Regulatory T Cells in Type 1 Diabetes

Subjects: Immunology

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Type 1 diabetes (T1D) is an autoimmune disease that typically presents in childhood and early adulthood that results in the destruction of insulin-producing pancreatic beta cells by T cells. One potential protective mechanism includes the suppression of immune responses by regulatory CD4 T cells (Tregs) that recognize self-peptides from islets presented by human leukocyte antigen (HLA) class II molecules.

Keywords: regulatory T cells ; type 1 diabetes ; islet autoimmunity

1. Introduction

The primary pathological presentation of T1D is inflammation of the pancreatic islets, termed insulitis, and it is due to the infiltration of immune cells, including CD4 and CD8 T cells along with B cells [1][2][3][4][5][6]. Therefore, there is a direct link between pathogenic T cells targeting insulin-producing pancreatic beta cells; however, regulatory T cells (Tregs) play an important role in protection from autoimmunity, including T1D. During T1D development, there is likely an imbalance between pathogenic T cells targeting pancreatic islets and Tregs that function to protect against targeting these cells.

The incidence of T1D continues to increase globally across racial and ethnic groups [7]. However, the measurement of T1D-associated antibodies in the peripheral blood allows for the prediction of disease progression through stages, as islet autoantibodies precede the development of clinical diabetes, in general, by a number of years [8][9][10]. Furthermore, a recent study has shown that short-term immunotherapy with an anti-CD3 monoclonal antibody can delay the onset of clinical disease [11], which is marked by hyperglycemia and the need for exogenous insulin treatment. Despite these clinically meaningful successes, there is a strong need in the field to specifically induce tolerance to islet antigens prior to and at disease onset.

2. T Cell Receptor–Peptide–MHC Interactions

T cells possess T cell receptors (TCRs) that recognize peptides bound to major histocompatibility complex (MHC) molecules on the surface of antigen-presenting cells (APCs) such as B cells, dendritic cells, and macrophages. TCRs consist of an alpha chain and beta chain that are generated via the recombination of noncontiguous gene segments for each chain. Both chains of the TCR include a variable (V), joining (J), and constant region, while the beta chain also contains a diversity (D) region. Therefore, the process by which TCRs are rearranged into fully functional receptors is known as V(D)J recombination. In this manner, each T cell generates a unique receptor for the recognition of a closely related set of peptides presented in the context of the MHC molecule. The interaction between TCR/peptide/MHC leads to activation of the T cells, and two major classes of T cells are involved in an adaptive immune response. Both classes recognize peptide/MHC, and cluster of differentiation 4 (CD4) T cells release cytokines to activate other immune cells and also interact with and activate B cells, while CD8 T cells act to directly kill target cells. CD4 cells can generally be subdivided into T helper type 1 (Th1) and type 2 (Th2) cells, which are distinguished by the functions and cytokine production. Th1 cells are considered proinflammatory due to the production of the cytokines interleukin-2 (IL-2) and interferon gamma (IFN-γ). Th1 cells function to direct immune responses against intracellular viral and bacterial pathogens. Th2 cells are considered more anti-inflammatory and produce the cytokines IL-4, IL-5, and IL-13 to generate immune responses directed against extracellular pathogens. Regulatory T cells (Tregs) are another subtype of CD4 T cells that suppress immune responses via multiple mechanisms, including the secretion of anti-inflammatory cytokines (e.g., IL-10 and transforming growth factor beta (TGF-β)), the expression of regulatory cell surface receptors (e.g., cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)), and the direct killing of APCs via perforin and granzyme B.

T cells are directly involved in the immunopathogenesis of T1D, and the link between autoimmunity and particular MHC alleles has been well established. In fact, numerous studies have demonstrated that MHC is the major genetic determinant for T1D [12][13][14][15][16][17][18][19][20]. Conversely, the presence of diabetes-resistant MHC alleles in transgenic mouse models can both induce the deletion of autoreactive T cells and also promote the development of Tregs [15][16][17].

Therefore, the MHC genotype may determine the balance between proinflammatory and anti-inflammatory responses to a given self-antigen. In humans, MHC proteins are encoded by the human leukocyte antigen (HLA) genes, and variants of these genes can confer significant disease risk or protection from autoimmune diseases, including T1D [21]. For example, individuals with the HLA-DQ8 (DQB1*03:02) allele have an odds ratio for T1D development of 11, while those with HLA-DQ6 (DQB1*06:02) are protected from T1D with an odds ratio of only 0.03 [22][23][24]. T1D research has primarily been focused on identifying peptides that activate T cells via presentation by diabetes risk MHC class II molecules [25]. However, little is known about the mechanisms by which protective MHC molecules provide dominant protection from T1D development.

