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Differential processing of self-antigens by subsets of thymic stromal cells

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The stromal network of the thymus provides a unique environment that supports the development of mature CD4⁺ and CD8⁺ T cells expressing a very diverse repertoire of T cell receptors (TCR) with limited reactivity to self-antigens. Thymic cortical epithelial cells (cTECs) are specialized antigen-presenting cells (APCs) that promote the positive selection of developing thymocytes while medullary thymic epithelial cells (mTECs) and thymic dendritic cells (tDCs) induce central tolerance to self-antigens. Recent studies showed that cTECs express a unique set of proteases involved in the generation of self-peptides presented by major-histocompatibility encoded molecules (pMHC) and consequently may express a unique set of pMHC complexes. Conversely, the stromal cells of the medulla developed several mechanisms to mirror as closely as possible the constellation of self-peptides derived from peripheral tissues. Here, we discuss how these different features allow for the development of a highly diverse but poorly self-reactive repertoire of functional T cells.

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Introduction

The progression through the discrete stages of intrathymic T cell development and the fate of thymocytes is determined by the affinity of their clonally distributed TCR for self-pMHC complexes. Immature double-negative CD4[−]CD8[−] thymocytes develop into double-positive (DP) CD4⁺CD8⁺ cells in the outer cortical regions of the thymus. DP cells expressing TCR capable

of interacting with self-pMHC complexes presented by cTECs are rescued from programmed cell death — the default fate of DP cells in the absence of TCR signaling — and mature into single-positive (SP) CD4⁺CD8[−] or CD4[−]CD8⁺ cells, a process called positive selection. In the thymic medulla, those SP cells expressing TCR that interact with high affinity with self-pMHC complexes presented by mTECs and tDCs are deleted, a process referred to as negative selection, or 'deviated' into unconventional T cell lineages (for instance regulatory T cells (Treg cells) [1]), whereas those that are weakly self-reactive mature further before their release into the blood circulation where they possibly benefit from repeated contact with self-pMHC complexes for their persistence and activation [2[•],3–5].

A wealth of *in vitro* models support the affinity model of thymocyte selection whereby the fate of the developing T cell, survival or deletion, is determined by the affinity of the TCR for self-pMHC complexes [6]. Those models that have defined a fixed affinity threshold that delimits positive and negative selection do not take, however, into account the fact that positive and negative selections occur in compartmentalized microenvironments populated with different, highly specialized antigen-presenting stromal cells. The present review summarizes the recent data showing that the distinct thymic stromal cells have distinct antigen-processing and antigen-presentation capacities and that they presumably express partly non-overlapping sets of self-antigens and discusses the possible *raison d'être* for minimizing the abundance of 'shared' peptides between cTECs and mTECs-DCs.

Antigen presentation in the cortex

The foremost antigen-presenting cells (APCs) of the cortex are cTECs that are devoted to positive selection of developing thymocytes. Sparse tDCs are also found in the cortex where they co-operate with cTECs to induce the negative selection of a few self-reactive T cells [7,8]. Accumulating evidences indicate that cTECs stand apart from other APCs. Hence, cTECs demonstrate unusually high constitutive macroautophagy [9], express a unique set of proteases involved in the MHC class II presentation pathway, cathepsin (Cat)-L and Thymus Specific Serine Protease (TSSP, *prss16*), and a unique subunit of the proteasome that promotes the presentation of low affinity peptides by MHC class I molecules, the β5T subunit (Table 1, [10,11[•],12]). The proteolytic specificity of

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Table 1

Expression pattern of different antigen-processing enzymes in cTECs, mTECs and DCs.

