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Self-antigen presentation by dendritic cells and lymphoid stroma and its implications for autoimmunity

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Summary

The induction and maintenance of T cell tolerance is essential to prevent autoimmunity. A combination of central and peripheral mechanisms acts to control autoreactive T cells. In secondary lymphoid organs, dendritic cells (DCs) presenting self-antigen were thought to play a major role in the induction of peripheral T cell tolerance. Multiple recent studies have demonstrated that DCs are not absolutely essential to induce and maintain tolerance. Furthermore, it has also been recently shown that non-hematopoietic stromal cells expressing peripheral tissue-restricted antigens can induce T cell tolerance, independently of DCs. Together these studies imply that peripheral tolerance is more complex than previously thought and a consequence of the tolerogenic functions of the hematopoietic and non-hematopoietic compartments within secondary lymphoid organs.

Introduction

Discrimination between self and non-self is vital to the induction and maintenance of T cell tolerance. Several mechanisms exist to induce and maintain T cell tolerance against self. In the thymus, central tolerance mechanisms mediated by medullary thymic epithelial cells (mTECs) and dendritic cells (DCs) eliminate self-reactive T cells from the developing T cell repertoire [1]. Despite the effectiveness of this process, self-reactive T cells, particularly those bearing low affinity TCR for self-antigens, can escape negative selection. To avoid autoimmunity, various mechanisms evolved in the periphery for their subsequent control and elimination. In this review, we will discuss the contribution of DCs and non-hematopoietic stromal cells of secondary lymphoid organs (SLOs) in these processes.

Dendritic cells in autoimmunity and maintenance of tolerance in secondary lymphoid organs

DCs are antigen presenting cells that capture, process and present antigens to T cells [2]. Within SLOs, naïve T cells continuously scan the surface of DCs for their cognate MHC-peptide ligand. Under steady state conditions, DCs exist in an immature state characterized

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by low surface expression of MHC-II and costimulatory molecules[2]. Engagement of such DCs with naïve T cells is thought to result in immunological tolerance. However, signals associated with inflammation or infection cause DCs to mature. This process involves complex phenotypic and functional changes such as upregulation of MHC-II and costimulatory molecules, changes in their cytokine expression pattern, and augmented motility [2]. Activation of naïve T cells by mature DCs generates effector T cells and establishes immunological memory [3–5]. Aside from their ability to mature, the role of DCs in immunity and tolerance is further complicated by the fact that they represent an extensive network of antigen presenting cells comprised of multiple subsets. DCs can be classified into two major groups: conventional (cDC) and plasmacytoid DCs (pDC) [6]. Each exhibits distinct developmental, phenotypic and functional characteristics [2,7]. Moreover, cDCs can be divided into multiple subsets on the basis of their migratory ability or expression of various surface molecules [6,8,9](Table 1). The exact functional differences among these subsets are under intense investigation and their known features have been recently reviewed by Steinman and Idoyaga [7].

DCs have been considered the major cell type in induction of peripheral tolerance. The dominance of DCs in these processes was based primarily on early experiments using transgenic mice in which model antigen expression was targeted to specific peripheral tissues. Transfer of self-reactive T cells into these transgenic animals resulted in the activation, proliferation and subsequent deletion or anergy of antigen-specific T cells [10,11]. To further elucidate the role of DCs in tolerance, antigen was targeted to DCs either via expression of self-antigen under the CD11c promoter [12] or via antigen coupled to antibody specific to surface receptors expressed in DCs [13]. In both scenarios, antigen presentation to CD4 and CD8 T cells led to profound peripheral T cell tolerance under non-inflammatory conditions. Thus, DCs were identified as the main antigen presenting cells inducing peripheral T cell tolerance. In addition, CD8 T cell tolerance involved the cross-presentation of self-antigen, predominantly by CD8 α DCs [14–16]. Despite of these observations, mice deficient in the transcription factor BATF-3, which lack CD8 α DCs, did not develop autoimmune disorders [17•]. Nevertheless, a recent study by Luckashenak et al. demonstrated that cross-presentation of apoptotic cells by DCs indeed promotes peripheral CD8 T cell tolerance in vivo [18••]. In this study, transgenic animals expressing a dominant negative mutant form of Rac1 under the CD11c promoter were crossed with RIP-mOVA transgenic mice in which a membrane-bound form of ovalbumin is expressed selectively in pancreatic beta cells and kidney tubular cells under the control of the rat insulin promoter. The Rac1 mutation in DCs blocked phagocytosis of apoptotic cells and cellular debris, thereby inhibiting crosspresentation of OVA in this model. Importantly, polyclonal CD8 T cells isolated from these transgenic animals caused autoimmune disease upon transfer into Rag $^{-/-}$ recipients [18••].

