



The thymus and rheumatology: should we care?

Emilie Cosway^a, Graham Anderson^a, Paul Garside^b, and
Catriona Prendergast^b

Purpose of review

The purpose of this review is to discuss the mechanisms of central and peripheral tolerance in relation to T-cell mediated autoimmunity in rheumatoid arthritis (RA).

Recent findings

The well established association between major histocompatibility complex class II and RA has led us to understand that T cells, and the adaptive immune response, are important in the pathogenesis of disease. In order for autoimmune disease to develop, there is a breach of tolerance to self antigen and the mechanisms of both central and peripheral tolerance aim to prevent this. Here, we review evidence from mouse models indicating that alterations in T-cell receptor signalling thresholds during thymic selection may be linked to the escape of T cells that mediate autoimmune arthritis. In addition, we summarize the role of dendritic cells and Foxp3⁺ regulatory T cells in both peripheral and thymic tolerance, and highlight their relevance to what we know about the aetiology of RA.

Summary

Mechanisms of central tolerance in the thymus and peripheral tolerance are in place to control autoreactive T cells and to prevent the development of autoimmune disease. We anticipate that a better understanding of these mechanisms will lead to the development of better, antigen-specific therapeutics to restore tolerance.

Keywords

dendritic cell, T cell, thymus, tolerance, Treg

INTRODUCTION

The immune system is heavily implicated in the pathogenesis of rheumatoid arthritis (RA) and there is a generally accepted view that some type of ‘breach of self-tolerance’ underlies this. However, the exact mechanisms involved, whether these relate to differences in central versus peripheral tolerance, and how this relates to the aetiology of the disease remain unclear. Here, we discuss how defects in central and peripheral tolerance may be involved, how they might be linked and how they relate to what we know about RA. The ultimate goal for treatment of autoimmune disease would be the development of therapies that promote and restore self-tolerance in patients. As a result, understanding central and peripheral tolerogenic mechanisms that have gone awry in RA will lead to the development of improved therapeutics that target the restoration of tolerance.

CENTRAL TOLERANCE MECHANISMS IN RHEUMATOID ARTHRITIS

The antigen receptors of T cells are randomly generated to provide the potential to recognize a wide

array of pathogens. As a consequence, self-reactive T cells are generated. To avoid autoimmunity, regulatory mechanisms must be in place to remove and/or control these cells. This ‘immunological tolerance’ is mediated at several levels and in several cell types. Here, we will focus on tolerance in T cells in the context of RA.

Thymic selection

The thymus supports the development of T cells expressing the $\alpha\beta$ form of the T-cell receptor.

^aMRC Centre for Immunology, Institute for Immunology and Immunotherapy, Medical School, University of Birmingham, Birmingham and ^bCentre for Immunobiology, Institute of Infection, Immunology and Inflammation, Glasgow Biomedical Research Centre, University of Glasgow, Glasgow, UK

Correspondence to Paul Garside, Centre for Immunobiology, Institute of Immunology, Infection and Inflammation, Glasgow Biomedical Research Centre, University of Glasgow, 120 University Place, Glasgow G12 8TA, UK. Tel: +44 01 413307251; e-mail: paul.garside@glasgow.ac.uk

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KEY POINTS

- In murine models of RA, alterations in $\alpha\beta$ TCR signalling thresholds have been linked to the escape of autoreactive T cells from central tolerance.
- Peripheral tolerance mechanisms sometimes fail to control autoreactive T cells leading to development of RA. Targeting peripheral tolerance mechanisms, as opposed to central tolerance mechanisms, is a feasible approach to restore tolerance therapeutically.
- Although both Foxp3⁺ Treg and dendritic cells enter the thymus from peripheral tissues to influence intrathymic tolerance mechanisms, whether alterations in this process are linked to T-cell mediated autoimmunity is not known.
- There has been a resurgence of interest in the treatment of RA via restoration of antigen-specific tolerance, thus a clearer understanding of the underlying mechanisms is required.