		Thymus cortex	Thymus medulla		Periphery
		cTECs	mTECs	tDCs	DCs
MHC class II processing	CatL	+++	–	–	–
	TSSP	+++	–	+	–
	CatS	+/-	++	+++	+++
	AEP	–	–	++	++
MHC class I processing	$\beta 5t$	+++	–	–	–
	$\beta 5i$	+/-	+++	+++	+++
	Autophagy	+++	++	+ ?	+
	Endocytosis	+/-	+/-	+++	+++

The relative expression level of different proteases involved in the MHC class II pathway and of the proteasome $\beta 5$ subunit in the different thymic stromal cells and peripheral DCs is presented. Also shown is the relative efficiency of autophagy or endocytosis in these different cell types. Note that in the case of DCs, TSSP presents a particular pattern since it is expressed by thymic but not peripheral DCs. Green: Enzymes/processes predominantly at work in cortical epithelial cell of the thymus. Red: Enzymes/processes predominantly at work in medullary epithelial cell of the thymus and thymic or peripheral DCs.

proteasomes expressing the $\beta 5t$ subunit is discussed in an accompanying paper and here we will discuss only results pertinent to the MHC class II pathway.

Macroautophagy in MHC class II presentation by cTECs

MHC class II $\alpha\beta$ heterodimers assemble in the endoplasmic reticulum where they bind the invariant chain (Ii). Ii allows proper routing of the MHC class II complexes along the endocytic pathway and prevents their premature loading with endogenous peptides. Therefore, the chaperone function of Ii dedicates MHC class II molecules to the presentation of exogenous antigens. Removal of Ii is accomplished by an ordered proteolysis mediated by the cysteine proteases Cat-L or Cat-S and by the asparaginyl endopeptidase (AEP), leading to the generation of MHC class II complexes in which the Ii chain peptide (CLIP) occupies the peptide-binding groove [13]. CLIP is subsequently replaced by peptides derived from proteolytic degradation of endosomal proteins.

TECs are poorly phagocytic and consequently mainly present self-peptides derived from endogenous proteins. Optimal delivery of cytosolic proteins into MHC class II loading compartments is achieved in TECs and mainly cTECs by enhanced constitutive macroautophagy, a generic protein degradation process that is induced by stress in many cell types [14]. Disruption of macroautophagy in mice deficient for the essential autophagy gene *Atg5*, impaired positive selection of some TCR specificities indicating that macroautophagy contributes to the routing of some endogenous self-proteins into lysosomal compartments involved in the generation of positively selecting pMHC class II complexes [9]. Interestingly, positive selection of some TCR specificities was also enhanced in *Atg5*^{-/-} mice. Consistent with the increased expression of

one given pMHC complex by *Atg5*-deficient cTECs (I-E α_{52-68} -I-A^b), these observations indicated that the generation of some self-pMHC class II complexes was more efficient in the absence of autophagy. Such rather counterintuitive observations emphasize the complexity of the MHC class II presentation pathway and the rather opposite outcomes that result from the absence of macroautophagy or of a given protease (see below).

Role of Cat-L and TSSP in T cell repertoire selection

Recent evidences indicate that the different thymic APCs are equipped with different sets of endosomal proteases (Table 1). Cat-L is only expressed by cTECs but not by mTECs, tDCs or peripheral DC [10]. Conversely, Cat-S is expressed by mTECs and by both thymic and peripheral DCs whereas AEP is only expressed at detectable levels in DCs [10,15]. Although TSSP was initially described as an evolutionarily conserved cTEC-specific protease [16–18], more sensitive RT-PCR analysis revealed that it is also expressed at low levels by all tDC subsets, but not by peripheral DCs [19••].

Consistent with its selective expression in cTECs and its major role in Ii processing, Cat-L-deficient cTECs mainly present CLIP-MHC class II complexes at their surface [10]. As a consequence, the number of thymic CD4⁺ T cells is reduced by 70% in those mice [10,20,21]. Several experimental evidence suggested that impaired differentiation of CD4⁺ T cells in Cat-L-deficient mice was not solely due to reduced diversity of pMHC complexes linked to CLIP-MHC class II peptide accumulation but also reflected a contribution of Cat-L in the generation of positively selecting ligands [20–22]. Interestingly, in Cat-L-deficient mice in which DC-dependent negative selection was impaired, the number of CD4⁺ SP

thymocytes was restored to levels comparable to that of Cat-L-sufficient mice [22]. The CD4⁺ T cell repertoire positively selected by Cat-L-deficient cTECs is therefore deleted by thymic DCs. This was the first indication that the repertoire of positively and negatively selecting ligands ought to be somehow different to minimize the extensive deletion induced by mTECs and tDCs. The differential expression of proteases involved in MHC class II presentation — such as CatL — allowed for such limited overlap between the pMHC complexes presented by cTECs and medullary TECs and DCs.

