Restoring regulatory T cells in type 1 diabetes

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Abstract

Genetic and cellular studies of type 1 diabetes in patients and in the non-obese diabetic mouse model of type 1 diabetes point to an imbalance between effector T cells and regulatory T cells (Tregs) as a driver of the disease. The imbalance may arise as a consequence of genetically encoded defects in thymic deletion of islet antigen-specific T cells, induction of islet antigen-specific thymic Tregs, unfavorable tissue environment for peripheral Treg induction, and failure of islet antigen-specific Tregs to survive in the inflamed islets secondary to insufficient IL-2 signals. These understandings are the foundation for rationalized design of new therapeutic interventions to restore the balance by selectively targeting effector T cells and boosting Tregs.

Introduction

Type 1 diabetes (T1D) is caused by immunological destruction of insulin-producing beta (β) cells in the islets of Langerhans in the pancreas. Studies of disease incidence in twins demonstrate that both genetic risk factors and the environment contribute to the development of this disease [1]. The non-obese diabetic (NOD) mice that spontaneously develop autoimmune diabetes share many of the features of the human disease including genetic risk factors, cellular mechanisms, and autoantigens targeted by the immune system [2]. This model has been used extensively to understand disease pathogenesis and to test novel therapeutics. Many cell types are involved in the pathogenesis of diabetes, including T cells, B cells, dendritic cells (DCs), and macrophages. Islet antigen-specific $CD4^+$ and $CD8^+$ T cells are the principal drivers of disease initiation and progression. These autoreactive T cells are normally deleted during thymic selection and the few escapees are held in check in the periphery by a multitude of peripheral tolerance mechanisms. In NOD mice and in patients with genetic risk factors for T1D, the layers of redundant immune tolerance mechanisms have been weakened to the point that a slight perturbation of the delicate balance between immune activation and regulation precipitates disease. As an essential gatekeeper of peripheral tolerance, the Foxp3-expressing $CD4^+$ regulatory T cells (Tregs) are central to T1D pathophysiology. Mechanistic dissection of the disease and therapeutic explorations cannot be complete without considering the involvement of and impact on Tregs. Here, we take a Treg-centric view in reviewing advances in understanding T1D pathogenesis and in developing therapeutic interventions.

Tregs are critical mediators of peripheral tolerance

Tregs represent less than 10% of peripheral $CD4^+$ T cells but serve an indispensable function in maintaining immune homeostasis. Tregs fail to develop in the absence of the master

transcription factor Foxp3 [3-5]. Humans and mice with mutation in the Foxp3 gene develop systemic lethal autoimmune diseases [6, 7].

Tregs can develop in the thymus when maturing $CD4^+$ T cells encounter cognate antigens and they are referred to as thymic Tregs (tTregs) [8-12]. Self-antigens present in the thymus, either through Autoimmune Regulator (AIRE)-mediated tissue specific antigen expression or circulating $Sirp1\alpha^+$ DCs, drive the development of tTregs [13-17]. Tregs can also develop in the periphery from mature $CD4^+$ cells through non-inflammatory exposure to low-dose antigens. These cells are referred to as peripheral Tregs (pTregs). pTregs develop in response to peripheral encounters with antigens such as those from food, microbiota and fetal alloantigens for pregnant females [18-22]. Thus tTregs and pTregs have distinct repertoires that complement each other to ensure tolerance to self-antigens and the changing environment. Alteration in antigen presentation in the thymus and in the periphery will likely lead to changes in Treg repertoire and their ability to control autoimmunity. Moreover, both tTregs and pTregs constitutively express CD25, the IL-2 receptor α (IL-2Rα) chain that confers high affinity binding to IL-2. Tregs depend on IL-2 for their development, lineage stability, and survival [23, 24] and perturbation in IL-2 signaling has significant impact on Treg homeostasis and function as highlighted by the severe systemic autoimmunity in IL-2 and CD25-deficient mice [25].