DCs in SLOs are also thought to play a critical role in the homeostasis of T regulatory (Tregs) cells. Presentation of self-antigen by cDCs can result in the induction of regulatory T cells that suppress effector T cell responses [19–21]. During EAE induction, pDCs can also present self-antigen in the context of MHC-II and promote the expansion of myelin-specific regulatory T cells and thereby dampen the pathogenic T cell response [22]. It was also recently reported that MHC-II expression by CD11c^{hi} DCs is required to sustain the number of natural regulatory T cell repertoire in vivo [23••]. Bone marrow chimeras in which bone marrow from transgenic animals expressing the Diphtheria toxin receptor (DTR) under the control of the CD11c promoter is transplanted into wild-type recipients, is a well-established model to transiently deplete CD11c^{hi} cells in mice [24,25]. Systemic injection of DT toxin leads to the depletion of DCs in SLOs within 24hrs [24,25]. In this model, the transient absence of CD11c^{hi} cells reduced the frequency of regulatory T cells and resulted in autoimmunity [23••]. Conversely an increase in the number of DCs, using Flt3L, promoted

the expansion of Tregs by a mechanism that required MHC-II expression by DCs [23••]. This feedback regulation between DCs and Tregs could ensure the reestablishment of regulatory T cell numbers and the maintenance of immune homeostasis. According to this concept, increased frequency of DCs was associated with autoimmune phenotype in *lpr* mice [26] and in transgenic animals where the apoptosis of DCs was selectively inhibited [27,28]. Interestingly, transgenic expression of the caspase inhibitor p35 in DCs [27], which prolongs life span of DCs, did not change the frequency of regulatory T cells. Moreover, mice deficient in DCIR (dendritic cell immunoreceptor) displayed an increased frequency of DCs and spontaneously developed sialadenitis and enthesitis associated with high serum autoantibody titers [29]. DCIR is a C-type lectin receptor expressed preferentially by DCs and signaling via its inhibitory ITIM motif impairs DC differentiation [29]. Interestingly, DCIR-deficient mice did not show an increase in Treg abundance despite elevated numbers of DCs in SLOs [29]. It is plausible that the number of DCs in SLOs has to exceed a relatively high threshold to significantly affect the size of regulatory T cell repertoire. Further studies are required to address also this possibility.

Given the importance of antigen presentation by DCs in peripheral tolerance, one of the most critical questions in the field has been whether T cell-mediated autoimmunity would develop in the absence of DCs. In the CD11c-DTR system the transient, short-term loss of CD11c^{hi} DCs resulted in increased number of Th1 and Th17 cells and hypergammaglobulinemia [23••]. Similarly, short-term depletion of pDCs exacerbated immunopathology in a murine model of experimental arthritis [30]. According to these short-term DC-depletion experiments (6–18 days), the absence of DCs was associated with a higher risk of autoimmune disease. However, to study DC contributions to T cell homeostasis and maintenance of tolerance requires long-term elimination of these cells in the steady state. To accomplish this, two research groups developed genetic models of constitutive DC depletion. Birnberg et al. crossed CD11c-Cre BAC transgenic mice [31] with mice that bear the conditional diphtheria toxin transgene (DTA) under the constitutively active, Rosa26 locus [32]. Efficient antiviral responses could not be mounted in mice constitutively lacking DCs, in agreement with data generated upon transient DC depletion [33••]. Surprisingly, T cell homeostasis, negative selection and generation of Tregs were unaffected. Instead, these mice developed a myeloproliferative disorder characterized by an elevated level of serum Flt3L and an increase in CD11b⁺ Gr1^{int} monocytes and Gr1^{hi} neutrophils in lymphoid and non-lymphoid organs [33••]. Ohnmacht et al. took a similar approach to constitutively deplete DCs by crossing CD11c-Cre mice with animals expressing the DTA under the Rosa26 locus [34••]. The DC-depleted mice showed impaired negative selection of CD4 T cells and developed spontaneous autoimmunity. The autoimmune phenotype was characterized by autoantibody formation, higher frequency of Th1 and Th17 cells and development of inflammatory bowel disease [34••]. Interestingly, the CD8 T cell and Treg compartments were unaffected. Additionally, Gr1^{hi} neutrophils and F4/80⁺ macrophages accumulated in SLOs and in peripheral organs [34••]. In both models, constitutive depletion of DCs led to alterations in myeloid cells, thus highlighting a previously unrecognized regulatory circuit that connects DCs to myelopoiesis. However, the role of DCs in regulating T cell-mediated autoimmunity remains controversial. The discrepancies between these two studies may be attributable to differences in the remaining DC subsets present within SLOs. While in the first case pDCs and Langerhans cells (LCs) were still present [33••], in the model by Ohnmacht et al. the pDCs were absent and only small numbers of LCs could be detected in the epidermis [34••]. Given the observation that pDCs can present antigen to CD4 and CD8 T cells [35], it is possible that in the absence of cDCs they might compensate and maintain peripheral tolerance. Moreover, CD11c is not an exclusive marker of DCs and can be expressed by other cell types such as macrophages and NK cells [24]. Therefore, it will be important to resolve this discrepancy and to evaluate the contribution of these other cell types in future studies.