The initial analysis of TSSP-deficient B6 mice revealed no significant alteration in the intra-thymic differentiation of CD4⁺ T cells expressing polyclonal $\alpha\beta$ TCR [23]. Subsequent analysis of the intra-thymic differentiation of CD4⁺ T cells expressing I-A^b restricted transgenic TCR, specific for an ovalbumin (OVA)-derived peptide (OT-II) or for a male specific peptide (Marylin) showed a major impairment of positive selection when TECs lacked TSSP [11[•]]. Consistent with the endosomal localization of TSSP, these results suggested that TSSP plays a role in the MHC class II Ag presentation pathway and the generation of ligands for positive selection.

Since *prss16* has been linked to a diabetes susceptibility locus in humans, we analyzed the impact of TSSP-deficiency on the development of CD4⁺ T cells reactive to islet antigens in the NOD mouse. As observed for B6 mice, differentiation of polyclonal CD4⁺ T cells or of CD4⁺ T cells expressing a GAD_{206–220} specific TCR proceeded normally in TSSP-deficient NOD mice [19^{••}]. In contrast, the differentiation of CD4⁺ T cells expressing a phogrin-specific TCR or a TCR denoted NY4.1 and specific for an as yet unknown pancreatic islet antigen was severely impaired in TSSP-deficient NOD mice. Mixed bone-marrow chimeras showed that differentiation of NY4.1-expressing CD4⁺ T cells was affected when either TECs or bone marrow derived APCs lacked TSSP expression. The phenotype and reduced thymic cellularity observed in bone-marrow chimeras with TSSP-deficient TECs strongly suggested that TSSP-deficient TECs and more likely cTECs cause, either directly or indirectly, the negative selection of developing NY4.1 thymocytes. These results suggest that both TSSP-deficient TECs and DC present a ligand that is recognized by the NY4.1 TCR and capable of inducing negative selection of thymocytes expressing the NY4.1 TCR. Accordingly, in WT mice, TSSP impairs the generation of this ligand in both TECs and tDCs.

Importantly, the enhanced deletion of CD4⁺ T cells specific for some β -cell antigens in TSSP-deficient NOD mice expressing a polyclonal TCR repertoire (estimated at 15% of the functional islet-reactive CD4⁺ T cell repertoire) correlated with a complete protection from spontaneous diabetes indicating that in the NOD mouse,

TSSP may limit, either directly or indirectly, the presentation by cTECs or tDCs of several epitopes derived from pancreatic islet antigens and consequently prevented induction of central tolerance to these self-antigens [19^{••}].

In addition to its role in the development of some CD4⁺ T cells reactive against pancreatic islet antigens, thymic expression of TSSP was also found necessary for the development of CD4⁺ T cells specific for hen egg lysozyme (HEL); the response against five additional foreign protein antigens was, however, unaffected [24^{••}]. The development of polyclonal HEL-specific CD4⁺ T cells and of CD4⁺ T cells expressing a TCR specific for HEL was reduced when TECs or tDCs lacked TSSP expression. These results are best explained by an enhanced negative selection driven by either TSSP-deficient TECs and/or tDCs presenting a HEL mimotope of sufficient affinity to delete the HEL-reactive CD4⁺ T cells, though a role for TSSP in generating a positively selecting ligands cannot be formally excluded akin to the observations made for the OT-II or Marylin CD4⁺ T cells.

