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Review article

Maintenance of peripheral tolerance to islet antigens

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ABSTRACT

Reestablishment of immune tolerance to the insulin-producing beta cells is the desired goal for type 1 diabetes (T1D) treatment and prevention. Immune tolerance to multiple islet antigens is defective in individuals with T1D, but the mechanisms involved are multifaceted and may involve loss of thymic and peripheral tolerance. In this review we discuss our current understanding of the varied mechanisms by which peripheral tolerance to islet antigens is maintained in healthy individuals where genetic protection from T1D is present and how this fails in those with genetic susceptibility to disease. Novel findings in regards to expression of neo-islet antigens, non-classical regulatory cell subsets and the impact of specific genetic variants on tolerance induction are discussed.

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1. Introduction

Maintenance of self-tolerance and immune regulation are critical functions of the immune system as a means of both preventing autoimmunity and terminating immune responses to pathogens. Self-tolerance is secured by multiple mechanisms involving both deletion and induction of unresponsiveness as well as

Abbreviations: APC, antigen presenting cell; DC, dendritic cell; *Idd*, insulin-dependent diabetes; NOD, non-obese diabetic; PcLN, pancreatic lymph node; T1D, type 1 diabetes; Treg, regulatory T cell; Teff, T effector cell.

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immunoregulation. In the thymus, developing T-cells with high avidity for self-antigens are deleted during negative selection. Some self-reactive T-cells still escape from the thymus after which they may be deleted in the peripheral lymphoid organs, be made unresponsive or be suppressed by regulatory T-cells. In type 1 diabetes (T1D), multiple disease associated genetic variants are thought to lead to defects in regulation of the immune response, tipping the immune system towards loss of self-tolerance to the insulin-producing beta cells in the pancreas. This review will focus on recent progress in our understanding of the failure of peripheral tolerance mechanisms in mouse models of T1D and how this informs our knowledge of human disease.

1.1. Normal peripheral tolerance mechanisms

While thymic tolerance greatly reduces the frequency of circulating, potentially autoreactive T cells, this tolerance mechanism is incomplete and additional mechanisms are required to prevent autoimmune disease. Following exit from the thymus, self-antigen specific T cell clones first encounter cognate antigen presented by dendritic cells in lymph nodes draining the site of antigen expression [1]. The outcome of this encounter in non-autoimmune prone mice and presumably in healthy humans is tolerance, which may be classified as either cell-intrinsic (deletion and anergy) or cell-extrinsic.

1.1.1. Deletion

The deletion or induction of functional unresponsiveness in an autoreactive T cell requires the interaction of the T cell with an antigen-presenting cell (APC) presenting cognate antigen. Full T cell activation leading to effector differentiation and formation of memory requires recognition of peptide−MHC complex on APC together with a second costimulatory signal such as CD80/CD86 presented by mature APC. APC maturation, induced for example in the presence of pathogen derived molecular patterns, results in third signals such as proinflammatory cytokine production that further reinforce T cell activation. The result of an unproductive encounter, in the absence of a strong co-stimulatory signal, between an autoreactive T cell and a quiescent APC presenting cognate antigen is either the deletion of that T cell or the T cell entering an unresponsive state [2]. Factors that can influence whether deletion rather than anergy result upon T cell encounter with quiescent APC include TCR affinity, antigen density and the duration of antigen exposure [3,4]. Peripheral deletion has been well characterized using antigen-specific TCR transgenic T cells specific for model self-antigens expressed in a variety of contexts such as under the expression of the insulin promoter amongst many others [5,6]. During deletional tolerance, there is an initial period of abortive T cell proliferation preceding the death of the self-reactive T cells via apoptosis [7]. The apoptotic pathway of deletional tolerance involves the Bcl-2 family member Bim, which induces the release of cytochrome *c* and entry into the caspase cascade [8]. $CD8^+$ T cells undergoing deletional tolerance exhibit a distinct gene profile, with reduced up-regulation of markers of functional ability, as well as marked up-regulation of death pathway genes including Bim, receptor activator of, NF-κB ligand (RANKL) and programmed death 1 (PD-1) [9,10].

