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Extrathymic mechanisms of T cell tolerance: Lessons from autoimmune gastritis

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A B S T R A C T

While the thymus plays a key role in the prevention of many autoimmune phenomena it is clear that robust mechanisms external to the thymus are also vital in controlling self-reactive T cells. Here we review the current concepts in the field of extrathymic tolerance and use recent studies of autoimmune gastritis to illustrate how T cells directed to a prominent, clinically relevant autoantigen, namely the gastric proton pump, can be silenced with little or no thymic involvement. Autoimmune gastritis represents one of the most thoroughly characterised autoimmune systems and the knowledge and tools available to study this disease will continue to allow a thorough assessment of the genetic, cellular and molecular events that underlie tolerance and autoimmunity.

1. Introduction

One of the hallmarks of the immune response is the ability of T cells to recognise and respond to foreign antigens derived from invading organisms, while at the same time avoiding excessive damage by ignoring self antigens and foreign antigens that do not pose a threat. Failure to maintain this tolerance to self can have pathogenic consequences, resulting in autoimmune disease. Therefore, a variety of intrathymic and extrathymic mechanisms exist to induce T cell tolerance. This review discusses some of the mechanisms by which T cell tolerance is maintained to protect from autoimmune disease, specifically using autoimmune gastritis as an example.

1.1. Central tolerance

The great majority of T cells develop in the thymus; therefore this is often the first site at which tolerance mechanisms are enacted to eliminate autoreactive cells from the developing T cell pool. Intrathymic editing of the TCR repertoire is a major mechanism of self-tolerance, ensuring that the thymocytes bearing a TCR with a high affinity for self peptide:MHC complexes are deleted prior to T cells emerging from the thymus and encountering self antigen in the periphery [1].

To ensure effective central tolerance to a variety of autoantigens, in addition to ubiquitously expressed proteins that are present in the thymus, developing thymocytes are also exposed to many organ or tissue-specific antigens. In the mouse, these promiscuously expressed antigens may represent as much as 10% of the entire genome [2]. Thymic antigen presenting cells with key roles in tolerance express aire, which regulates the ectopic expression of tissue-restricted antigens, although not all peripheral antigens depend on aire for their expression in the thymus [3]. The AIRE gene was identified as the gene mutated in patients with the autosomal recessive autoimmune condition autoimmune polyendocrinopathy, candidiasis and ectodermal dystrophy (APECED) syndrome, which is one of the few autoimmune diseases that is essentially monogenic in its aetiology.

However, expression of individual proteins in the thymus may be insufficient for effective central tolerance [4]. For autoreactive T cells to be deleted, antigenic peptides derived from these proteins must be effectively processed and presented to developing thymocytes [5]. Furthermore, not every self antigen can be expressed in the thymus, and environmental non-self antigens such as those derived from food or commensal microorganisms are not available to developing thymocytes.

1.2. Peripheral tolerance

Due to the incomplete nature of central tolerance, there is a constant risk that autoreactive T cells that have escaped thymic selection might become activated, with potentially pathogenic consequences. Therefore, further mechanisms of T cell tolerance are enacted in the periphery to ensure that autoaggressive T cells are either controlled by the actions of regulatory T cells (T_{REG}), or purged from the repertoire by extrathymic deletion or inactivation. The immunological outcome of TCR engagement in the periphery – tolerance or activation – is determined by the context in which the T cell encounters antigen. Therefore, much research has focussed on

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the role of the antigen-presenting cell in tolerance induction, with particular attention given to dendritic cells (DC).

In the steady state, DC are able to traffic freely through tissues and capture antigen, sampling their environment before migrating to the lymph nodes and presenting antigen to T cells [6]. It has been proposed that the maturation state of the dendritic cell defines its tolerogenic function, with immature or semi-mature DC inducing T cell tolerance rather than immunity [7]. However, a tolerogenic phenotype may also be actively induced in DC, by specific signals delivered either through cytokines [8], antigen receptors [9], or the uptake of apoptotic cells [10].

Although the short half life of apoptotic cells *in vivo* makes it difficult to directly measure T cell deletion in an experimental setting [11], the disappearance of antigen-specific cells following an initial period of activation and expansion is generally used as an indication that these cells are being purged from the repertoire [12]. This transient T cell activation and subsequent decline in the number of antigen-specific cells is observed when antigens are targeted to steady state DC, for example via antigen-loaded apoptotic cells [10] or by using antibodies to the endocytic receptor DEC-205 [13]. In these studies, although the majority of antigen-specific T cells are deleted, repertoire purging is incomplete and a small population of T cells remains. Despite the persistence of antigen-specific cells, T cell tolerance is maintained. Although these cells are not deleted, many have been rendered anergic; an actively induced process of T cell tolerance in which the cells are unable to respond to antigenic stimulation via their TCR [14].

2. $CD4^+Foxp3^+$ regulatory T cells

Potentially damaging autoimmune responses can also be prevented in the periphery through the actions of regulatory T cells, which suppress the activation and effector functions of other T cells. A number of T cell lineages with regulatory functions have been described that differ with respect to their development and mode of action [15]. One of the best characterised of these populations are the Foxp3-expressing T_{REG} cells, which develop in the thymus [16], and can also be induced in the periphery by conversion of conventional naïve T cells [17,18]. Expression of the Foxp3 transcription factor is essential for the development and maintenance of functionally suppressive T_{REG} cells [19–21]. However, the development of T_{REG}-like cells in the absence of functional Foxp3 indicates that factors upstream of Foxp3 are responsible for commitment of T cells to the T_{REG} lineage [20].