In the NOD mouse, TSSP appears to have a rather 'destructive' effect on the generation of some self-MHC class II binding peptides that is not necessarily due to a direct proteolytic cleavage of the corresponding epitopes. Such a 'destructive' potential likely results in opposite effects in that it prevents and allows the generation of some MHC class II ligands. This is suggested by the effect of TSSP-deficiency on the positive and negative selection of some T cell specificities and may in fact counterbalance the exacerbated autophagy of cTECs. These observations also raised the intriguing possibility that TSSP's primary function may be to limit self-antigen presentation and consequently limit self-tolerance induction to increase the diversity of the functional CD4⁺ T cell repertoire. While providing a selective advantage to control pathogens, such a function may, as observed in the NOD mouse, lead to autoimmunity.

Antigen presentation in the medulla

Consistent with a primary role in inducing self-tolerance, the different APCs found in the medulla have evolved several independent and concerted strategies to purge the repertoire of strongly self-reactive T cells. The pool of thymic self-pMHC complexes available in the medulla for T cell tolerance induction derives from proteins expressed by tDCs and mTECs [25]. The ability of mTECs to ectopically express tissue-restricted antigens (TRAs) that represent most of the extra-thymic tissues [8,26], a mechanism partly controlled by the autoimmune regulator *Aire* gene extends the scope of central tolerance [27]. Peripheral self-antigens can also gain access to the thymus via the blood supply as freely circulating proteins [28,29^{••},30]. Additionally, extra-thymic self-antigens can be picked up by differentiated, peripheral DCs that are capable of migrating via the blood to the thymus

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[31,32,33]. Therefore, the medulla displays a wide constellation of self-peptides. However, to compensate for incomplete representation of self-peptides in the thymus, mTECs and tDCs are also capable of inducing the development of Treg cells that suppress the activity of conventional autoreactive T cells that have escaped central tolerance [34–36].

Thymic DCs are heterogeneous and three subsets of DCs coexist in the mouse thymus [37]. One subset expresses CD45R and corresponds to plasmacytoid DCs, whereas the two other tDC subsets are CD45R[−] and can be distinguished based on the differential expression of CD8 α and CD172 α (Sirp α). The CD8 α^{low} CD172 α^+ tDCs arise from quasi-differentiated blood precursors that continuously enter the thymus [31,32]. In contrast, the CD8 α^{high} CD172 $\alpha^−$ tDCs develop intrathymically and have been thought to have a lymphoid origin [32,38,39]. Consistent with fate mapping experiments that showed that thymic DCs have no lymphoid past [40], it has been recently shown that CD8 α^{high} CD172 $\alpha^−$ tDCs are of myeloid origin [41]. Whether the different types of tDCs sample distinct pools of self-antigens and show functional specialization remains to be determined.

Consistent with a major role in tolerance induction, the antigen-presenting machinery of tDCs and mTECs mirror that of peripheral APCs (Table 1). One exception is the selective expression of TSSP by tDCs but not peripheral DCs [19]. It remains to be determined whether this reflects a lineage specification of tDCs or is induced by the thymic microenvironment. mTECs also show exceptionally high levels of macroautophagy, a mechanism likely permitting direct presentation of endogenous antigens in the class II pathway [42]. The autonomous contribution of mTECs to deletional tolerance has been evaluated using bone marrow chimeras designed to result into thymi containing MHC-positive mTECs and MHC-negative tDCs. These studies showed that mTECs can induce central tolerance in an autonomous manner for CD8⁺ but not CD4⁺ T cells [43]. Subsequent observations showed that intact membrane domains, harbouring functional surface self pMHC complexes can be unidirectionally transferred between mTECs and DCs and questioned the autonomous role of mTECs [42,44]. Further attempts to determine whether mTECs can autonomously achieve central tolerance involved transgenic mice expressing the A subunit of diphtheria toxin under the control of a minimal *cd11c* promoter (*cd11c*-DTA mice) [45,46]. Lack of CD11c^{high} tDCs did not affect negative selection of CD8⁺ T cells. Conflicting results were observed for CD4⁺ T cells with one study showing normal negative selection [46] and the other impaired negative selection [45,47]. Considering that deletion of self-reactive SP cells can still be achieved when the frequency of thymic APCs was lowered to 1% of normal numbers [48], it is possible that the *cd11c*-DTA