3. Immunologic Tolerance by Regulatory T Cells

Two types of Tregs include thymus-derived natural Tregs (nTregs) and peripherally induced Tregs (iTregs). nTregs express the transcription factor forkhead box P3 (Foxp3) and suppress other T cell subsets during inflammation, whereas iTregs are conventional T cells that are induced to express Foxp3 and become regulatory in peripheral lymphoid tissues. A subtype of iTreg cells, known as type 1 regulatory (Tr1) cells, do not express high levels of Foxp3 but rather CD49b and lymphocyte-activation gene 3 (LAG-3) and are able to produce high levels of both IL-10 and TGF-β [26][27]. Thus, Tr1 cells are potent suppressors of other T cell subsets via cytokine-mediated mechanisms. However, Tr1 cells can also mediate suppression via granzyme-mediated killing in a cell contact-dependent fashion [28]. Distinct subtypes of Tr1 cells have now been identified in both humans and mice, and the subtypes differ primarily in the cytokine secretion profiles [29].

Tregs are not only important for the return to a homeostatic state after an immune response, but they are also critical for the prevention of autoimmunity. For example, Tregs induce tolerance in the periphery by suppressing autoreactive T cells that are specific for self-tissues. However, the precise molecular mechanisms by which Tregs suppress auto-antigen-specific T cells are unknown, and it is of interest to determine which antigens are being recognized by Tregs to prevent autoimmunity. It is appreciated that T1D patients do not lack overall numbers of Tregs but likely have a functional defect in bulk Tregs, which contributes to disease development [30][31][32][33]. However, researchers will focus on studies that identified and evaluated islet antigen-specific Tregs in autoimmune T1D.

4. Antigen-Specific Tregs in Type 1 Diabetes

Although the deletion of most autoreactive T cells occurs in the thymus, self-reactive T cells do escape and are able to circulate in the periphery [34]. These escaped self-reactive T cells can be controlled by Tregs as one mechanism of peripheral tolerance. Determining the specificity of these Tregs may aid in understanding the mechanisms involved in the loss of tolerance to self-antigens that occurs during autoimmunity and identifying specific antigens and epitopes that may be utilized for antigen-specific immunotherapy to treat the underlying autoimmunity.

In T1D, Kwok et al. showed that islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP)-specific T cells from healthy individuals and those with T1D could produce both proinflammatory (i.e., IFN-γ) and anti-inflammatory (i.e., IL-10) cytokines, indicating that antigen-specific Th1 cells and Tregs are present in the peripheral blood [35]. The IGRP-specific T cells were detected using HLA-DR4 and -DR3 tetramers, and therefore, they were of high avidity for peptide/MHC. However, because both pro- and anti-inflammatory IGRP-specific T cells were present in healthy individuals in addition to T1D patients, the results indicate that the escape of high avidity self-reactive T cells is not sufficient to cause disease. Likely, there is an imbalance in the pathogenic CD4 T cells and protective Tregs, as measured by pro- and anti-inflammatory cytokine responses, to islet self-antigens that skews the response during T1D development with a threshold needing to be met for autoreactive T cells to target pancreatic islets.

Data in support of a cytokine imbalance toward self-antigens come from studies in the researchers' laboratory that measured cytokine responses to native and mutated insulin B chain peptides using a cytokine enzyme linked immunospot (ELISPOT) assay with peripheral blood immune cells from new-onset T1D patients as well as non-diabetic controls [36]. Importantly, the majority of individuals in both groups were carrying at least one diabetes-risk HLA allele (e.g., DQ8 or DQ2). The strongest proinflammatory T cell responses were found in response to a mutated insulin peptide—much more than the native peptide sequence. The amino acid substitution in the mutated insulin B chain peptide (B22R→E) allows the peptide to bind in an otherwise unfavorable register to T1D-risk HLA molecules (i.e., DQ8 and DQ2). In addition, anti-inflammatory responses were present in the vast majority of the non-diabetic subjects and several T1D patients. Both the IFN-γ and the IL-10 responses were greater in non-diabetic individuals who carried at least one non T1D-risk DQ allele. These results indicate that in non-diabetic individuals, the presence of a diabetes protective or neutral HLA-DQ molecule may lead to a regulatory T cell response to insulin, whereas in T1D individuals, this ability may be muted or absent. Other studies using cytokine ELISPOT assays with epitopes from beta cell specific self-antigens, proinsulin, and insulinoma-

associated antigen 2 (IA-2) provide similar results with a proinflammatory response in disease and regulatory response in health [37][38].