Suppressive mechanisms used by Tregs are subject of many previous reviews [26, 27] and we will limit our discussion here to CD25 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) because of their link to T1D susceptibility. Constitutive expression of CD25 contributes to Treg suppressive activity by functioning as an IL-2 sink, depriving conventional T (Tconv) cells of this important factor for differentiation into effector T cells. Tregs also constitutively express CTLA-4, a checkpoint inhibitor of T cell activation. CTLA-4 can inhibit T

cell activation in the cells that express it by reducing T cell receptor (TCR) signaling [28]. It can also inhibit activation of other T cells by reducing costimulation ligands CD80 and CD86 on antigen presenting cells (APCs) by stripping CD80 and CD86 off APCs via trans-cytosis [29], inhibiting DC maturation [30], or, in its soluble form, by serving as a decoy receptor for CD80 and CD86 [31]. CTLA-4 expression on a subset of Tregs called T follicular regulatory cells is essential for controlling B cell responses and autoantibody production [32, 33]. Although some of these functions are not exclusively mediated by Tregs, selective deletion of CTLA-4 in Tregs leads to systemic autoimmunity, demonstrating an important role of CTLA-4 for Treg function [34].

Genetic evidence linking Treg imbalance to T1D

By analyzing single nucleotide polymorphisms in T1D patients in comparison with control subjects, genome wide association studies (GWAS) have identified more than 60 genetic loci associated with T1D [35]. Further investigations into the genes at these loci and their functions have allowed us to better understand the pathogenesis of this disease and point to ways of developing new interventions.

MHC class II

The strongest locus associated with T1D is the major histocompatibility complex (MHC) locus, also known as human leukocyte antigen (HLA) locus in humans, with an odds ratio of 6.8. HLA class II DR3/DR4 and DQ8 are associated with higher disease risks while DQ6 is strongly protective against diabetes. Similarly, the I-A^{g7} allele of mouse MHC class II is essential for disease development in the NOD mice and I-E^{k} allele is protective of disease [36, 37]. I-A^{g7} and its human homolog HLA DQ8 share features of self-peptide presentation [38]. I-A^{g7} has a

shallow and wide peptide-binding grove that loosely accommodates many peptides. Insulin B chain peptide 9 to 23 (Ins B:9-23) can bind to I-A^{g7} in 4 different registers and many diabetogenic T cells are found to recognize Ins B:9-23 in the weak register 3. Although insulin is expressed in the thymus to delete insulin-specific T cells, the poor binding of Ins B:9-23 in register 3 allows register 3 specific T cells to escape negative selection [39, 40]. Tregs develop in the thymus in a multi-stepped process involving sequential TCR and IL-2 signaling [41]. Inefficient presentation of self-antigen in the thymus can potentially affect tTreg development. It is also conceivable that diabetes-protective MHC alleles may prevent disease by deleting autoreactive cells or promoting Tregs. More comprehensive analysis of self-antigen specific T cells is still needed to decipher how HLA alleles impact Treg versus diabetogenic T cell development.

Insulin

The locus with the second strongest association with T1D in humans is the insulin gene itself with an odds ratio of 2.38. A repetitive sequence in the 5' regulatory region of the insulin gene is linked to disease risk. The variant associated with T1D has a shorter stretch of repeats and lower expression of insulin in the thymus and thus less efficient deletion of insulin-specific T cells [42, 43]. Mice have two insulin genes with insulin 1 exclusively expressed in the pancreas and insulin 2 expressed in both thymus and pancreas. Deletion of insulin 2 leads to loss of central tolerance to insulin and exacerbation of diabetes [44]. As discussed above for HLA, altered expression of self-antigens in the thymus will most likely impact the development of tTregs specific for those self-antigens. Comparative analysis of insulin-specific effector T cells and Tregs will help to understand how the insulin gene variants in humans contribute to the balance of the pathogenic and protective T cells.

PTPN22

The third most influential locus for diabetes susceptibility is protein tyrosine phosphatase, non-receptor type 22 (PTPN22), with an odds ratio of 2.05 [45-47]. PTPN22 encodes a tyrosine phosphatase, LYP, a negative regulator of T and B cell activation. A knockin of the disease-associated sequence into PEP, the mouse homolog of LYP, leads to hyperactivation of T and B cells and spontaneous development of autoimmune diseases [48]. Overexpression of wild type PEP in NOD mice prevented diabetes. The protection is associated with reduced TCR signaling in both Tconv cells and Tregs that results in selective reduction of effector differentiation without impacting Treg development or function [49]. This result suggests that LYP-mediated dampening of TCR activation preferentially impairs effector T cells and tilts the balance toward regulation. It has been reported that Tregs can be activated to suppress by low affinity ligands whereas activation of effector function in Tconv cells requires higher affinity engagement with antigens [50]. Strikingly, the differential requirement can be three orders in magnitude. Moreover, TCR overstimulation in Tregs can lead to their destabilization and adoption of an effector phenotype [51]. Together, these results suggest that tuning of TCR signaling strength has the potential to dramatically affect the function of effector T cells versus Tregs. It is possible that reduced function of LYP encoded by the disease allele leads to enhanced TCR signaling, selectively boosting effector cells and possibility impairing Tregs.