mice still contained enough tDCs capable of both co-operating with mTECs and of presenting their proper set of self-antigens to mediate negative selection [49]. It remains thus a daunting challenge to determine the fraction of CD4⁺ and CD8⁺ T cells that are purged from the repertoire by tDCs and mTECs and in the case of mTECs to evaluate their autonomous versus DC-dependent contribution [50].

Conclusions

Recent studies have highlighted the specific features of the different stromal cell population of the thymus (reviewed in [25,51] and here). The emerging concept is that the expression of highly specialized antigen-processing machinery by cTECs reduces the total amount of shared pMHC complexes presented by cTECs and mTECs/tDCs and consequently minimize the impact of negative selection on T cell repertoire diversity. A future challenge is certainly to evaluate whether predominant expression of Cat-L, TSSP or the $\beta 5T$ subunit by cTECs leads to substantial qualitative or quantitative differences of the pMHC repertoire presented by these stromal cells as compared to mTECs or DCs.

The stromal cells of the medulla have evolved several distinct antigen-presenting features to reflect the ‘peripheral immunological self’. The seminal demonstration that mTECs express a variety of TRAs provided the first conceptual framework for how tolerance to peripheral tissues is achieved in the thymus. The recent demonstration that peripheral immature DCs gain access to the thymus and induce tolerance to peripheral tissue antigens further extended the scope of tissue self-antigens seen by developing T cells. Still much work needs to be done to understand the interplay between these different stromal populations in deleting autoreactive CD4⁺ T cells and inducing the differentiation of natural T regulatory cells.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Hinterberger M, Aichinger M, da Costa OP, Voehringer D, Hoffmann R, Klein L: **Autonomous role of medullary thymic epithelial cells in central CD4(+) T cell tolerance.** *Nat Immunol* 2010, **11**:512–519.
2. Zachariah MA, Cyster JG: **Neural crest-derived pericytes promote egress of mature thymocytes at the corticomedullary junction.** *Science* 2010, **328**:1129–1135.

This article shows that pericytes produce S1P that promotes mature thymocytes egress from the thymus and entry into the blood circulation.