Recently, researchers identified both pro- and anti-inflammatory cytokine T cell responses to hybrid insulin peptides (HIPs) from longitudinal peripheral blood samples of individuals genetically at risk for T1D who either developed islet autoantibodies or remained seronegative [39]. HIPs are neoantigens that form in the lysozymes of beta cells via a covalent bond between a fragment of C-peptide and another peptide from a beta cell protein [40]. In this manner, autoreactive T cells that are specific for HIPs may escape into the periphery because these neoantigens are not presented in the thymus during T cell education. In this entry, individuals who became autoantibody positive or who progressed to clinical T1D (high blood sugars requiring exogenous insulin treatment) had a predominantly proinflammatory response to the HIPs, and these responses correlated to worsening measurements of blood glucose control. Separate studies have also found T cell responses to HIPs in newly diagnosed T1D patients [41][42][43].

As islet antigen-specific Tregs are present within healthy individuals, these cells have been cloned after culture with IA-2 (IA-$2_{709–736}$) or proinsulin peptides (B:11–30, B:9–28) [44]. Interestingly, although the cells produced IL-10 in response to the islet antigen and were able to suppress the proliferation of T cells, the study found that direct cell-to-cell contact was required for the suppression to occur. The autoantigen-specific Tregs were further able to express cytotoxic molecules (i.e., granzyme A and granzyme B) and directly kill islet autoantigen-loaded antigen-presenting cells in a perforin/granzyme-dependent manner. These results indicate that antigen-specific Tregs are potent regulators of pathogenic T cells as well as antigen presenting cells in healthy individuals.

The age of T1D onset may also help direct T cell responses to beta cell proteins, as Ueno et al. measured CD4 T cell responses to glutamic acid decarboxylase (GAD), preproinsulin, and IGRP in adult-onset versus childhood-onset T1D patients [45]. In adult patients, there was predominantly a Th1 response to IGRP, whereas those with childhood onset had a Th2 immune response with Tr1 regulatory cells. In fact, the frequency of Tr1 cells responding to IGRP in adult-onset patients was much lower than in childhood-onset patients and non-diabetic controls. These results indicate that distinct subsets of CD4 T cells may respond to IGRP differently and could influence the timing of disease onset.

Taken together, these studies indicate that during T1D disease development, there are anti-inflammatory responses to islet autoantigens; however, there is either a defect in that response or the proinflammatory immune response predominates. This concept is highlighted by data from a spontaneous animal model of autoimmune diabetes in which there are regulatory T cells within the inflamed pancreatic islets that are directed against an immunodominant peptide, insulin B:9–23, but insulitis and diabetes still develop [46]. At the current time, it is unknown and under investigation whether islet antigen-specific Tregs are present in human insulitis.

5. Structural Basis of a Treg T Cell Receptor Recognizing Peptide/MHC

To better understand the structural basis of a proinsulin-specific Treg, Rossjohn et al. solved the crystal structure of a Treg T cell receptor (TCR) binding to proinsulin/DR4 and compared this to the canonical TCR docking pattern for a proinsulin-specific effector T cell [47]. Proinsulin-specific Tregs were cloned and then induced to become iTregs via interaction with tolerogenic dendritic cells. Typically, the α chain of the TCR docks over the β chain of the MHC class II molecule, while the β chain of the TCR docks over the MHCα. However, this Treg crystal structure determined that the TCRα chain overlaid the MHCα chain, while the TCRβ chain overlaid the β chain of the proinsulin/DR4 complex. Thus, a possible structural mechanism exists with reversed polarity docking of the TCR that may account for the difference in response when Tregs recognize a self-antigen versus effector T cells for the same peptide/MHC complex. Although the study identified a novel docking motif for proinsulin-specific Tregs in the context of DR4, it is unknown whether this unusual docking motif extends to other Treg cell specificities at this time.

In a separate study, two murine CD8 T cell receptors specific for a nucleoprotein epitope (NP_{366}) presented by the MHC class I molecule, H-$2D^b$, docked with a 180 degree position relative to other CD8 TCR-peptide-MHC class I complexes [48]. Although not a Treg TCR–peptide–MHC class II interaction, the authors found that the responses were defective in downstream signal transduction and proliferative capacity. Therefore, irregular docking of a TCR on a peptide–MHC complex may play an important role in how T cells respond to various peptides, including self-peptides.

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