IL-2Ra

The fourth most influential locus of T1D is IL-2Rα, aka CD25, with an odds ratio of 1.61 [52]. Genetic variations in the IL-2Rα locus are all outside the protein-coding region of the gene; thus the influence on disease is most likely through regulation of CD25 expression. While the connection between IL-2R and Tregs seems obvious, variant alleles in IL-2R mostly affect

CD25 expression on naïve $CD4^+$ T cells, memory $CD4^+$ T cells, and stimulated monocytes, but not in Tregs [53]. CD25 also confers heightened sensitivity to IL-2 in Tconv cells. Expression of CD25 by DCs can enable high affinity IL-2 binding *in trans* to cognate T cells that do not express CD25 [54]. Therefore, higher expression of CD25 on naïve T cells and activated monocytes found in people carrying the diabetes susceptibility variants may make their T cells more easily activated. This combined with unchanged expression of CD25 on Tregs could tip the balance toward immune activation than regulation.

IL-2 and CTLA-4

The remaining diabetes susceptibility loci confer relatively lower risks to the disease, all with odds ratio of less than 1.3. A few of these genes are particularly noteworthy in the context of Tregs. Variants in the IL-2 gene have been found to increase diabetes susceptibility in both human patients and in NOD mice. The NOD IL-2 gene contains variants that reduce IL-2 expression by 50% and this subtle change can lead to immune dysregulation [55, 56]. The diabetes-prone allele of the protein tyrosine phosphatase, non-receptor type 2 (PTPN2) is associated with impaired IL-2 signaling that can potentially directly affect Treg homeostasis [57]. An integrated enrichment analysis of gene variants in T1D identifies significant enrichment of genes related to IL2 signaling [58]. Thus, although each variant singly may have a mild impact on disease susceptibility, their combined effects in a pathway can compound and exert strong influence on the disease.

Another prominent Treg-related diabetes susceptibility locus in both NOD mice and T1D patients is CTLA-4 [46, 59-61]. In humans, the variant is located in the 3' non-coding region of the CTLA-4 gene resulting in reduced production of the soluble splice form. Selectively decreasing soluble CTLA-4 in NOD mice leads to reduced Treg function and increased diabetes

[31]. These results suggest that the CTLA-4 polymorphism may impact Treg function and diabetes development through releasing soluble CTLA-4.

Together, many large-scale GWAS studies have provided a blueprint for probing the pathogenesis of T1D. Finer mapping and follow-up analyses of causal variants point to a loss of balance between immune activation and regulation during thymic selection and peripheral immune activation as the immunological basis of the disease. Tconv cells and Tregs share many common pathways in development and activation, and variants associated with T1D affect these common mechanisms in ways that shift the balance to favor immune activation.

Treg impact on T1D progression

Treg defects in T1D

Analyses of Tregs in T1D patients reported numerical and functional defects by some [62-66], but refuted by others [67, 68]. Some of these discrepancies may be due to the difficulties in identifying and isolating a pure population of Treg in humans. Activated effector T cells in humans can also express CD25 and Foxp3, making it difficult to distinguish the two populations of cells [69]. CD25 expression level on $CD45RA^+$ Tregs is lower than on $CD45RA^-$ Tregs [69]. Thus, different CD25 thresholds used to isolate Tregs in various labs may account for differences in the frequencies and composition of Tregs studied and different activities observed. The addition of the CD127 marker greatly improves the purity and yield for human Treg isolation [70, 71]. When using the surface phenotype $CD4^+CD25^+CD127^{lo/-}$ to identify Tregs, T1D subjects and controls have a similar frequency of Tregs. The $CD4^+CD25^+CD127^{lo/-}$ Tregs from T1D subjects have higher propensity to produce IFN□□[72]. In addition, Tregs in T1D subjects are prone to apoptosis, which is associated with a gene expression signature similar

to that of Tregs undergoing IL-2 deprivation [73-75], consistent with the reported defects in IL-2R signaling in T1D patients [57, 76].