3. Ebert PJ, Jiang S, Xie J, Li QJ, Davis MM: **An endogenous positively selecting peptide enhances mature T cell responses and becomes an autoantigen in the absence of microRNA miR-181a.** *Nat Immunol* 2009, **10**:1162-1169.
 4. Lo WL, Felix NJ, Walters JJ, Rohrs H, Gross ML, Allen PM: **An endogenous peptide positively selects and augments the activation and survival of peripheral CD4+ T cells.** *Nat Immunol* 2009, **10**:1155-1161.
 5. Janeway CA Jr: **How the immune system works to protect the host from infection: a personal view.** *Proc Natl Acad Sci U S A* 2001, **98**:7461-7468.
 6. Palmer E, Naeher D: **Affinity threshold for thymic selection through a T-cell receptor-co-receptor zipper.** *Nat Rev Immunol* 2009, **9**:207-213.
 7. Goldman KP, Park CS, Kim M, Matzinger P, Anderson CC: **Thymic cortical epithelium induces self tolerance.** *Eur J Immunol* 2005, **35**:709-717.
 8. McCaughy TM, Baldwin TA, Wilken MS, Hogquist KA: **Clonal deletion of thymocytes can occur in the cortex with no involvement of the medulla.** *J Exp Med* 2008, **205**:2575-2584.
 9. Nedjic J, Aichinger M, Emmerich J, Mizushima N, Klein L: **Autophagy in thymic epithelium shapes the T-cell repertoire and is essential for tolerance.** *Nature* 2008, **455**:396-400.
 10. Nakagawa T, Roth W, Wong P, Nelson A, Farr A, Deussing J, Villadangos JA, Ploegh H, Peters C, Rudensky AY: **Cathepsin L: critical role in II degradation and CD4 T cell selection in the thymus.** *Science* 1998, **280**:450-453.
 11. Gommeaux J, Gregoire C, Nguessan P, Richelme M, Malissen M, Guerder S, Malissen B, Carrier A: **Thymus-specific serine protease regulates positive selection of a subset of CD4(+) thymocytes.** *Eur J Immunol* 2009, **39**:956-964.
- This was the first study to demonstrate that TSSP is necessary for positive selection of some CD4 but not CD8 T cells, suggesting that TSSP plays a role in the generation of some class II binding peptides in cTEC.
12. Ziegler A, Muller CA, Bockmann RA, Uchanska-Ziegler B: **Low-affinity peptides and T-cell selection.** *Trends Immunol* 2009, **30**:53-60.
 13. Hsing LC, Rudensky AY: **The lysosomal cysteine proteases in MHC class II antigen presentation.** *Immunol Rev* 2005, **207**:229-241.
 14. Munz C: **Antigen processing via autophagy – not only for MHC class II presentation anymore?** *Curr Opin Immunol* 2010, **22**:89-93.
 15. Manoury B, Mazzeo D, Fugger L, Viner N, Ponsford M, Streeter H, Mazza G, Wraith DC, Watts C: **Destructive processing by asparagine endopeptidase limits presentation of a dominant T cell epitope in MBP.** *Nat Immunol* 2002, **3**:169-174.
 16. Carrier A, Nguyen C, Victorero G, Granjeaud S, Rocha D, Bernard K, Miazek A, Ferrier P, Malissen M, Naquet P et al.: **Differential gene expression in CD3epsilon- and RAG1-deficient thymuses: definition of a set of genes potentially involved in thymocyte maturation.** *Immunogenetics* 1999, **50**:255-270.
 17. Cheunsuk S, Sparks R, Noveroske JK, Hsu T, Justice MJ, Gershwin ME, Gruen JR, Bowlus CL: **Expression, genomic structure and mapping of the thymus specific protease prss16: a candidate gene for insulin dependent diabetes mellitus susceptibility.** *J Autoimmun* 2002, **18**:311-316.
 18. Boehm T: **The adaptive phenotype of cortical thymic epithelial cells.** *Eur J Immunol* 2009, **39**:944-947.
 19. Viret C, Leung-Theung-Long S, Serre L, Lamare C, Vignali DA, Malissen B, Carrier A, Guerder S: **Thymus-specific serine protease controls autoreactive CD4 T cell development and autoimmune diabetes in mice.** *J Clin Invest* 2011, **121**:1810-1821.
- This study shows that on the type 1 diabetes susceptible NOD background, the lack of TSSP protects against spontaneous disease. The resistance correlated with altered selection of several islet antigen-specific CD4 T cells mediated by either radioresistant or hemopoietic thymic cells.