Following their entry at the corticomedullary junction [1], CD4⁻ CD8⁻ lymphoid precursors randomly rearrange their T-cell receptor (TCR) genes to maximize variation within the developing T-cell repertoire. Following the induction of CD4 and CD8 expression [2], these processes generate a large cohort of CD4⁺CD8⁺ thymocytes that express a wide range of $\alpha\beta$ TCR specificities. Owing to the random nature of TCR gene rearrangement, such cells undergo selection processes to ensure the thymus is biased toward the generation of self-tolerant T cells capable of self-major histocompatibility complex (MHC) recognition.

During positive selection, recognition of self-peptides bound to MHC II or MHC I molecules on cortical thymic epithelial cells ensures the generation of single positive CD4⁺ and CD8⁺ thymocytes, respectively [3]. During this process, newly selected thymocytes undergo Chemokine Receptor 7 (CCR7)-mediated migration to the thymic medulla (Fig. 1), an important site for tolerance induction [4]. Here, CD4⁺ and CD8⁺ thymocytes are screened further for their reactivity to self-antigens, including those expressed intrathymically as a result of the Autoimmune regulator (AIRE)-mediated transcription of tissue-restricted antigens in medullary thymic epithelial cells [5,6]. In the medulla, dendritic cells also play a key role in thymic tolerance through both cross-presentation of self-antigens from medullary thymic epithelial cells as well as migration of self-antigen bearing dendritic cells from the periphery [7]. Importantly, the outcome of TCR-MHC interactions within the medulla is highly dependent upon how strongly the TCR recognizes

self-antigen. High affinity TCR-mediated interactions result in negative selection through apoptosis induction, whereas medium affinity interactions induce expression of the transcription factor Foxp3, and the emergence of the natural T-regulatory (Treg) lineage [8]. Collectively, T-cell development involves intrathymic selection events based upon self-antigen interaction, to maximize the spectrum of antigen recognition by newly selected T cells and also minimize the risk of generating autoreactive T cells. As a consequence, factors with the potential to alter TCR signal strength in response to self-antigen during intrathymic development can lead to alterations in thymic tolerance and potentially skew the T-cell repertoire, leading to autoimmune disease.

Rheumatoid arthritis and altered T-cell selection

As noted above, RA is a common autoimmune disease with the cause being linked to a combination of both genetic and environmental factors [9]. Various murine models have improved our understanding of the genetic aspects of the disease with a particular focus on mutations that alter central and peripheral T-cell tolerance. For instance, under circumstances where mutations dampen the perception of TCR affinity strength during selection, high affinity autoreactive TCRs may escape negative selection. These instead are allowed to enter into the peripheral T-cell pool, where they have a greater propensity to react with self and elicit an autoimmune phenotype [8]. There are a number of models and clinical examples which illustrate this.

The murine SKG model of autoimmune arthritis

Mice with a recessive point mutation in the gene encoding the TCR signalling protein Zeta-chain-associated protein kinase 70 (ZAP-70), are known as SKG mice and are frequently used as a model for autoimmune arthritis. SKG mice have suppressed TCR signal perception allowing selection of a more autoreactive T-cell repertoire in the thymus that peripherally contributes to the development of autoimmune arthritis [10]. To illustrate this, autologous mixed lymphocyte reactions were used to examine the reactivity of peripheral SKG T cells. These were shown to have high levels of proliferation and activation to autologous Antigen Presenting Cells (APCs) when compared with control T cells, highlighting their autoreactive nature [11]. In addition, analysis of TCR Variable Beta subunit usage showed preferential expression of certain