Besides determining the overall contribution of DCs in peripheral tolerance, it is important to pinpoint the specific subsets that influence the development of autoimmune diseases and to define their precise contributions. A recent report showed that elevated numbers of CD11c+CD11b[−]/lo CD8α[−] DCs in SLOs coincided with the appearance of insulinitis in NOD mice [36•]. These DCs, termed merocytic DCs, can acquire apoptotic cellular materials and induce T cell activation. Presentation of apoptotic cellular material by merocytic DCs to self-reactive T cells did not result in tolerance but rather reversed the deletion of self-reactive T cells and promoted their activation. Furthermore, transfer of antigen-loaded merocytic DCs induced early onset diabetes in young NOD recipients [36•]. In another study, Hamilton-Williams et al. demonstrated that expression of the protective alleles *Idd3/5* exclusively by CD11c^{hi} DCs led to the deletion of autoreactive T cells and protects NOD mice from diabetes [37•]. It remains to be elucidated whether among the CD11c^{hi} cells, the merocytic DCs carry these protective alleles which would diminish their immunogenic potential or whether other functional links might exist between merocytic DCs and the well-established cross-presenting CD8α DC subset during development of diabetes. Moreover, it is of interest whether merocytic DCs play a role in other autoimmune conditions.

Presentation of self by non-hematopoietic stromal cells of secondary lymphoid organs

The complex architecture of SLOs is organized and supported by a network of stromal cells. These stromal cells provide the structural framework upon which immune cells move and interact with one another [38]. The SLO stromal compartment can be divided into four well-defined subsets, follicular dendritic cells (FDCs), fibroblastic reticular cells (FRCs), lymphatic endothelial cells (LECs) and blood endothelial cells (BECs) (Table 2) [38,39]. Besides these cell types, there is a so far poorly characterized subset referred to as double-negative cells (DN), which do not express the common stromal surface markers, CD31 and gp38 (podoplanin) (Table 2). Additionally, Katakai et al. identified a novel subset termed the marginal reticular cells (MRCs) based on histological findings. MRCs express MadCAM-1 and localized exclusively beneath the subcapsular sinus [40]. Moreover, in NOD mice Gardner et al [41•] identified a highly motile stromal cell type, the Aire-expressing stromal cells (eTACs) which they found to present Aire-regulated peripheral tissue-restricted antigens (PTAs) and induce deletion of autoreactive T cells (Table 2). Some of the characteristic features of stromal subsets are summarized in Table 2.

FRCs, BECs and LECs comprise 80–85% of the non-hematopoietic cellular content of the LN [39,42••]. FRCs reside primarily in the T cell zone where they secrete extracellular matrix components and form the lymph-draining conduit system of LNs [43,44]. Furthermore, FRCs orchestrate naïve T cell and DC migration and promote naïve T cell homeostasis [38,39]. Upon migrating in and out of SLOs, lymphocytes come into direct contact with BECs (lining blood vessels) and LECs (lining lymphatics) [38,45]. Both cell types possess various chemokines, integrins, and glycoproteins that guide the migration and adhesion of immune cells [38,45]. Moreover, LECs as a cellular source of S1P control the egress of activated T cells from the LN [46].