Treg studies in T1D patients are limited by the lack of access to Tregs in the pancreatic tissue and the draining pancreatic lymph nodes where the function and potential defects may manifest. In the NOD mice, the importance of Tregs in controlling diabetes has been clearly established. Although Foxp3-deficient NOD mice succumb to multi-organ autoimmune diseases before developing diabetes, NOD mice with milder Treg defect, such as that seen in CD28-deficient mice and NOD mice treated with IL-2 neutralizing antibodies, develop accelerated diabetes [77-80]. Yet, at population level, no systemic defect in Treg development or function has been observed in the standard NOD mouse stock [81, 82]. In the pancreatic lymph nodes of NOD mice, Treg numbers increase as inflammation in the pancreatic islets intensifies with age, demonstrating that Tregs in NOD mice can mount an appropriate regulatory response; however, the proportion of Tregs in the pancreatic islets progressively declines with inflammation [83]. This highly localized defect of intra-islet Tregs is associated with reduced CD25 expression and low level of anti-apoptotic protein Bcl-2, consistent with their apoptosis due to IL-2 deprivation. IL-2 therapy rescues intra-islet Tregs and prevents diabetes. Thus, NOD mice have a diabetes-protective Treg repertoire, but they fail to keep up with effector cells in chronically inflamed islets leading to progressive loss of cell mass and eventual development of diabetes.

Antigen specificity of diabetes-protective Tregs

Adoptive transfer of Tregs can prevent diabetes in NOD mice and islet antigen-specific Tregs are 50 times more potent than polyclonal Tregs [84]. However, the specificities of Tregs among the natural NOD Treg repertoire that are responsible for delaying diabetes are currently unknown. Antigen specificities of diabetogenic effector T cells have been extensively

investigated. Among numerous islet autoantigens, insulin is the primary driver and required for the initiation anti-□-cell autoimmunity [85-88]. Pathogenic T cells can arise by escaping thymic deletion as discussed above and by recognizing neoantigens formed in the □□cells not present in the thymus [89, 90]. TCR sequencing analysis suggests that TCRs used by Tregs and autoreactive T cells share structural similarities at MHC-peptide contact sites [91], thus defective self-antigen presentation responsible for escape of autoreactive T cells from thymus may also impair tTreg development. pTreg differentiation results from high-affinity TCR engagement by persistent low-dose antigen in non-inflamed conditions [92]. A □ cell can produce 1 million insulin molecules per minute. Thus, insulin and other proteins supportive of insulin manufacturing and release are likely present at very high concentrations in the islets. T cells with a high affinity TCR for these antigens are more likely to develop into pathogenic effector cells than pTregs in the islets [93, 94]. It is tempting to speculate that islets, and other endocrine organs specialized in high amount of protein production, are not conducive for induction of pTreg specific for the self-antigens they express. Since insulin is secreted and present at low concentration systemically, insulin-specific pTregs may develop if □ cells are not destroyed too soon. For example, the BDC12-4.1 Rag knockout TCR transgenic mice, where all T cells are specific for insulin, develop pTregs that are responsible for a partial protection against diabetes [95, 96]. It is noteworthy that proinsulin-specific Treg activity can be detected in healthy human subjects in *in vitro* Treg specificity screens [97]. Using I-A^{g7}-insulin tetramer staining and TCR sequencing analysis, we have found that insulin-specific Tregs are readily detectable in pre-diabetic NOD mice, particularly in inflamed islets (*Spence et al*, manuscript in preparation).

Overall, current evidence in T1D patients and NOD mice show no general systemic defect of Tregs, but point to a loss of Treg and effector T cell balance in the islets likely confined

to T cells specific to islet self-antigens. This imbalance may arise as a consequence of a genetically encoded defect in thymic deletion of islet antigen-specific T cells, induction of islet antigen-specific tTregs, unfavorable tissue environment for pTreg induction, and failure of islet antigen-specific Tregs to survive in the inflamed islets secondary to insufficient IL-2 signals.

Restoring Treg and effector T cell imbalance to treat T1D

Therapies that selectively enhance β cell protective Tregs while decreasing diabetogenic effector T cells may restore the imbalance in T1D patients and stall the progression of the disease. Many of these therapeutic concepts are being tested in NOD mice and in early phase clinical trials.