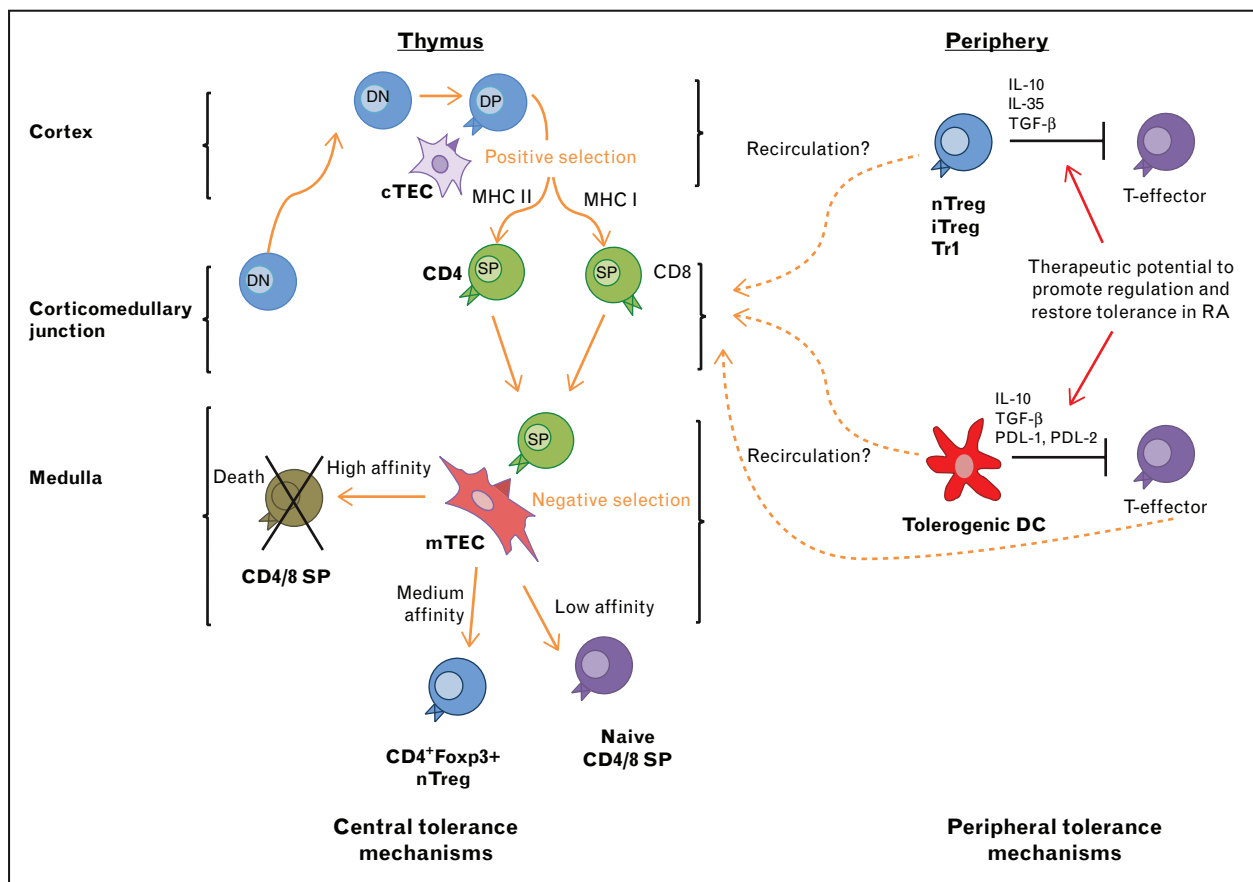


FIGURE 1. Regulation of central and peripheral tolerance. The organization of the thymus into cortex and medulla ensures that thymic selection events take place in a stepwise fashion. In the medulla, tolerance-inducing events coordinated by medullary thymic epithelial cells and dendritic cells help to remove potentially autoreactive specificities from the newly produced T-cell pool, and also result in the generation of Foxp3⁺ regulatory T cells. In peripheral tissues, several immunoregulatory products of Dendritic cells and Treg have been linked to the control of effector T cells that mount responses to self-antigens. Perhaps significantly, the thymus also acts as a site that supports T-cell and dendritic cell populations that enter from peripheral tissues, and evidence suggests a role for thymus recirculation in the control of T-cell tolerance. DN: CD4⁺CD8⁺; DP: CD4⁺CD8⁺; SP CD4⁺ or CD8⁺.