Emerging evidence indicates that the stromal compartment of SLOs possesses additional roles beyond scaffolding and guidance of immune cells. In transgenic mice, where model antigen is expressed under the control of tissue specific promoter, direct presentation of self-antigen by non-hematopoietic cells to autoreactive T cells resulted in activation, proliferation and subsequent deletion of CD8 T cells [47•,48•]. Furthermore, Nichols et al. found similar deletion of autoreactive CD8 T cells where a melanocyte specific self-antigen, tyrosinase, was presented by radioresistant cells of peripheral LNs [49•]. Importantly, in these models, DCs and other hematopoietic cells were dispensable for the induction of tolerance. The

surprising capacity of non-hematopoietic stromal cells to present self-antigen was due to their expression of various peripheral tissue antigens [41•,48•,49•] and transcription factors [41•,50]. Notably, in the iFABP model where truncated OVA (tOVA) is expressed under the control of tissue specific promoter for intestinal fatty acid binding protein (iFABP), it seems that expression of FABP proteins especially FABP7 is highly specific for FRCs within SLOs [51]. Additionally, FABP7 deficient mice exhibit increased percentage of CD4 T cell in LNs indicating that FRC might regulate lymphocyte homeostasis via expression of these proteins [51].

In the absence of genetic tools that trace specific stromal subsets, identification of the tolerizing cell type required high purity sorting of the 4 major subsets identified by flow cytometry [39] (Table-2). In the iFABP model FRCs were wholly responsible for the direct presentation of OVA to self-reactive T cells [42••,47•]. In parallel, Cohen et al. using a similar approach, identified LECs as the primary source of tyrosinase among lymph node stromal subsets [52••]. Since tyrosinase-specific T cells escape thymic selection [49•], this novel mechanism of tolerance induction at the periphery is crucial to avoid autoimmunity. Upon further examination of PTA expression by lymph node stromal cells it became evident that multiple subsets were involved as each had its own characteristic antigen display. Furthermore, several PTAs were expressed by more than one stromal cell subset such as alpha fetoprotein (liver antigen) and proteolipid protein (central nervous system antigen) [42••,52••]. Among the transcription factors involved in regulating the expression of self-antigens, Aire (Autoimmune regulator) was expressed only in the DN subset and Deaf-1 (Deformed epidermal autoregulatory factor-1) in all stromal subset examined [42••]. In the thymus, Aire regulates the transcription of various PTAs and plays essential role in central tolerance. Deaf-1 drives PTA expression in pancreatic LNs and reduced expression of Deaf-1 is associated with type-I diabetes in humans and mice [50].

It is of specific interest how stromal cells operate under inflammatory conditions and whether they can maintain their tolerance-inducing capacity similar to DCs [2]. TLR ligands altered the ability of DCs to stimulate T cells without compromising their capability to induce deletion of autoreactive T cells [53]. TLR stimulated DCs induced autoimmunity only in the presence of antigen specific CD4 T cell help [53]. Stromal cells express various TLRs and could respond to TLR ligands by upregulating co-stimulatory and co-inhibitory molecules [42••]. The major hallmark of their response to a TLR3 ligand was the upregulation of PD-L1 [42••,54], a principal co-inhibitory molecule involved in negative regulation of T cell activation and in homeostasis of regulatory T cells [55]. Importantly, systemic PDL-1 blockade could break T cell tolerance and cause autoimmunity in the iFABP model [54]. Yet, injection of TLR ligands alone, was not sufficient to break tolerance in these mice. This could be attributable to additional yet undetermined factors associated with tolerance induction by stromal cells. Besides, systemic injection of anti-CD40 antibody together with oral cholera toxin could induce colitis in iFABP mice [56,57]. This indicates that distinct stimuli can result in qualitatively different activation states of DCs which might influence the overall outcome of the immune response despite the coexistence of tolerogenic, antigen-presenting stromal cells.