Low-dose IL-2

The crucial importance of IL-2 in Treg homeostasis and apparent Treg survival defects in NOD mice and T1D patients provide a rationale for using IL-2 therapy to enhance Tregs. IL-2 has pleotropic effects on a variety of cell types and heightened sensitivity of Tregs to IL-2 support the use of low-dose IL-2 to selectively target Tregs [98]. IL-2 therapy increases Bcl-2 expression in Tregs and boosts Treg numbers and function in NOD mice leading to diabetes prevention and even reversal [83, 99, 100]. The efficacy of the therapy is highly dose dependent, with high dose driving effector T cell and NK cell activation and acceleration of diabetes [83].

Improved clinical outcomes for patients using low-dose IL-2 therapy has been observed in chronic graft-versus-host disease [101, 102], hepatitis C virus-induced vasculitis [103], and alopecia areata [104]. Despite reported IL-2 signaling defects, Tregs from T1D patients treated with low-dose IL-2 therapy can respond to IL-2, supporting the use of this therapy for enhancing Tregs in T1D subjects [105]. In a T1D IL-2 and rapamycin combination trial, Tregs increased, but so did $CD56^{hi}$ NK cells and $CD4^{+}$ memory T cells, with a concomitant decrease in β cell

function [106]. Another CD25 expressing population, the group 2 innate lymphoid cells, are activated in patients receiving low dose IL-2 therapy and contribute to eosinophilia observed in these patients [107]. These studies highlight the importance of dosing as well as potential side effects of this treatment. Thus, defining the optimal dose is vital for the efficacy of IL-2 therapy. Clinical trials aimed at addressing IL-2 dosing in T1D are ongoing [108].

CTLA-4Ig

CTLA-4Ig binds to costimulatory ligands CD80 and CD86, preventing CD28-mediated costimulation. Treg development and homeostasis also require CD28 signaling. However, there is a quantitative difference between the amount of costimulation needed for effector T cell differentiation and for Treg homeostasis. Partial blockade of costimulation using CTLA-4Ig can effectively prevent effector cell activation while minimally affecting Tregs in mice and human patients [109, 110]. A trial of abatacept, a fusion protein of human CTLA-4 extracellular domain and human IgG1, in newly diagnosed T1D patients found a decrease in the rate of β cell loss [111]. This beneficial effect in the β cell function and HbA1c lasted for at least one year after abatacept treatment ended [112]. A phase II clinical trial for individuals at risk for T1D is currently enrolling patients (NCT01773707, clinicaltrials.gov).

LFA-3Ig

CD2 on T cells binds to lymphocyte function antigen (LFA)-3 on APCs to enhance T cell activation. Memory T cells express higher levels of CD2 than naïve T cells and Tregs, making CD2 an attractive target to alter the balance of autoantigen-experienced memory T cells and Tregs without global immunosuppression. Anti-CD2 antibodies bind to CD2 with high affinity and cannot distinguish the different levels of CD2 expression on different T cells and NK cells.

Prolonged deletion of T cells and NK cells have been observed in psoriasis patients treated with an anti-CD2 antibody Siplizumab [113]. In patients with $CD2^+$ lymphoma, the degree of T cell and NK cell depletion is associated with higher rate of EBV reactivation and EBV^+ B cell lymphoma [114]. On the other hand, Alefacept is a LFA-3Ig fusion protein that binds to CD2 on T cells with much lower affinity [115]. When used in a multicenter clinical trial in T1D, alefacept treatment resulted in selective depletion of memory T cells while preserving naïve T cells and Tregs [116]. Alefacept treatment also showed promising efficacy in improving metabolic outcomes in T1D patients, including C-peptide preservation, lower insulin use, and reduction in hypoglycemic events [117].

Treg cell therapy

Direct Treg infusion to patients is another approach to increase Tregs to treat T1D. In animal models, a single infusion of Tregs prevents and reverses diabetes by suppressing lymph node priming of effector cells and restrains effector T cell function in the inflamed tissue [84, 118, 119]. A highly pure population of Tregs can be isolated from T1D patients using fluorescence activated cell sorting based on cell surface phenotype of $CD4^+CD25^+CD127^{lo/-}$. These cells can be expanded *in vitro* with short-term polyclonal stimulations [120]. Billions of Tregs can be produced from one unit of blood (~450 ml) under good manufacturing process compliant conditions, making it feasible to evaluate this therapy in clinical trials.