subfamilies with more autoreactive tendencies in SKG mice than controls [12]. When these TCRs were isolated, transfected into bone marrow cells and transferred into Rag2^{-/-} hosts, autoimmune arthritis was induced, reinforcing the idea that SKG mice have a skewed T-cell repertoire toward specificities that are more autoreactive [13^{***}]. Collectively, such observations suggest that alterations in the perception of TCR signal strength may drive the development of arthritis in SKG mice by altering intrathymic selection of the developing T-cell repertoire. In addition, intrathymically generated Treg from SKG mice show a defective suppressive capacity when transferred along with SKG conventional T cells into nude hosts [12,14], again suggesting that altering the strength of TCR signalling during intrathymic T-cell selection events directly impacts upon the T-cell repertoire that is selected, and increases susceptibility to abnormality [15].

As a consequence of altered thymic selection, autoreactive peripheral T cells can respond to self-antigens expressed by dendritic cells, triggering their activation and differentiation into functionally distinct T-cell subsets. In SKG mice, as with human RA, the main effector cells have been shown to be Th17 cells driven by APC-derived IL-6, with IL-6 deficient SKG mice being devoid of IL-17-producing CD4 T cells [11]. Interestingly, Th17 cells can be recruited to joints through CCR6 expression, attracted by the high CCL20 levels found in arthritic joints where they are thought to mediate innate cell activation and joint destruction [16].

Rheumatoid arthritis and PTPN22 variants

Protein tyrosine phosphatase, non-receptor type 22 (PTPN22) encodes lymphocyte tyrosine phosphatase (Lyp) or the mouse ortholog Pep, and is a critical

negative regulator of TCR signal transduction upstream of ZAP-70. A single nucleotide polymorphism in the protein tyrosine phosphatase N22 producing a PTPN22 variant (R620W) has been shown to support the progression of autoimmune disease such as type I diabetes and it was further suggested to be the second strongest genetic risk factor for RA, second to HLA variants in humans [17,18]. Interestingly, this PTPN22 variant (R620W) has been suggested to be a gain of function mutation, linked to reduced TCR signalling during intrathymic T-cell selection and high affinity self-reactive T cells in the periphery [17,19]. However, other studies have reported that the consequence of PTPN22 polymorphisms may be a decrease in phosphatase activity and an increase in TCR signalling [20].

Despite this discrepancy, a variant of Lyp (Lyp620W) which is encoded by PTPN22 has been associated with autoimmune disease in humans. Its effect on disease development can be studied using mice expressing the Lyp variant homolog Pep619W. Analysis of these mice carrying OT-II TCR or male H-Y antigen TCR transgenes showed increased CD4⁺ T-cell positive selection, proliferation of the memory/effector T-cell pool, but no alteration in negative selection indicating a complex requirement for functional Lyp in ensuring appropriate TCR signalling during T-cell selection [20]. In addition, a lack of PTPN22 expression was investigated in mice with regard to T-regulatory cells, which have been shown to express higher levels of PTPN22 than conventional T cells [21]. Interestingly, when compared with WT mice, Ptpn22^{-/-} mice showed significant increases in Treg and their precursor populations [21], suggesting alterations in TCR signalling are able to affect the balance between conventional and Treg development, which might further affect autoimmune status.