20. Maeher R, Minter JD, Herman AE, Lennon-Dumenil AM, Mathis D, Benoist C, Ploegh HL: **Cathepsin L is essential for onset of autoimmune diabetes in NOD mice.** *J Clin Invest* 2005, **115**:2934-2943.
 21. Hsing LC, Kirk EA, McMillen TS, Hsiao SH, Caldwell M, Houston B, Rudensky AY, LeBoeuf RC: **Roles for cathepsins S, L, and B in insulinitis and diabetes in the NOD mouse.** *J Autoimmun* 2010, **34**:96-104.
 22. Honey K, Nakagawa T, Peters C, Rudensky A: **Cathepsin L: regulates CD4+ T cell selection independently of its effect on invariant chain: a role in the generation of positively selecting peptide ligands.** *J Exp Med* 2002, **195**:1349-1358.
 23. Cheunsuk S, Lian ZX, Yang GX, Gershwin ME, Gruen JR, Bowlus CL: **Prss16 is not required for T-cell development.** *Mol Cell Biol* 2005, **25**:789-796.
 24. Viret C, Lamare C, Guiraud M, Fazilleau N, Bour A, Malissen B, Carrier A, Guerder S: **Thymus-specific serine protease contributes to the diversification of the functional endogenous CD4 T cell receptor repertoire.** *J Exp Med* 2011, **208**:3-11.
- This work shows that the reduced capacity of CD4 T cells from TSSP NOD mice to react to HEL reflects variation in their TCR repertoire. Together with [19], this study suggests that TSSP deficiency promotes the expression of peptide:MHC II complexes that narrow the functional CD4 T cell repertoire through modified thymic selection.
25. Klein L, Hinterberger M, Wirnsberger G, Kyewski B: **Antigen presentation in the thymus for positive selection and central tolerance induction.** *Nat Rev Immunol* 2009, **9**:833-844.
 26. Derbinski J, Gabler J, Brors B, Tierling S, Jonnakuty S, Hergenahn M, Peltonen L, Walter J, Kyewski B: **Promiscuous gene expression in thymic epithelial cells is regulated at multiple levels.** *J Exp Med* 2005, **202**:33-45.
 27. Mathis D, Benoist C: **Aire.** *Annu Rev Immunol* 2009, **27**:287-312.
 28. Volkman A, Zal T, Stockinger B: **Antigen-presenting cells in the thymus that can negatively select MHC class II-restricted T cells recognizing a circulating self antigen.** *J Immunol* 1997, **158**:693-706.
 29. Baba T, Nakamoto Y, Mukaida N: **Crucial contribution of thymic Sirp alpha+ conventional dendritic cells to central tolerance against blood-borne antigens in a CCR2-dependent manner.** *J Immunol* 2009, **183**:3053-3063.
- The authors show that many CD11c+CD8α–Sirpα+ thymic DCs that can take up blood-circulating antigens are localized in the vicinity of vessels within the cortical region suggesting an important role in tolerance induction to circulating peripheral antigens.
30. Atibalentja DF, Byersdorfer CA, Unanue ER: **Thymus-blood protein interactions are highly effective in negative selection and regulatory T cell induction.** *J Immunol* 2009, **183**:7909-7918.
 31. Proietto AI, van Dommelen S, Zhou P, Rizzitelli A, D'Amico A, Steptoe RJ, Naik SH, Lahoud MH, Liu Y, Zheng P et al.: **Dendritic cells in the thymus contribute to T-regulatory cell induction.** *Proc Natl Acad Sci U S A* 2008, **105**:19869-19874.
 32. Li J, Park J, Foss D, Goldschneider I: **Thymus-homing peripheral dendritic cells constitute two of the three major subsets of dendritic cells in the steady-state thymus.** *J Exp Med* 2009, **206**:607-622.
- The authors show that in mice, major subsets of thymic DCs are of peripheral origin. Both immature plasmacytoid DCs and Sirpα+CD11b+CD8α– conventional DCs that are able to capture and transport circulating antigens constantly home to the thymus.
33. Bonasio R, Scimone ML, Schaerli P, Grabie N, Lichtman AH, von Andrian UH: **Clonal deletion of thymocytes by circulating dendritic cells homing to the thymus.** *Nat Immunol* 2006, **7**:1092-1100.
 34. Aschenbrenner K, D'Cruz LM, Vollmann EH, Hinterberger M, Emmerich J, Sweet LK, Rolink A, Klein L: **Selection of Foxp3+ regulatory T cells specific for self antigen expressed and presented by Aire+ medullary thymic epithelial cells.** *Nat Immunol* 2007, **8**:351-358.