Another intriguing question is whether stromal cells maintain their PTA expression under inflammatory or immunogenic conditions. We recently reported that sorted FRCs derived from iFABP-tOVA mice could induce the activation and proliferation of OT-I T cells *ex vivo* [42••]. Importantly, stimulation of FRCs with the TLR3 ligand, polyI:C, reduced their ability to activate OT-I T cells. Interestingly, FRCs expressed lower levels of OVA following polyI:C stimulation [42••]. This, together with the marked increase in PD-L1 level might contribute to maintain their tolerogenic potential. The underlying mechanisms remain to be elucidated. Interestingly, systemic injection of TLR ligands resulted in altered

expression of PTAs primarily by the DN subset with some going up or down and others remaining unchanged [42••]. Why the expression of some PTAs changed and others did not and how their expression is regulated remain unclear. It is possible that this function could be linked with hitherto unrecognized tasks of stromal subsets during inflammation.

Conclusions

That T cell-mediated autoimmunity did not develop in the absence of DCs [33••] is surprising and challenges assumptions about how T cell tolerance is induced and maintained (Fig. 1). An increasing number of studies have demonstrated intercellular cross-talk between distinct DC subsets [8]. These interactions either in a synergistic or antagonistic way exerted a potent influence on the outcome of immune responses, suggesting that the DC network is most likely a cooperative unit rather than just a group of distinct and unrelated subsets. Additionally, most studies so far have focused on the CD8 α ⁺ and CD11b⁺ DC subsets, whereas our understanding of other DCs including the double and triple negative DC subsets are extremely limited (Table 1.). Thus, new models or techniques are required to adequately address the role of each subset and their possible interplay in the future.

PTA expression by stromal cells seems to be shared among subsets. Moreover, microarray analyses of Aire-expressing stromal cells in SLOs demonstrated that the Aire-regulated PTA expression in these cells is complementary rather than overlapping with mTECs [41•]. It is unclear whether this is also true for the other stromal subsets and for non-Aire regulated genes as well. In addition to this, DC-mediated self-antigen presentation was not absolutely necessary for tolerance. This could indicate that stromal cells are not simply a backup mechanism but for certain PTAs the exclusive cell type capable of inducing peripheral tolerance. Moreover, it is also possible that between stromal cells and DCs in SLOs, similar division of labor exists as in the thymus between mTECs and DCs [1]. Taken together, peripheral tolerance is more complex than previously thought and both the non-hematopoietic and the hematopoietic compartment contribute to the induction and maintenance of these processes (Fig. 1).

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Figure 1. Division of labour between dendritic cells and lymphoid stromal cells

DCs are involved in the induction of T cell immunity and tolerance. Moreover DCs can regulate the homeostasis of regulatory T cells and they are involved in a feedback loop affecting myelopoiesis. Stromal cells within SLOs are capable of inducing T cell tolerance independently of DCs via their expression of PTAs and the transcriptional regulators, Aire and Deaf-1. However, their possible roles in immunity and induction or homeostasis of regulatory T cells remain to be elucidated.

Table 1

Dendritic cell subsets in secondary lymphoid organs

Markers	Resident DC			Migratory DC			Inflammatory	pDC
	CD8 DC	CD4 DC	DN	TN	CD11b+ (classic dermal DC)	CD103+ (langerin+ dermal DC)	Langerhans cells	
CD4	–	+	–	–	?	?	–	–
CD8	+	–	–	–	–	–	–/+	+/-
CD11b	–	+	+	–	+	–	+	–
B220	–	–	–	–	–	–	–	+
Langerin	–	–	–	–	–	+	+	–
Ep-CAM	–	–	–	–	–	+/-	+	–

DN – double negative

TN – triple negative

DC – dendritic cells

pDC – plasmacytoid dendritic cells

Langerhans cells can derive from monocytes (Ref. 57)

Table 2

Subsets of stromal cells in secondary lymphoid organs

Markers	FRC	LEC	BEC	DN	eTAC	MRC	FDC
gp38	+	+	-	-	-	+	-
CD31	-	+	+	-	?	?	-
CD140a	+	-	-	-	-	?	-
CD35	-	-	-	-	?	-	+
MadCam-1	-	+	-	-	?	+	+
Ep-CAM	-	-	-	-	+	?	-
Localization within LN	Mainly in the T zone	T zone, medullary and subcapsular region	HEV	Unknown	T-B-zone junction	Subcapsular region	B cell follicle

FRC – fibroblastic reticular cells
LEC – lymphatic endothelial cells
BEC – blood endothelial cells
DN – double negative subset
eTAC – extra-thymic Aire expressing cells
MRC – marginal reticular cells
FDC – follicular dendritic cells