PERIPHERAL TOLERANCE MECHANISMS IN RHEUMATOID ARTHRITIS

Although mutations within the thymus can alter T-cell selection and skew the T-cell repertoire toward an autoreactive nature, under normal circumstances, the selection processes that are in place to prevent autoreactive T cells escaping from the thymus are still not 100% effective. As a result, there are always some autoreactive T cells that do escape, undetected, into the periphery. For this reason, the mechanisms of peripheral tolerance are vital to prevent the development of autoimmune disease. In this section, various mechanisms of peripheral tolerance will be discussed. These include regulation via Treg, and tolerogenic dendritic cells as well as

their relevance to RA, and how these mechanisms may fail in RA patients, leading to a breakdown of tolerance.

T-regulatory cells

Treg are known to be vital for the maintenance of immunological tolerance (reviewed in [22,23]). Along with natural CD4⁺CD25⁺Foxp3⁺ Treg (nTreg), other regulatory populations include inducible CD4⁺Foxp3⁺ Treg (iTreg), which develop in the periphery after induction of Foxp3⁺ expression, and CD4⁺Foxp3⁺ type 1 regulatory T cells (Tr1). Foxp3⁺ has been shown to be vital for the development and suppressive function of Treg [24] as in its absence aggressive autoimmune disease develops as self-tolerance is broken [25–29]. Treg manifest their suppressive function through various mechanisms including direct cell-to-cell contact, or indirectly through the secretion of anti-inflammatory cytokines (e.g. IL-10, IL-35, or TGF-β) as IL-10 or IL-35 deficient have an impaired suppressive capacity [30,31]. The secretion of TGF-β can induce the development of further Treg from CD4⁺CD25⁻ naïve T cells [32,33]. Treg can also act by reducing the functions of APCs by inhibiting CD80 and CD86 expression, via a CTLA-4 dependent mechanism [34–36].

Murine studies, using collagen-induced arthritis as a model of RA, have shown that the depletion of Treg using anti-CD25 resulted in increased disease severity [37], and reduced disease severity was observed when Treg were introduced by adoptive transfer [38]. Treg from RA patients have been shown to be defective in their ability to suppress proinflammatory cytokine production by CD4⁺ T cells, although they can efficiently suppress their proliferation; this defect in their suppressive abilities may be because of a defect in CTLA-4 expression and function [39–41]. B cells are known to have a key pathogenic role in the aetiology of RA, as evidenced by the efficacy of rituximab treatment in RA patients [42,43]. In relation to this, Treg have been shown to regulate the pathogenic function of B cells during inflammation both in murine models and in RA itself [44,45].

Tr1 cells mediate their suppressive function through the secretion of IL-10 and TGF-β and are known to suppress both immune and autoimmune responses [46]. A population of antigen-specific IL-10-producing Tr1 cells has been identified in the blood of RA patients [47] indicating they may have a role in maintenance of peripheral tolerance in RA. The transfer of Tr1 cells in murine models of RA was found to be beneficial, reducing the incidence and severity of arthritis when administered before and after induction of disease [48]. The Tr1

cell population could, therefore, be a promising target for the restoration of tolerance in autoimmune diseases, including RA.

Role of dendritic cells in the regulation of peripheral tolerance

Dendritic cells are vital for the induction of the inflammatory immune response against, for example, invading pathogens. In addition to this, dendritic cells are also central to immune regulation and inducing and maintaining tolerance (Fig. 1), as demonstrated by the development of fatal spontaneous autoimmune disease following dendritic cell depletion [49].

'Tolerogenic' dendritic cells are generated through the incomplete maturation of dendritic cells, as occurs during the steady state. These tolerogenic dendritic cells present antigen to T cells in the absence of adequate costimulatory signals and result in the maintenance of peripheral tolerance through mechanisms including T-cell deletion, unresponsiveness or anergy [50], and the induction of regulatory T cells [51]. These effects are mainly attributed to the production of anti-inflammatory cytokines, for example, IL-10 and TGF- β , and the expression of downregulatory/inhibitory markers, for example, Programmed death-ligand 1 and 2 (PDL-1, PDL-2) (reviewed in [52,53]).