Two clinical trials of Treg cell therapy in T1D have been published thus far. A trial conducted in Poland evaluated the safety and efficacy of *ex vivo* expanded $CD3^+CD4^+CD25^{hi}CD127^-$ Tregs in 12 children within two months after the diagnosis of T1D [121, 122]. Among the 12 patients, 3 patients received single infusion of 10×10^6 Tregs/kg body weight, 3 received 20×10^6 Tregs/kg, and 6 received two infusions of total 30×10^6 Tregs/kg

with a 6–9 month interval between infusions. Treg-treated patients had significantly elevated C-peptide 6 months after disease onset when compared with untreated children followed at the same center during the trial. One year after inclusion to the study, 8 out of 12 patients still met the criteria of clinical remission and two remained insulin independent. A positive correlation between C-peptide levels and percentage of Tregs in peripheral blood was observed. No serious infections, acute hyperglycemia, or hypoglycemia was associated with the treatment. The investigators concluded that Treg therapy in children was well tolerated and potentially efficacious.

In a separate trial conducted at the University of California, San Francisco, patients 18 to 40 years of age within 3 to 24 month after T1D diagnosis received a single infusion of 5 $x10^6$ (3 patients), 40 x 10^6 (3 patients), 320 x 10^6 (4 patients), and 2.6 x 10^9 (4 patients) polyclonally expanded $CD4^+CD25^+CD127^{lo/-}$ Tregs [123]. This study also found the therapy well tolerated with no infusion reactions and no increased rate of infection. Metabolic parameters remained stable in most patients after Treg infusion, although this study was not powered to address efficacy. To enable the assessment of the pharmacokinetics of the infused Tregs, deuterated glucose was used in the expansion medium, which resulted in the incorporation of deuterium into the deoxyribose moiety in replicating DNA through the *de novo* purine nucleotide synthesis pathway. The level of infused Tregs can then be monitored by measuring deuterium enrichment in purine deoxyribonucleosides in Tregs using gas chromatography/mass spectrometry. Infused Tregs peaked in the first two weeks and declined sharply to ~25% of the peak level by 28 days. The level of Tregs then remained relatively stable with a half-life of approximately 2.5 months. Deuterium remained detectable at 1 year after infusion among circulating Tregs in all patients that received 2.6 x 10^9 Tregs. These results demonstrate that autologous Treg therapy in T1D

patients is safe and at least a portion of the Tregs can be long-lived. Currently, a phase II study of Treg therapy in T1D (NCT02691247) and a phase I study of combination of Treg cells and low-dose IL-2 (NCT02772679) are ongoing.

Conclusions and Future directions

Genetic and cellular studies in mice and human patients indicate heightened effector T cell activity and defective Treg function and survival as a driver of the disease. New interventions aimed at correcting imbalance between effector T cells and Tregs are under active development and show promising preliminary results. None of the therapies currently tested are specific for islet cells. These polyclonal approaches may achieve selective effect by the virtue of differential sensitivity of activated effector T cells to the therapeutic agents. For example, anti-CD3 targets all T cells, but its effect on naïve T cells is transient and T helper 1 cells are particularly sensitive to its deletional effects. In Treg cell therapy, islet antigen specific clones may preferentially home to inflamed islets and expand locally to exert site-specific effects. Nonetheless, targeting therapy to cells is much needed. In this regard promising results have been observed using tolerogenic islet antigens to inactivate effector T cells in animal models [124]. In Treg cell therapy, antigen specific Tregs produced by selective expansion *in vitro* has already been tested in liver transplantation and Crohn's disease [125, 126]. The low frequency of islet antigen specific Tregs and the propensity of Tregs to destabilize after repeated *in vitro* stimulation makes selective expansion of islet antigen specific Tregs challenging. However, Treg specificity may be engineered using genetic tools. These new interventions are currently being evaluated in preclinical models. In the future, precision repair of islet-specific autoimmunity may be achieved using a combination of inactivation of islet-specific effector cells to halt cell destruction and islet antigen-specific Tregs to restore tolerance.

Conflicts of Interest

Allyson Spence declares no conflict of interest. Qizhi Tang is a recipient of grants from Caladrius for the development of Treg cell therapy and speaker honorarium from Merck. She also has a patent issues (US Patent #7,722,862.B2) and a patent pending (US Patent #20150110761.A1).

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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