In the periphery, $Foxp3^+$ T_{REG} cells constitute approximately 5–10% of all $CD4^+$ cells [18,22]. This population is not dependent on constant thymic output of T cells [23], and maintenance of Foxp3 expression in the periphery is dependent on both IL-2 and TGFβ [23,24]. Both these cytokines are also essential for de novo generation of $Foxp3^+$ T_{REG} cells from conventional naïve T cells [25]. At mucosal surfaces such as the gastrointestinal tract, lymphocytes are exposed to an enormous variety of environmental antigens, which may or may not pose a threat to the host. Therefore, the ability to regulate immune responses at these sites is imperative, and mucosal DC appear to be specially equipped for tolerance induction [26]. The gut associated lymphoid tissue (GALT) is thus emerging as a major site for the peripheral generation of $Foxp3^+$ T_{REG}. DC isolated from the lamina propria and mesenteric lymph nodes are able to specifically induce Foxp3 expression in naïve T cells through the production of TGFβ and the vitamin A metabolite retinoic acid [27–30]. In addition to promoting the generation of regulatory T cells, retinoic acid also inhibits the TGFβ-mediated differentiation of the pro-inflammatory T_H17 lineage [27]. IL-6 has the opposite effect to that of retinoic acid, inhibiting T_{REG} differentiation and promoting T_H17 polarization, thus the development of T_H17 cells and T_{REG} cells is linked by a reciprocal developmental pathway [27,31].

Regulatory T cells can suppress effector cell responses by a variety of mechanisms, including inhibition of cell cycling and IL-2 production in effector cells by cell contact dependent mechanisms, and secretion of immunomodulatory cytokines such as IL-10 and TGFβ [32]. Although T_{REG} cells require TCR stimulation in order to suppress effector T cell responses, their action is not antigen-specific, and the T_{REG} cell need not recognise the same antigen as its target cell [33]. However, data from a number of autoimmune disease studies suggests that disease-protective T_{REG} recognise antigens that are at least derived from the same tissue as that targeted by the pathogenic cells. Peripheral T_{REG} from adult rats lose their ability to protect against thyroiditis, but not diabetes, when the thyroid of the T_{REG} donor is ablated in utero [34]. In the mouse, diabetes-protective T_{REG} are found preferentially in the pancreatic lymph node [35], and islet-specific T_{REG} cells are more effective than polyclonal T_{REG} at preventing diabetes in NOD mice [36]. The ability of polyclonal T_{REG} to suppress both prostatitis and autoimmune ovarian disease increases with exposure to antigen in the periphery, and the protective cells localise to the lymph node draining the site of antigen expression [37]. Collectively, these data suggest that recognition of tissue-specific antigens by T_{REG} cells may contribute to their ability to localise to and become activated in the lymph nodes draining that tissue, thus preventing autoimmunity at that site.

3. Pernicious anemia and autoimmune gastritis

Pernicious anemia is the end stage of autoimmune gastritis. It is one of the commonest autoimmune diseases, with a prevalence in Western populations over the age of 60 of 1.9%, and it represents the commonest cause of vitamin B12 deficiency [38]. Autoimmune gastritis shares many features with autoimmune diabetes, autoimmune thyroiditis and autoimmune diseases of the gonads. The disease is characterised by a monocytic infiltrate of the gastric mucosa, depletion of differentiated mucosal cells, and an immune response to the gastric H^+/K^+ ATPase, the proton pump expressed by parietal cells responsible for acidification of the gastric lumen [38,39].

Experimental autoimmune gastritis in mice is one of the best characterised autoimmune diseases at the molecular, cellular and genetic levels, and thus represents a highly defined model of human pernicious anemia [40]. The disease is characterised by an influx of $CD4^+$ T cells and macrophages into the gastric mucosa, and this inflammation is accompanied by a loss of the parietal and zymogenic cells of the stomach. The causative autoantigen in mouse autoimmune gastritis is also the H^+/K^+ ATPase. The H^+/K^+ ATPase is a heterodimeric protein consisting of an α- and a β-subunit (H/Kα and H/Kβ, respectively), both of which are targeted by the autoaggressive $CD4^+$ T cells [39].

Our current level of understanding of disease susceptibility, initiation and pathogenesis has been facilitated through the availability of a range of transgenic mice that are deficient in or overexpress either subunit of H^+/K^+ ATPase, or that express a transgenic TCR specific for epitopes derived from either H/Kα or H/Kβ [41–45].

3.1. Genetic susceptibility to autoimmune gastritis

There were two early unsuccessful attempts at identifying gastritis susceptibility genes in mice. A study utilising a (BALB/cBy × C57BL/6By) cross found that a gene/s near the minor histocompatibility antigen 27 (*H27*) on chromosome 5 was genetically linked with susceptibility to autoimmune gastritis [46]. However, this association could not be confirmed in subsequent studies [47,48]. The second study utilised a (BALB/cCrSlc × DBA/2CrSlc) cross and found genetic linkage of the *Mls-1* gene on chromosome 1 with susceptibility to autoimmune gastritis [49]. However, the study relied on a low-resolution genetic map and subsequent