Restoring tolerance through the use of immunomodulatory tolerogenic dendritic cells in RA has become an exciting line of therapeutic potential. Studies in murine CIA have shown tolerogenic dendritic cells to be highly effective. Introducing in-vitro derived type-II collagen-pulsed tolerogenic dendritic cells into arthritic CIA mice reduced the severity of the disease, reduced the inflammatory environment (lower levels of Th17 cells) and increased the anti-inflammatory environment through increased levels of IL-10 producing T cells [54]. The generation of tolerogenic dendritic cells from RA patients has been investigated [55] and their safety and efficacy as a therapy are currently being determined in clinical trials [56]. Cellular therapies like tolerogenic dendritic cells or the manipulation of dendritic cells *in vivo* to induce a more tolerogenic population could be a potential and feasible therapy for RA patients in the future.

In addition to tolerogenic dendritic cells being able to regulate immune responses, different subsets of dendritic cells may have different roles in autoimmune disease. Using a novel breach of self-tolerance murine model of arthritis [57], we have shown that plasmacytoid dendritic cells have an anti-inflammatory role [58]. By contrast, conventional dendritic cells have a more proinflammatory role.

Their depletion resulted in reduced severity of disease as well as reduced anticollagen responses [59]. Interestingly, peripheral dendritic cell homing to the thymus provides a source of peripheral antigens for tolerance induction in the steady state [60,61]. Whether the onset of autoimmune reactions alters this process, and so impacts on intrathymic tolerance mechanisms is not known.

Recirculation of peripheral T cells back to the thymus

It has been known for some time now that peripheral T cells, including both conventional and Foxp3⁺ Treg, can home back to the thymus, which challenges the view that movement out of the thymus is unidirectional (Fig. 1). The first evidence showed labelled lymph node cells transferred into syngeneic hosts could be found within the thymus in both adult and neonatal hosts [62]. In the mouse, mature peripheral T cells that migrate into the thymus resemble activated, or previously activated, CD44^{hi} T cells which appear to preferentially enter over naive T cells [63–65] (reviewed in detail [66]). The question remains as to what function these recirculating peripheral lymphocytes have in the thymus. There is emerging evidence that these cells are able to alter central tolerance and induce the deletion of thymic APC populations in an antigen-specific manner [67]. In addition, very recently it has been shown that peripheral Treg can also recirculate back to the thymus and once there they suppress the development of new Treg through the inhibition of IL-2 [68^{***}]. In the same study, evidence of the reentry of mature T cells and Treg into the human thymus was also found. In the setting of autoimmune disease and RA, this could be an interesting mechanism for silencing auto-reactive T cells. Moreover, despite having sufficient numbers of progenitor cells [69–71], RA patients exhibit impaired thymic function as indicated by fewer recent thymic emigrants. Whether this is linked to changes in peripheral T-cell recirculation back to the thymus caused by ageing and/or RA is not clear.

CONCLUSION

The thymus represents a key site for the generation of $\alpha\beta$ T cells that play an essential role in immune responses. However, the removal of autoreactive T cells from the developing TCR repertoire via intrathymic selection mechanisms is incomplete, which is a significant factor in relation to the onset of T-cell mediated autoimmune diseases. To combat this, peripheral tolerance mechanisms involving

modulation of dendritic cell function and Foxp3⁺ Treg are in place. In RA, evidence suggests that a breakdown in T-cell tolerance takes place. However, whether this maps to altered T-cell responses in either the thymus or within peripheral tissues is not clear. Perhaps significantly, both sites are linked not only by the conventional T cells, Foxp3⁺ Treg and dendritic cell subsets they contain, but also by trafficking of these cell types between each site. How such processes impact on the maintenance of tolerance, and its breakdown, is not understood. We propose that adopting an overarching approach to studying tolerance regulation at sites of T-cell production and effector function will provide new opportunities to better understand tolerance maintenance and breakdown, and inform future strategies for immune intervention.

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Conflicts of interest

There are no conflicts of interest.

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