6 Antigen processing

35. Roman E, Shino H, Qin FX, Liu YJ: **Cutting edge: hematopoietic-derived APCs select regulatory T cells in thymus.** *J Immunol* 2010, **185**:3819-3823.
36. Chinen T, Volchkov PY, Chervonsky AV, Rudensky AY: **A critical role for regulatory T cell-mediated control of inflammation in the absence of commensal microbiota.** *J Exp Med* 2010, **207**:2323-2330.
37. Wu L, Shortman K: **Heterogeneity of thymic dendritic cells.** *Semin Immunol* 2005, **17**:304-312.
38. Ardavin C, Wu L, Li CL, Shortman K: **Thymic dendritic cells and T cells develop simultaneously in the thymus from a common precursor population.** *Nature* 1993, **362**:761-763.
39. Corcoran L, Ferrero I, Vremec D, Lucas K, Waithman J, O'Keeffe M, Wu L, Wilson A, Shortman K: **The lymphoid past of mouse plasmacytoid cells and thymic dendritic cells.** *J Immunol* 2003, **170**:4926-4932.
40. Schlenner SM, Rodewald HR: **Early T cell development and the pitfalls of potential.** *Trends Immunol* 2010, **31**:303-310.
41. Luche H, Ardouin L, Teo P, See P, Henri S, Merad M, Ginhoux F, Malissen B: **The earliest intrathymic precursors of CD8alpha(+) thymic dendritic cells correspond to myeloid-type double-negative 1c cells.** *Eur J Immunol* 2011, **41**:2165-2175.
This study demonstrates that under physiological conditions myeloid-restricted progenitors generate the whole constellation of DCs present in the body including the thymus.
42. Koble C, Kyewski B: **The thymic medulla: a unique microenvironment for intercellular self-antigen transfer.** *J Exp Med* 2009, **206**:1505-1513.
Together with Ref. [44], this study documents the constitutive transfer of a self-antigen between medullary epithelial cells and thymic dendritic cells. Such a transfer allowed for the efficient deletion of developing thymocytes specific for the model antigen used.
43. Gallegos AM, Bevan MJ: **Central tolerance to tissue-specific antigens mediated by direct and indirect antigen presentation.** *J Exp Med* 2004, **200**:1039-1049.
44. Millet V, Naquet P, Guinamard RR: **Intercellular MHC transfer between thymic epithelial and dendritic cells.** *Eur J Immunol* 2008, **38**:1257-1263.
See comments for Ref. [42].
45. Birnberg T, Bar-On L, Sapozhnikov A, Caton ML, Cervantes-Barragan L, Makia D, Krauthgamer R, Brenner O, Ludewig B, Brockschneider D *et al.*: **Lack of conventional dendritic cells is compatible with normal development and T cell homeostasis, but causes myeloid proliferative syndrome.** *Immunity* 2008, **29**:986-997.
46. Ohnmacht C, Pullner A, King SB, Drexler I, Meier S, Brocker T, Voehringer D: **Constitutive ablation of dendritic cells breaks self-tolerance of CD4 T cells and results in spontaneous fatal autoimmunity.** *J Exp Med* 2009, **206**:549-559.
47. Bar-On L, Birnberg T, Kim KW, Jung S: **Dendritic cell-restricted CD80/86 deficiency results in peripheral regulatory T-cell reduction but is not associated with lymphocyte hyperactivation.** *Eur J Immunol* 2011, **41**:291-298.
48. Merckenschlager M, Graf D, Lovatt M, Bommhardt U, Zamoyska R, Fisher AG: **How many thymocytes audition for selection?** *J Exp Med* 1997, **186**:1149-1158.
49. Brocker T, Riedinger M, Karjalainen K: **Targeted expression of major histocompatibility complex (MHC) class II molecules demonstrates that dendritic cells can induce negative but not positive selection of thymocytes in vivo.** *J Exp Med* 1997, **185**:541-550.
50. Klein L, Hinterberger M, von Rohrscheidt J, Aichinger M: **Autonomous versus dendritic cell-dependent contributions of medullary thymic epithelial cells to central tolerance.** *Trends Immunol* 2011, **32**:188-193.
51. Derbinski J, Kyewski B: **How thymic antigen presenting cells sample the body's self-antigens.** *Curr Opin Immunol* 2010, **22**:592-600.