1.1.2. Anergy

Anergy can occur when T cells persist in an unresponsive state following antigen encounter in the absence of a costimulatory signal [11]. The development of anergic-like T cells *in vivo* tends to be greatest when high amounts of antigen are ubiquitously present on both quiescent APC and parenchymal cells, for example following high-dose peptide injection or global transgene expression [4,12–14]. This anergic state is characterized by reduced TCR signalling and IL-2 expression [15]. The molecular features of anergic cells include upregulation of immunosuppressive molecules such as FR4 and CD73 [16]. Anergy induction can occur via signalling through alternate receptors such as Cytotoxic T lymphocyte antigen-4 (CTLA-4), which competes with CD28 for binding of its ligands CD80/CD86 [17]. For $CD8^+$ T cells, continual exposure to a high affinity TCR signal resulted in anergy [4,18]. Following TCR ligation with sub-optimal antigen doses, potent phosphorylation of activation-related TCR signalling molecules (e.g. Lck, CD3ζ, ZAP70, LAT and SLP-76) occurred [19]. However, phosphorylation of anergy-associated inhibitory signalling molecules (e.g. c-cbl, SHP-2 or CrkL) did not sharply increase until higher antigen doses were used [19]. Thus a 'molecular switch' linked to TCR signal strength controls cell fate. Removal of anergic cells from their antigen source allowed cells to later be reactivated [18,20,21], posing a potential problem for the maintenance of self-tolerance if high-affinity self-reactive T cells are still present in the repertoire.

1.1.3. Cell-extrinsic peripheral tolerance mechanisms

Cell-extrinsic peripheral tolerance mechanisms involve suppression of effector cells by cells with regulatory properties. There are a number of cell types with regulatory abilities including conventional regulatory T cells (Treg), T helper 3 (Th3) and type 1 regulatory T cells (Tr1). Conventional Tregs, as characterized by CD4, CD25 and FoxP3 expression are critical in maintaining peripheral tolerance and thereby preventing the development of autoimmune disease [22,23]. Treg deficiency in humans occurs in individuals with mutations in the *FOXP3* gene and results in severe, multi-organ autoimmunity including T1D [24]. Tregs can suppress through contact-dependent or −independent mechanisms. Contact-dependent suppression mechanisms include ligation of CTLA-4 on the surface of Treg with CD80/CD86 on APCs, which acts to inhibit co-stimulation and cytokine secretion of APC [25]. Tregs may also cause direct cytolysis of effector cells as a result of perforin and granzyme secretion by Treg [26]. Contact-independent mechanism of Treg function include the release of the regulatory cytokines interleukin (IL)-10, IL-35 and transforming growth factor (TGF)-β [27–29] as well as depriving effector cells of IL-2 [30].

Contact-dependent effector T cell suppression can also be mediated through the PD1/PD-L1 pathway independently of Tregs. PD-1 is an inhibitory costimulatory molecule upregulated on T cells following their activation and is characteristically maintained at high levels on exhausted T cells. APCs as well as epithelial and vascular endothelial cells express PD-L1, which delivers an inhibitory signal to the T cell, suppressing the effects of TCR signalling. Recently, PD-1/PD-L1 pathway blockade has become a promising new therapy for the treatment of cancer. Strikingly, several case reports have described the rapid onset of type 1 diabetes following PD-1 pathway blockade in older patients [31–33], suggesting that autoreactive T cells present in these patients are held in check by PD-1. It was thought that PD-1 blockade could cause the reactivation of anergic T cells present in these patients. However, recent studies in NOD mice have shown that although both effector and anergic populations of islet-specific T cells expressed PD-1, it was the effector phenotype T cells that expanded and increased their IFNγ production following anti-PD-L1 treatment [34]. Thus, PD-1 treatment could invigorate effector T cell populations without reversing the functional state of previously tolerized populations.

1.2. Failure of peripheral tolerance mechanisms in T1D

In order for autoimmunity to occur, it is believed that central tolerance, peripheral tolerance and immunoregulation are all defective in genetically predisposed individuals (Fig. 1A). This is

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