Vitamin B9 or folic acid derived from diet and commensal bacteria showed marked reduction in gut FoxP3+ Treg cells.\textsuperscript{141} In addition to induction approaches, homing of T lymphocytes to the gut is also important in induction of cTregs and impaired homing of T-cells is implicated in many inflammatory diseases. For example, GPR15, an orphan heterotrimeric guanine nucleotide-binding protein (G protein) coupled receptor, controls the specific homing of FoxP3 Tregs, to the large intestine lamina propria, and its expression can be modulated by gut microbiota and TGF-β.\textsuperscript{142} Despite the difficulties in characterization of induced Tregs, there is an increasing awareness about the importance of induction of immune tolerance via gut and generation of cTregs have come to the forefront as an actively pursued area of research, in prevention of autoimmune diseases like T1D.

CONCLUSIONS

Many immunotherapeutic approaches including self-antigens and immune modulating agents have been tried to tackle autoimmunity observed in T1D. Most of these treatment strategies have failed to prevent or improve the clinical outcome of the disease. There are multiple etiologies that are known to cause β cell destruction in T1D. Hence targeting a single factor may not provide a lifelong preservation of the β cell mass in T1D. While the defects in number and function of Tregs in T1D were known long ago, research on application of Tregs in T1D has picked up more in recent years. Today there are several choices available in immunotherapeutic approaches with Tregs, ranging from their type (natural versus induced), source (peripheral versus colonic) or specificity (polyclonal versus antigen-specific) or methods of induction (direct versus indirect) and expansion (in-vitro versus in-vivo), each of which has its specific advantages and limitations. Regardless of the variety, Tregs have opened up new vistas in treatment of T1D. With growing understanding about the generation of different types of Tregs and their clinical applications, the use of Tregs in future treatment of T1D looks quite promising. We believe, Tregs might provide benefit in the form of a combination therapy that attenuates autoimmunity towards the pancreas, ultimately preserving β cell mass.

CONFLICTS OF INTEREST: None.

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